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ORIGINAL PAPER

Vagal tone regulates cardiac shunts during activity and at low temperatures in the South American rattlesnake, *Crotalus durissus*

Renato Filogonio[1,2] · Tobias Wang[2] · Edwin W. Taylor[1,3] · Augusto S. Abe[1] · Cléo A. C. Leite[4]

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Abstract The undivided ventricle of non-crocodilian reptiles allows for intracardiac admixture of oxygen-poor and oxygen-rich blood returning via the atria from the systemic circuit and the lungs. The distribution of blood flow between the systemic and pulmonary circuits may vary, based on differences between systemic and pulmonary vascular conductances. The South American rattlesnake, *Crotalus durissus*, has a single pulmonary artery, innervated by the left vagus. Activity in this nerve controls pulmonary conductance so that left vagotomy abolishes this control. Experimental left vagotomy to abolish cardiac shunting had no effect on long-term survival and failed to identify a functional role in determining metabolic rate, growth or resistance to food deprivation. Accordingly, the present investigation sought to evaluate the extent to which cardiac shunt patterns are actively controlled during changes in body temperature and activity levels. We compared hemodynamic parameters between intact and left-vagotomized rattlesnakes held at different temperatures and subjected to enforced physical activity. Increased temperature and enforced activity raised heart rate, cardiac output, pulmonary and systemic blood flow in both groups, but net cardiac shunt was reversed in the vagotomized group at lower temperatures. We conclude that vagal control of pulmonary conductance is an active mechanism regulating cardiac shunts in *C. durissus*.

Keywords Reptiles · Snakes · Cardiac shunt · Vagus nerve · Arterial pressure · Blood flow · Vascular regulation

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✉ Renato Filogonio
renatofilogonio@gmail.com

[1] Department of Zoology, State University of São Paulo (UNESP), Rio Claro, SP, Brazil

[2] Zoophysiology, Department of Bioscience, Aarhus University, 8000 Aarhus C, Denmark

[3] School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK

[4] Department of Physiological Sciences, Federal University of São Carlos (UFSCar), São Carlos, SP, Brazil

Introduction

The undivided ventricle of the non-crocodilian reptile heart enables variable proportions of cardiac output to bypass the systemic or pulmonary circulations, resulting in either left-to-right (L–R) or right-to-left (R–L) cardiac shunts (Wang et al. 2001). The direction and magnitude of the cardiac shunts affect arterial blood gases, and it has been suggested that active control of these shunts is as important as pulmonary ventilation in regulating arterial oxygen levels (Wang and Hicks 1996a; Wang et al. 1997). However, the functional role of cardiac shunts remains largely unresolved (Hicks and Wang 2012) and recent experimental evidence, based on chronic manipulation of cardiac shunt patterns in rattlesnakes, questions whether R–L shunts influence metabolic regulation, measured as long-term changes in metabolic rate, growth and resistance to food deprivation (Leite et al. 2013, 2014). Similar conclusions have been reached on crocodilians where permanent ligature of the left aortic arch, which normally allows for R–L shunts, is without effects on growth, diving metabolism or respiratory patterns (Eme et al. 2009, 2010).

Despite failure to demonstrate a clear physiological role for cardiac shunting in these organisms, it has been shown

that shunt patterns in turtles change consistently with metabolic rate. For instance, during activity, pulmonary vascular conductance (G_{pul}) and blood flow ($\dot{Q}_{pul}$) increase more than systemic blood flow ($\dot{Q}_{sys}$) causing a reduction in net R–L shunts (Krosniunas and Hicks 2003; West et al. 1992). Similar hemodynamic changes occur during pulmonary ventilation (Wang and Hicks 1996b), hypoxia (Wang et al. 1997; Herman and Smatresk 1999) and when temperature increases (Galli et al. 2004).

