

RESEARCH ARTICLE

Winter metabolic depression does not change arterial baroreflex control of heart rate in the tegu lizard *Salvator merianae*

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ABSTRACT

Baroreflex regulation of blood pressure is important for maintaining appropriate tissue perfusion. Although temperature affects heart rate (f_H) reflex regulation in some reptiles and toads, no data are available on the influence of temperature-independent metabolic states on baroreflex. The South American tegu lizard *Salvator merianae* exhibits a clear seasonal cycle of activity decreasing f_H along with winter metabolic downregulation, independent of body temperature. Through pharmacological interventions (phenylephrine and sodium nitroprusside), the baroreflex control of f_H was studied at $\sim 25^\circ\text{C}$ in spring–summer- and winter-acclimated tegus. In winter lizards, resting and minimum f_H were lower than in spring–summer animals (respectively, 13.3 ± 0.82 versus 10.3 ± 0.81 and 11.2 ± 0.65 versus 7.97 ± 0.88 beats min^{-1}), while no acclimation differences occurred in resting blood pressure (5.14 ± 0.38 versus 5.06 ± 0.56 kPa), baroreflex gain (94.3 ± 10.7 versus $138.7 \pm 30.3\%$ kPa^{-1}) or rate–pressure product (an index of myocardial activity). Vagal tone exceeded the sympathetic tone of f_H , especially in the winter group. Therefore, despite the lower f_H , winter acclimation does not diminish the f_H baroreflex responses or rate–pressure product, possibly because of increased stroke volume that may arise because of heart hypertrophy. Independent of acclimation, f_H responded more to hypotension than to hypertension. This should imply that tegus, which have no pressure separation within the single heart ventricle, must have other protection mechanisms against pulmonary hypertension or oedema, presumably through lymphatic drainage and/or vagal vasoconstriction of pulmonary artery. Such a predominant f_H reflex response to hypotension, previously observed in anurans, crocodylians and mammals, may be a common feature of tetrapods.

KEY WORDS: Adrenergic receptors, Baroreceptor, Blood pressure, Cholinergic tone, Hibernation, Seasonal changes

INTRODUCTION

Baroreflex regulation of arterial blood pressure is important to the maintenance of adequate tissue perfusion and thus matching oxygen and nutrient delivery to metabolic demands (Bagshaw, 1985; Hagensen et al., 2010; Zena et al., 2015). With respect to ectothermic reptiles, there is evidence of baroreceptors that monitor blood pressure in turtles (Millard and Moalli, 1980;

Stephens et al., 1983; Crossley et al., 2015), snakes (Lillywhite and Seymour, 1978; Lillywhite and Gallagher, 1985), the lizard *Tiliqua rugosa* (formerly known as *Trachydosaurus rugosus*; Berger et al., 1980) and crocodylians (Altimiras et al., 1998; Crossley et al., 2003; Hagensen et al., 2010).

Baroreflex regulation in crocodylians is characterized by a pronounced heart rate (f_H) reflex response to hypotension, as has been reported for the saltwater crocodile, *Crocodylus porosus*, and the broad-snouted caiman, *Caiman latirostris* (Altimiras et al., 1998; Hagensen et al., 2010). It is interesting to note that baroreflex sensitivity is enhanced at a high temperature (30°C) in *C. latirostris* (Hagensen et al., 2010), and also in the toad *Rhinella schneideri* (Zena et al., 2015), but the ability to respond mainly to hypotension is preserved regardless of temperature in both species. Temperature is known to directly influence metabolic rate and the cardiovascular adjustments to the new metabolic demands seem to be a proportional response (Piercy et al., 2015). In contrast, there are situations when metabolic rate can be downregulated independent of temperature in ectotherms; for example, when they are seasonally exposed to adverse environmental conditions and enter the physiological/behavioural state of hibernation or aestivation (Abe, 1995; Glass et al., 1997; Bicego-Nahas et al., 2001; Andrade et al., 2004; Milsom et al., 2008; Navas and Carvalho, 2010). Seasonal reductions in metabolic rate of ectotherms is marked by profound temperature-independent suppression of many physiological and biochemical processes (Navas and Carvalho, 2010), including reduction in f_H (Delaney et al., 1974; Glass et al., 1997; Bicego-Nahas et al., 2001; da Silva et al., 2008; Sanders et al., 2015).