It is clear that several mechanisms may influence cardiac shunt patterns. For amphibians, it was recently argued that the rise in L–R shunt at high cardiac output (CO) is mainly driven by inherent physical properties of the vascular system, where the high distensibility of the pulmonary circuit allows for increased pulmonary blood flow (Hillman et al. 2014; Kohl et al. 2013). In reptiles, activity in the vagal innervation of the pulmonary artery can massively reduce G_{pul} and increased vagal tone can mediate large net R–L shunts (Burggren 1987; Luckhardt and Carlson 1921; Milsom et al. 1977; Taylor et al. 2009). Adrenergic control of systemic conductance (G_{sys}) may alter blood flow relations (Hicks 1994; Galli et al. 2007), and many humoral factors such as nitric oxide (Crossley et al. 2000; Galli et al. 2005a), bradykinin (Galli et al. 2005b) and adenosine (Joyce and Wang 2014) have also been demonstrated to affect cardiac shunt patterns. Thus, as the relative importance of central regulation of cardiac shunt patterns through vagal innervation of the pulmonary artery remains uncertain, it is pertinent to re-evaluate whether the vagus accounts for significant changes in cardiac shunts to respond to alterations in metabolic demand in conscious organisms. This is a fundamental question in the current debate on whether or not the regulation of cardiac shunts provide adaptive advantages to reptiles (Hicks 2002).

Given that the persistence of R–L shunt leads to arterial blood desaturation, high metabolic demands should cause reduced R–L shunts or even generate L–R shunts (Hicks and Wang 2012). In the present study, we investigate whether the vagus actively regulates cardiac shunt patterns in South American rattlesnakes (*Crotalus durissus* Linnaeus 1758). This species possesses a typical reptilian heart (Jensen et al. 2010), but only the left branch of the vagus controls the smooth muscle of its single pulmonary artery (Taylor et al. 2009). Hence, unilateral left vagotomy abolishes the control of G_{pul} without affecting regulation of heart rate (f_H), which is maintained via the right vagus (Leite et al. 2013; Taylor et al. 2009). We studied rattlesnakes with and without left vagotomy to verify whether cardiac shunts are actively regulated under different temperatures and activity.

Materials and methods

Experimental animals

Thirty-two South American rattlesnakes, *Crotalus durissus* (1.2 ± 0.1 kg), were obtained from the Butantan Institute (São Paulo, Brazil) and transported to Universidade Estadual Paulista in Rio Claro (São Paulo, Brazil), where they were maintained in 0.5 × 0.5 × 0.5 m cages at 28 ± 5 °C on a natural light regime with free access to water. They were fed rodents approximately once a week, but food was withheld 15 days prior to experiments. All incisions were accompanied by subcutaneous injection of 2 % Lidocaine (Pearson, São Paulo). At completion of the experimental protocol, snakes were euthanized by an intravenous overdose of lidocaine. All procedures were performed in accordance with the guidelines for animal experimentation (CEUA, 023/2011, UNESP, Rio Claro).

Surgery and instrumentation

The snakes were initially anesthetized with CO_2 until righting reflexes disappeared (Wang et al. 1993) and intubated for mechanical ventilation with 1–4 % isoflurane in air, at a rate of 5 breaths min^{-1} with a tidal volume of 30 ml kg^{-1}. Snakes were then defanged and their mouth sutured with two lateral stitches to render them harmless during activity trials (see experimental protocol below). The left branch of the vagus nerve was exposed through a 2 cm rostro-caudal incision caudally from the 7th line of the ventral scales. Half of the experimental group ($n = 15$) had the left vagus sectioned by removal of 1–2 cm of the nerve, as previously described (Leite et al. 2013, 2014), whereas the other half ($n = 17$) underwent a sham operation. Following trial instrumentation, blood flows and pressures were measured in separate groups of snakes, to avoid excessive instrumentation of individual animals. Flows were measured on 20 snakes (1.4 ± 0.1 kg), either intact or vagotomized (10 in each group), while pressures were measured on 12 snakes (0.8 ± 0.1 kg; 6 in each group).