The South American tegu lizard *Salvator merianae* (Harvey et al., 2012), formerly known as *Tupinambis merianae* (Duméril and Bibron 1938), exhibits a pronounced seasonal cycle of activity/inactivity. During spring and summer, tegus are diurnally active (Klein et al., 2006). As winter approaches, animals retreat to underground burrows and actively suppress metabolism (Lopes and Abe, 1999; Sanders et al., 2015) even though ambient temperatures in winter in southeastern Brazil are relatively benign (tegu burrows can reach $\sim 17^\circ\text{C}$; Abe, 1995; Andrade et al., 2004; Sanders et al., 2015). Investigations of seasonal energy metabolism, ventilation and cardiac morphology in tegus have focused on the underlying mechanism(s) of metabolic suppression (Abe, 1983; Andrade and Abe, 1999; de Souza et al., 2004; Milsom et al., 2008; Toledo et al., 2008; da Silveira et al., 2013) and have elucidated reduced thermal sensitivity of metabolic rate in dormant tegus (Abe, 1995; Milsom et al., 2008; Sanders et al., 2015). Therefore, this species is especially important as a model for investigating f_H baroreflex responses independent of temperature because it may undergo changes in cardiovascular autonomic balance across seasons.

In the present study, we hypothesize that the metabolic reduction of winter-acclimated *S. merianae* decreases f_H baroreflex sensitivity along with the suppression of other cardiovascular variables in

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List of symbols and abbreviations

f_H	heart rate
PAP	pulsatile arterial pressure
P_D	diastolic pressure
PE	phenylephrine
P_{MA}	mean arterial blood pressure
P_S	systolic pressure
RPP	rate-pressure product
SNP	sodium nitroprusside

comparison to the spring–summer-acclimated animals at the same ambient temperature ($\sim 25^\circ\text{C}$). To this end, peripheral injections of sodium nitroprusside (SNP), a vasodilator, and the $\alpha 1$ -adrenergic agonist phenylephrine (PE), a vasoconstrictor, were performed to evaluate the reflex responses of f_H against changes in blood pressure. In addition, adrenergic and cholinergic tones on the heart were determined, and a full autonomic blockade was performed to test the relative role of sympathetic and parasympathetic modulation on the tegu f_H response to hypotension and hypertension. In addition, the underlying influences of α - and β -adrenergic receptors in the tegu cardiovascular system were measured.

MATERIALS AND METHODS**Animals**

Experiments were conducted on unanaesthetized tegu lizards *S. merianae*. Tegus of both sexes had been captive bred at the Jacarezário, São Paulo State University, in Rio Claro, São Paulo state (IBAMA permit no. 02001-000412/94-28 and SISBIO-ICMBio/n. 26677-1). Different young animals, during their second year cycle, were used for the spring–summer (from October to February; 586.2 ± 72.2 g body mass) versus the winter (July; 392 ± 30.67 g) experiments, which were performed in two consecutive years. At least 3 weeks before the experiments were initiated, animals were group-housed indoors in plastic boxes ($70 \times 70 \times 100$ cm) lined with wood shavings and containing PVC tubes for sheltering. During the spring–summer season, tegus were maintained under a natural photoperiod and fed with chicken eggs and fruits three times per week until 7 days before surgery. Water was available *ad libitum*. A source of heat for thermoregulation was provided by a heating lamp programmed to turn on at 09:00 h and turn off at 17:00 h, except for the week before surgery. Temperatures varied from 23 – 25°C (lamp off) to 33 – 35°C (lamp on). During winter months, tegus were maintained in darkness at 23 – 25°C with no source of heat and no food. These animals, even in captivity, voluntarily stop eating at the beginning of winter (K.C.B., personal observations; Andrade and Abe, 1999; de Souza et al., 2004). This study was conducted with the approval of the local ethical committee of São Paulo State University (CEUA; protocol no. 021802-07).

Drugs

SNP (nitric oxide donor), PE (agonist of $\alpha 1$ -adrenergic receptors), atropine (antagonist of muscarinic receptors), sotalol (antagonist of β -adrenergic receptors), prazosin (antagonist of $\alpha 1$ -adrenergic receptors) and isoproterenol (agonist of β -adrenergic receptors) were purchased from Sigma (St Louis, MO, USA). All drugs were dissolved in sterile saline.