A 5 cm ventrolateral incision cranial to the heart provided access to the systemic and pulmonary arteries. For measurements of mean systemic pressure (P_{sys}), a catheter was inserted occlusively into the vertebral artery and forwarded into the right aortic arch. Another catheter, for measurement of mean pulmonary pressure (P_{pul}), was inserted occlusively into a branch of the pulmonary artery. Both catheters (PE50) contained heparinized saline (100 IU/ml) and were connected to Baxter Edward (model PX600, Irvine, CA, USA) disposable pressure transducers. Signals were amplified with an in-house

built preamplifier and transducers were calibrated daily with a static water column. Blood flow was measured using probes (2.0RB or 1.5RB, Transonic Systems, Inc, NY, USA) placed around the left aortic arch or the pulmonary artery to record respective blood flows ($\dot{Q}_{LAo}$ and $\dot{Q}_{pul}$). Ultrasound gel was infused around each blood flow probe to enhance the signal. The probes were connected to a dual-channel blood flow meter (Transonic T206, Transonic Systems, Inc, NY, USA). Signals from pressure transducers and flow probes were recorded via a Biopac MP100 data acquisition system (Biopac Systems, Inc., Goleta, CA, USA) at a rate of 400 samples s^{-1}. After surgery, each animal was placed in a plastic box 30 × 50 × 10 cm ($W \times L \times H$) maintained within a temperature-controlled chamber at a range of experimental temperatures (see protocol).

Experimental protocol

Cardiovascular parameters were found to have stabilized 24 h after surgery held in any of the three experimental temperatures: 15, 20 or 30 °C. At the end of these equilibration periods, blood pressures or flows were recorded for several hours from each snake while it remained undisturbed and following 5 min of uninterrupted activity. Activity was enforced by handling the snake within the temperature-controlled chamber to entice movement and consisted in immobilizing the animal's head while the body freely moved in response to continuous manipulation. After 5 min, snakes appeared fatigued as they no longer responded to handling. After each recording, a change in temperature was randomly assigned within the chamber and snakes were let to equilibrate to the new temperature for a further 24 h, when the protocol was repeated.

Calculation of blood flows, stroke volumes, vascular conductances and shunt

Systemic blood flow ($\dot{Q}_{sys}$) was calculated from $\dot{Q}_{LAo}$ using the pooled correction factor relating both flows as $\dot{Q}_{sys} = 2.6 \times \dot{Q}_{LAo}$ (Filogonio et al. 2014). Cardiac output (CO) was calculated as the sum of $\dot{Q}_{sys}$ and $\dot{Q}_{pul}$. $f_H$was derived from the pulsatile signals of either blood pressure or flow. Systemic and pulmonary stroke volumes (Vs_{sys} and Vs_{pul}, respectively) were calculated from the integration of the area under the blood flow signal in systemic and pulmonary circulations, respectively. Total stroke volume (Vs) was the sum of Vs_{sys} and Vs_{pul}. Vascular conductances (G_{sys} and G_{pul}) were calculated as $\dot{Q}_{sys}/P_{sys}$ and $\dot{Q}_{pul}/P_{pul}$, respectively, assuming that venous blood pressures could be neglected (Crossley et al. 1998; Galli et al. 2004, 2007). When the ratio $\dot{Q}_{pul}/\dot{Q}_{sys}$ was >1, this indicated an L–R cardiac shunt while a ratio <1 indicated an R–L shunt.

Statistical analyses

One-way ANOVA for repeated measurements, followed by a post hoc Student–Newman–Keuls test, was used to verify the effects of temperature within each experimental group: intact snakes either inactive or following a bout of activity, left-vagotomized snakes, inactive or following activity. *T* tests were used to assess the effects of vagotomy on inactive or active animals at each temperature, and paired *T* tests to assess the effects of activity within both intact and vagotomized groups, at each temperature. This statistical approach was chosen because our set of data had two repeated measure factors (temperature and activity) and one non-repeated measure factor (vagotomy), precluding us from using a three-way ANOVA design. For G_{sys} and G_{pul} and data derived from $\dot{Q}_{LAo}$, variances of products and ratios were calculated as proposed by Goodman (1969) and Kempen and Vliet (2000). Corresponding nonparametric tests were used whenever homoscedasticity or normality assumptions were not met. The assumption of sphericity was met for all repeated measure tests. A significance level was assigned at 95 % probability ($P < 0.05$) in all cases. Data are presented as mean ± SD.

Results

Effects of temperature change in intact snakes

Heart rate and CO increased with temperature (Fig. 1a, c). Vs_{sys} decreased between 20 and 30 °C, although neither Vs nor Vs_{pul} was affected (Table 1). Both P_{sys} and $\dot{Q}_{sys}$ rose proportionally from 15 to 30 °C (Table 1; Fig. 1e); thus, G_{sys} remained unchanged (Fig. 2). In contrast, P_{pul} was unaffected by temperature (Table 1), and the threefold elevation of $\dot{Q}_{pul}$ between 15 and 30 °C (Fig. 1g) was mediated by a significant rise in G_{pul} (Fig. 2a). Despite this differential regulation of G_{pul} and G_{sys}, the R–L shunt was unaffected (Fig. 3).