Surgery

Lizards were anaesthetized with 5% isoflurane in 100% oxygen, via face mask, in an open circuit. After induction, the trachea was intubated with an endotracheal tube inserted through the mouth; the endotracheal tube was fitted for the animal's trachea. Isoflurane (~ 1 Vol%) was

Table 1. Resting cardiovascular and baroreflex variables derived from sigmoidal baroreflex–heart rate (f_H) curves generated after bolus injections of phenylephrine and sodium nitroprusside (Eqn 1) for spring–summer- and winter-acclimated tegus, *Salvator merianae*

	Spring–summer	Winter
Resting variables		
P_{MA} (kPa)	5.14 ± 0.38	5.06 ± 0.56
f_H (beats min^{-1})	13.3 ± 0.82	$10.3 \pm 0.81^*$
RPP (kPa min^{-1})	86.0 ± 10.5	70.6 ± 12.4
Baroreflex variables		
Minimum f_H (beats min^{-1})	11.2 ± 0.65	$7.97 \pm 0.88^*$
Maximum f_H (beats min^{-1})	35.9 ± 2.10	33.5 ± 5.43
f_H range (beats min^{-1})	24.7 ± 1.97	25.5 ± 4.67
R^2	0.92 ± 0.02	0.86 ± 0.06
Slope coefficient (beats min^{-1} kPa^{-1})	10.7 ± 1.21	10.2 ± 1.63
$P_{MA,50}$ (kPa)	3.38 ± 0.17	3.86 ± 0.29
G_{50} (beats min^{-1} kPa^{-1})	20.1 ± 3.21	17.4 ± 4.56
Normalized gain (% kPa^{-1})	94.3 ± 10.7	138.7 ± 30.3

*Indicates a significant difference between seasons (Student's *t*-test; $P < 0.05$). P_{MA} , mean arterial blood pressure; RPP, rate-pressure product; R^2 , coefficient of determination; slope coefficient, slope of the linear portion of the curve; $P_{MA,50}$, mean arterial blood pressure at the midpoint of the curve; G_{50} , maximum gain. Winter ($N=5$); spring–summer ($N=11$).

delivered with 0.5 l min^{-1} oxygen flow from an agent-specific vaporizer (VetCase, Brasmed, São Paulo, Brazil). The depth of anaesthesia was monitored during the surgical procedure by maintaining breathing frequency at 1 – 2 breaths min^{-1} . A polyethylene cannula (Clay Adams, Parsippany, NJ, USA) made from a connection between a PE-10 cannula and a PE-50 cannula filled with heparinized 0.9% NaCl solution (100 i.u. ml^{-1} heparin) was occlusively inserted into the femoral artery for the measurement of pulsatile arterial pressure (PAP). The same procedure was followed to cannulate the femoral vein for drug injections. After surgery, animals received intramuscular injections of an analgesic (flunixin meglumine; 1 mg kg^{-1}) and an antibiotic (enrofloxacin; 5 mg kg^{-1}) agent according to recommended dosages for reptiles (Martinez-Jimenez and Hernandez-Divers, 2007; Mosley, 2011). After recovery from anaesthesia, each animal was individually placed in an experimental chamber, where it was kept for a minimum of 24 h without disturbance until the beginning of the experimental procedure.

Measurements of blood pressure and heart rate

The arterial cannula was connected to a pressure transducer (TSD 104A, Biopac Systems, Santa Barbara, CA, USA) calibrated against a mercury column. The signal from the pressure transducer was collected by an acquisition system (Biopac Systems model MP 100A-CE). Heart rate was derived from the PAP signal and mean arterial blood pressure (P_{MA}) was calculated as $P_{MA} = P_D + 1/3(P_S - P_D)$, where P_D is the diastolic pressure and P_S is the systolic pressure. Rate-pressure product (RPP), as a measure of myocardial activity, was calculated as $f_H \times P_S$ (kPa min^{-1}).