Cardiovascular responses to activity in intact snakes

Both CO and f_H increased during activity at all temperatures (Fig. 1a), but Vs increased only at 15 °C (Table 1). Although P_{sys} increased (Table 1), G_{sys} was only affected at high temperatures (Fig. 2), such that $\dot{Q}_{sys}$ rose by 80 % at 30 °C, but did not change at the lower temperatures (Fig. 1). The rise in $\dot{Q}_{sys}$ was due to tachycardia, since Vs_{sys} did not change (Table 1). P_{pul} increased during activity at 30 °C (Table 1), whereas G_{pul} increased threefold at 15 °C and maintained very similar values at all temperatures (Fig. 2). Consequently, $\dot{Q}_{pul}$ was consistently higher than inactive values (Fig. 1g). Vs_{pul} did not change at

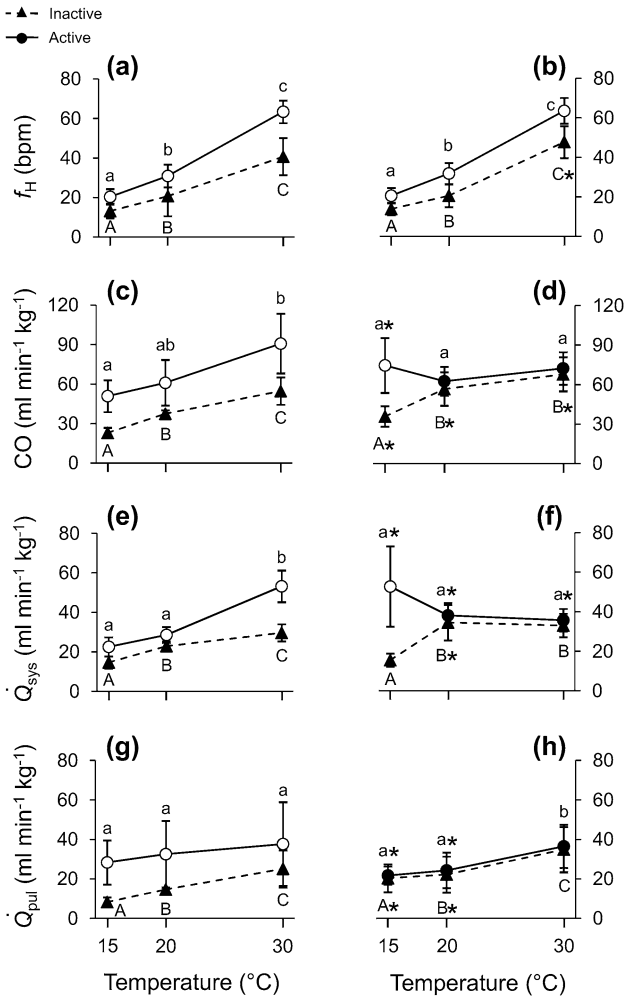

Fig. 1 Effects of temperature on either intact (**a**, **c**, **e**, **g**) or left-vagotomized (**b**, **d**, **f**, **h**) rattlesnakes (*Crotalus durissus*), inactive (*triangle*) or during activity (*circle*). Variables are: heart rate (f_H), cardiac output (CO), systemic blood flow ($\dot{Q}_{sys}$) and pulmonary blood flow ($\dot{Q}_{pul}$). *Different capital letters* indicate differences due to temperature change within inactive groups; *different small letters* indicate differences due to temperature change within active groups; *open symbols* indicate significant differences between the mean values from active and inactive snakes; an *asterisk* indicates an effect of left vagotomy. Values are mean ± SD. Statistical significance was $P < 0.05$. Sample sizes were: intact and inactive, $n = 10$; intact and active, $n = 9$; vagotomized and inactive, $n = 10$; vagotomized and active, $n = 9$

30 °C, but increased at lower temperatures (Table 1). L–R shunt decreased (Fig. 3) as $\dot{Q}_{sys}$ increased (Fig. 1e) and $\dot{Q}_{pul}$ remained stable (Fig. 1g).

Effect of left vagotomy in inactive snakes

The lack of control of pulmonary artery vasoconstriction in left-vagotomized snakes did not affect f_H except for a 17 % rise at 30 °C (Fig. 1b), but CO increased at all temperatures (Fig. 1d). *Vs* rose at 15 and 20 °C but decreased at 30 °C, when it was similar to intact snakes (Table 1). Increased *Vs* and CO related to higher Vs_{pul}, as Vs_{sys} was unaltered (Table 1). P_{pul} increased at 30 °C and P_{sys} was unaffected (Table 1).

G_{pul} increased compared to intact snakes at 15 °C, but remained constant with changes in temperature, whereas G_{sys} was unaltered at all temperatures (Fig. 2). $\dot{Q}_{sys}$ was similar to intact animals at 15 and 30 °C (Fig. 1f), and $\dot{Q}_{pul}$ increased at 15 and 20 °C (Fig. 1h). Thus, vagotomy elicited an L–R shunt that reduced with temperature (Fig. 3).

Cardiovascular responses of left-vagotomized snakes to activity

In the vagotomized group, exercise tachycardia was similar to intact animals (Fig. 1b). The increase in f_H with temperature was matched by a reduction in *Vs* (Table 1), such that CO increased with activity at 15 °C, but remained unchanged at 20 and 30 °C (Fig. 1d). P_{sys} in active vagotomized snakes was identical to those recorded in intact animals, whereas P_{pul} was higher than in intact animals at 30 °C (Table 1). Both P_{sys} and P_{pul} diverged from inactive left-vagotomized values only at 30 °C (Table 1). The constant G_{pul} was similar to that presented by active intact snakes (Fig. 2). G_{sys} increased 2.6-fold compared to inactive vagotomized snakes at 15 °C, but drastically decreased with temperature (61 %, see Fig. 2). This is the inverse hemodynamic alteration to that observed in active intact snakes (Figs. 3, 4).

Discussion

Critique of the method

It is important to note that the methods used to calculate variances (Goodman 1969; Kempen and Vliet 2000) are predicted to inflate the apparent standard deviation. However, we opted to calculate conductances using pressures and flows obtained from different individuals to reduce the stress imposed on each individual animal by insertion of probes (two cannulae or two blood flow probes placed in central vessels). Nevertheless, the qualitative patterns we found are in accordance with previous studies in *C. durissus*, with an increase of G_{pul} and slight decrease of G_{sys} with injected epinephrine (Galli et al. 2005a, 2007). Also, the reported values for mean arterial pressures and flows are in accordance with previous studies (Galli et al. 2005a, b, 2007; Leite et al. 2013; Taylor et al. 2009).

Table 1 Effects of temperature change (15, 20 or 30 °C), activity (inactive or active) and vagotomy (intact or vagotomized) on mean blood pressures and stroke volumes (mean ± SD) of rattlesnakes

Treatment	P_{sys} (kPa)	P_{pul} (kPa)	Vs (ml kg^{-1})	Vs_{sys} (ml kg^{-1})	Vs_{pul} (ml kg^{-1})
Intact					
Inactive					
15 °C	2.6 ± 0.2^{a}	2.2 ± 0.2^{a}	1.7 ± 0.4^{a}	1.1 ± 0.4^{a}	0.6 ± 0.1^{a}
20 °C	3.5 ± 0.2^{ab}	2.6 ± 0.2^{a}	1.7 ± 0.6^{a}	1.1 ± 0.6^{a}	0.6 ± 0.1^{a}
30 °C	4.4 ± 0.5^{b}	2.3 ± 0.2^{a}	1.3 ± 0.3^{a}	0.7 ± 0.2^{b}	0.6 ± 0.1^{a}
Active					
15 °C	4.0 ± 0.4^{a}*	2.9 ± 0.3^{a}	2.3 ± 0.5^{a}*	1.1 ± 0.3^{a}	1.2 ± 0.2^{a}*
20 °C	4.6 ± 0.2^{a}*	3.2 ± 0.2^{a}	1.8 ± 0.5^{ab}	0.9 ± 0.2^{ab}	0.9 ± 0.2^{b}*
30 °C	6.0 ± 0.5^{b}*	4.0 ± 0.3^{b}*	1.4 ± 0.3^{b}	0.8 ± 0.1^{b}	0.6 ± 0.1^{c}
Vagotomized					
Inactive					
15 °C	3.2 ± 0.5^{a}	2.9 ± 0.4^{a}	**2.4 ± 0.5^{a}**	1.1 ± 0.3^{a}	**1.3 ± 0.1^{a}**
20 °C	4.2 ± 0.5^{a}	3.3 ± 0.5^{a}	**2.6 ± 0.7^{a}**	1.7 ± 0.6^{b}	**0.9 ± 0.2^{b}**
30 °C	4.6 ± 0.3^{a}	**3.6 ± 0.3^{b}**	1.4 ± 0.3^{b}	0.7 ± 0.2^{c}	0.7 ± 0.1^{c}
Active					
15 °C	3.6 ± 0.3^{a}	3.2 ± 0.3^{a}	**3.5 ± 1.1^{a}***	**2.6 ± 1.1^{a}***	0.9 ± 0.1^{a}
20 °C	4.4 ± 0.3^{b}	3.6 ± 0.5^{a}	1.9 ± 0.4^{b}	1.2 ± 0.3^{b}	**0.7 ± 0.1^{b}***
30 °C	6.2 ± 0.2^{c}*	**4.6 ± 0.4^{b}***	1.1 ± 0.2^{c}*	**0.6 ± 0.1^{b}**	0.6 ± 0.1^{b}

Different letters denote differences after temperature change

P_{sys} mean systemic pressure, P_{pul} mean pulmonary pressure, Vs total stroke volume, Vs_{sys} systemic stroke volume, Vs_{pul} pulmonary stroke volume

* Difference due to activity; bold values indicate differences due to vagotomy. Statistical significance was assigned as $P < 0.05$ for all cases. For P_{sys} and P_{pul}, sample sizes were $n = 6$; for Vs, Vs_{sys} and Vs_{pul}, sample sizes were: intact and inactive, $n = 10$; intact and active, $n = 9$; vagotomized and inactive, $n = 10$; vagotomized and active, $n = 9$

Effects of temperature on intact snakes

The rise in CO with temperature of intact, inactive snakes was proportional to the elevation of O_2 uptake reported by Leite et al. (2013) and almost exclusively achieved by increased f_H as in other squamates (Clark et al. 2005; Stinner 1987; Tucker 1966; Wood et al. 1977) and chelonians (Kinney et al. 1977; Krosniunas and Hicks 2003). The combination of altered autonomic tones and the direct effect of temperature on the cardiac pacemaker tissue may be the primary reason for the observed tachycardia (Lillywhite et al. 1999; Seebacher 2009). However, in contrast to other reptiles (Galli et al. 2004; Stecyk et al. 2004; Stinner 1987), G_{sys} did not increase with temperature because of concomitant elevations of both P_{sys} and $\dot{Q}_{sys}$. The maintenance of R–L shunts at different temperatures indicates that resting metabolic rate changes with temperature observed in *C. durissus* (Leite et al. 2013) are unrelated to shunt patterns. Rather, O_2 demand is probably matched by a combination of CO increase and adjustments in blood O_2-carrying capacity, which is evidenced by the rise of arterial P_{O_2} and HbO_2 saturation in *C. durissus* (Wang et al. 1998).

Cardiovascular responses to activity in intact snakes

Leite et al. (2013) demonstrated that 5 min of forced activity elevates O_2 uptake four to sixfold at 30 °C in *C. durissus*. The present experiments show that f_H and CO increase 56 and 66 % during a bout of activity, which implies that the arterial–venous (AV) O_2 concentration difference was increased during activity as described in other reptiles (Clark et al. 2005; Frappell et al. 2002; Gleeson et al. 1980). Increased G_{pul} during activity in all temperatures is probably a result from reduced vagal tone, which resulted in $\dot{Q}_{pul}$ and L–R shunt increase and is in agreement with previous studies of reptiles during exercise (Frappell et al. 2002; Hicks and Krosniunas 1996).

Effect of left vagotomy in inactive snakes

Cardiac shunts were related to the ratio between vascular conductances (Fig. 4a; Crossley et al. 1998; Galli et al. 2007; Hicks et al. 1996). Left vagotomy induced loss of control of G_{pul} that resulted in elevated $\dot{Q}_{pul}$ and L–R shunts in inactive rattlesnakes at 15 °C, which suggests that

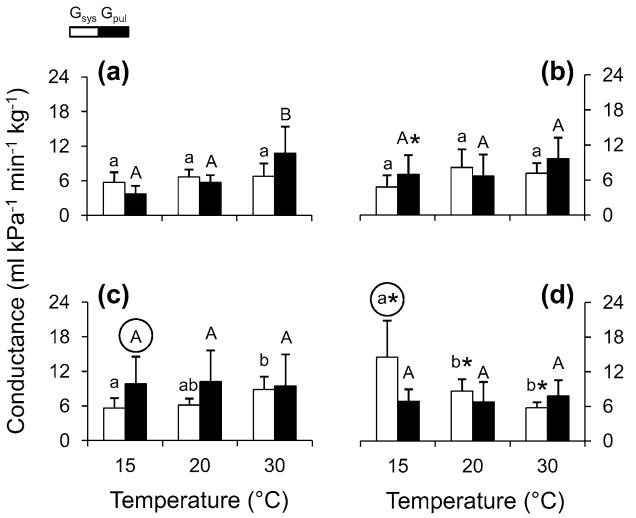

Fig. 2 Variation of systemic and pulmonary vascular conductances (G_{sys} = open bars; G_{pul} = closed bars, respectively—ml kPa^{-1} min^{-1} kg^{-1}) according to different temperatures (15 °C, 20 °C and 30 °C) in the experimental groups: **a** inactive and intact; **b** inactive and vagotomized; **c** active and intact; **d** vagotomized and active. *Different small letters* indicate differences due to temperature change in G_{sys}; *different capital letters* indicate differences due to temperature change in G_{pul}; the ringed letter indicates a difference between inactive and active snakes; an *asterisk* indicates an effect of left vagotomy. Values are mean ± SD. Statistical significance was $P < 0.05$. Sample sizes were: intact and inactive, $n = 16$; intact and active, $n = 15$; vagotomized and inactive, $n = 16$; vagotomized and active, $n = 15$

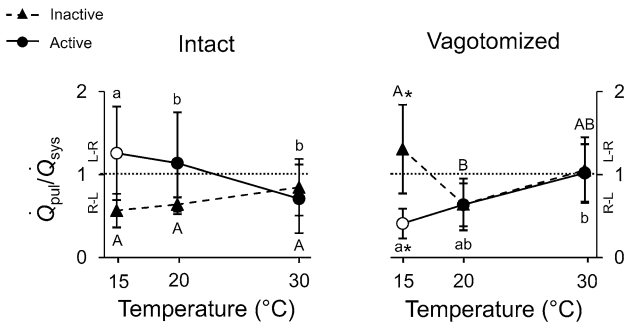

Fig. 3 Effects of temperature and activity in cardiac shunt direction ($\dot{Q}_{pul}/\dot{Q}_{sys}$) on either intact or left-vagotomized snakes, when inactive (*triangle*) or during activity (*circle*). *Different capital letters* indicate differences due to temperature change within inactive groups; *different small letters* indicate differences due to temperature change within active groups; an *asterisk* indicates an effect of left vagotomy. Values are mean ± SD. Statistical significance was $P < 0.05$. Sample sizes were: intact and inactive, $n = 10$; intact and active, $n = 9$; vagotomized and inactive, $n = 10$; vagotomized and active, $n = 9$

increased vagal activity sustains the R–L shunt at reduced temperatures. This finding confirms numerous reports outlining the importance of vagal tone in determining cardiac shunt direction in reptiles (Burggren 1977; Milsom et al. 1977; Hicks 1994; Comeau and Hicks 1994; Hicks and Comeau 1994).

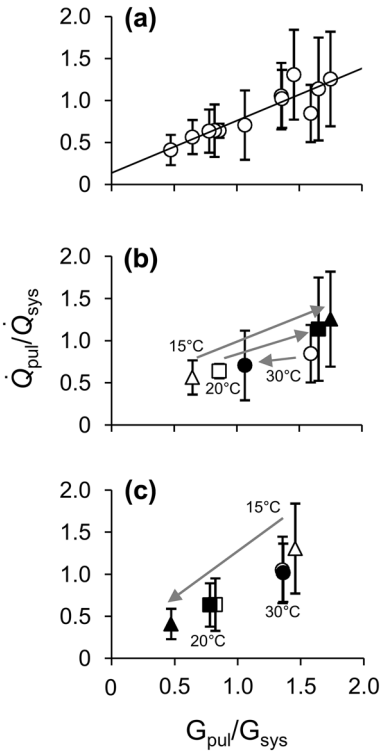

Fig. 4 **a** Positive regression between cardiac shunt direction ($\dot{Q}_{pul}/\dot{Q}_{sys}$) and ratio of vascular conductances (G_{pul}/G_{sys}) in the overall data set ($r^2 = 0.82$; $P < 0.001$). Variations in $\dot{Q}_{pul}/\dot{Q}_{sys}$ as a function of G_{pul}/G_{sys} on either intact (**b**) or vagotomized (**c**) snakes at 15 °C (*triangle*), 20 °C (*square*) or 30 °C (*circle*). *Open symbols* represent inactive and *closed symbols* active snakes. *Arrows* represent the direction of the variation on cardiac shunts due to activity at a given temperature. Values are mean ± SD. Sample sizes were: intact and inactive, $n = 16$; intact and active, $n = 15$; vagotomized and inactive, $n = 16$; vagotomized and active, $n = 15$

Cardiovascular responses of left-vagotomized snakes to activity

The pooled effects of activity and left vagotomy altered $\dot{Q}_{sys}$, CO, Vs_{sys}, $\dot{Q}_{pul}/\dot{Q}_{sys}$ and G_{sys} when compared to active intact individuals. We speculate that the growing AV difference during activity and the inability to properly direct O_2-rich blood to the systemic circulation led to a drastic regulation of G_{sys} during activity, which raised far above G_{pul} and allowed O_2-rich blood to circulate at the systemic circuit. Hence, vagotomized rattlesnakes clearly displayed inverse shunt patterns in response to activity at 15 °C.

The fact that oxygen consumption ($\dot{V}O_2$) was unaffected by left vagotomy during activity at 20 and 30 °C (Leite et al. 2013) is consistent with our observations that

shunt patterns are not significantly different at these same temperatures (Fig. 3). Furthermore, similar shunt patterns displayed by both intact and vagotomized inactive snakes are in accordance with the observations of Wang and Hicks (2008) that found no effect of shunt patterns on both $\dot{V}O_2$ and $\dot{V}CO_2$ in *T. scripta*. Therefore, our data does not support the hypothesis that cardiac shunt patterns change according to metabolic demands on these temperatures. However, the observed L–R shunt at 15 °C during activity shows that at lower temperatures, shunt patterns do respond to metabolic changes. Unfortunately, Leite et al. (2013) do not report $\dot{V}O_2$ during activity at 15 °C, so we cannot make comparisons with their data. Nevertheless, the O_2-carrying capacity in *C. durissus* is markedly reduced at 15 °C compared to 25 and 35 °C (Wang et al. 1998). In this scenario, increased L–R shunts could be tremendously beneficial to blood oxygenation during elevated metabolic demands due to activity by directing a higher portion of CO to the pulmonary circulation.

Although many mechanisms may influence cardiac shunts—humoral factors (Crossley et al. 2000; Galli et al. 2005a, b; Joyce and Wang 2014), adrenergic tone (Hicks 1994; Galli et al. 2007) and intrinsic differences between systemic and pulmonary vascular distensibilities (Kohl et al. 2013; Hillman et al. 2014)—left vagotomy completely inverted the shunt patterns observed at 15 °C. This is in accordance with the observations of cholinergic control of R–L shunts in *T. scripta* (Hicks and Comeau 1994). Further evidence of vagal influence on shunt patterns may also include its effects on the direction of heart depolarization during lung ventilation (Burggren 1978). Based on the present data, it is clear that vagal regulation of pulmonary vascular tone is an essential active mechanism controlling cardiac shunt patterns in *C. durissus*.

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