Experimental protocols

All experiments were conducted in tegus at an ambient temperature of $25.5^\circ\text{C} \pm 1.5^\circ\text{C}$, measured by a temperature sensor connected to an acquisition system (Biopac Systems model MP 100A-CE). After a 30–40 min basal recording of PAP, 0.5 ml kg^{-1} of saline was injected into the femoral vein to verify the possibility that the injection per se may influence P_{MA} and f_H . Serial increasing doses of SNP (2.5 , 5.0 , 10.0 and $25.0 \mu\text{g kg}^{-1}$) and PE (5.0 , 10.0 , 25.0 and $50.0 \mu\text{g kg}^{-1}$) were then injected (0.25 ml kg^{-1} followed by

0.25 ml kg⁻¹ of saline during 1 min). Each dose was injected only after the recorded cardiovascular variables had returned to the pre-injection values. This baroreflex protocol was performed in both spring–summer- and winter-acclimated animals. Because no difference in baroreflex sensitivity was observed between groups (Table 1), the pharmacological autonomic blockade of the f_H reflex responses was only performed in spring–summer tegus. To this end, atropine (1.5 mg kg⁻¹; muscarinic antagonist) plus sotalolol (6.0 mg kg⁻¹; β -adrenergic antagonist) were injected before PE (25.0 μ g kg⁻¹) or SNP (10.0 μ g kg⁻¹). Spring–summer animals were further tested to verify the influence of α 1- and β -adrenergic receptors on P_{MA} and f_H (the mechanism that underlies the hypertensive response to PE, the tachycardic response to isoproterenol, as well as the effectiveness of drugs): (1) injection of prazosin (0.2 mg kg⁻¹; α 1-adrenergic antagonist) alone or combined with PE (25.0 μ g kg⁻¹) and (2) injection of sotalolol (6.0 mg kg⁻¹) alone or combined with isoproterenol (2.5 μ g kg⁻¹; β -adrenergic antagonist). All doses were chosen on the basis of preliminary experiments and previous reports on reptiles (Altimiras et al., 1998; Skovgaard et al., 2005; Hagensen et al., 2010).

Analysis of the baroreflex

f_H (beats min⁻¹) was plotted against P_{MA} (kPa) data for each serial increasing dose of SNP and PE for each individual animal. The relationship between P_{MA} and f_H for each tegu was analyzed using a four-variable sigmoidal logistic function as described previously by Reid (1996):

$$f_H = \frac{(A - D)}{1 + (P_{MA}/C)^B} + D, \quad (1)$$

where A is the maximum f_H (beats min⁻¹), D is the minimum f_H of the curve, B is the slope coefficient (beats min⁻¹ kPa⁻¹), and C is the P_{MA} at the midpoint of the f_H range ($P_{MA,50}$; kPa), which represents the set-point of the baroreflex. The range of the baroreflex was calculated as the difference between the maximum and minimum f_H ($A - D$). The maximum gain (G_{50} ; in beats min⁻¹ kPa⁻¹) of the curve, which occurs when P_{MA} equals C , was determined by taking

the first derivative of the Eqn 1 resulting in the gain equation below:

$$G_{50} = \frac{-B(A - D)}{4C}. \quad (2)$$

In addition, to establish meaningful comparisons between groups (spring–summer versus winter), gain was normalized by recalculating it as the percentage change in f_H per unit change in P_{MA} relative to the minimum heart rate (D) (Berger et al., 1980; Altimiras et al., 1998):

$$G = \frac{100B}{D}. \quad (3)$$

Calculation of adrenergic and cholinergic tones

The adrenergic and cholinergic tones on the heart were calculated based on the cardiac interval ($1/f_H$; in s) derived from the P_S pulse. For this, changes in the cardiac interval induced by atropine (1.5 mg kg⁻¹) or sotalolol (6.0 mg kg⁻¹) were expressed relative to the intrinsic cardiac interval obtained after full autonomic blockade (atropine+sotalolol) of the heart (Altimiras et al., 1997).

Statistical analysis

Data are shown as means \pm s.e.m. The effect of the pharmacological treatments on P_{MA} and f_H between winter and spring–summer animals was analyzed through two-way repeated-measures ANOVA (factors: PE or SNP serial doses and season), and the differences among means were assessed by Tukey's *post hoc* test. A two-way ANOVA was used to compare adrenergic and cholinergic tonus between seasons. The effect of season on resting P_{MA} and f_H as well as on all baroreflex parameters derived from sigmoidal baroreflex– f_H curves was analyzed through a Student's *t*-test. The full autonomic blockade on baroreflex responses and the influence of α 1- and β -adrenergic receptors on P_{MA} and f_H were analyzed through one-way ANOVA or one-way repeated-measures ANOVA (factor: treatment), respectively, and the differences among means were assessed by Tukey's *post hoc* test. Means were considered to be different at $P \leq 0.05$.

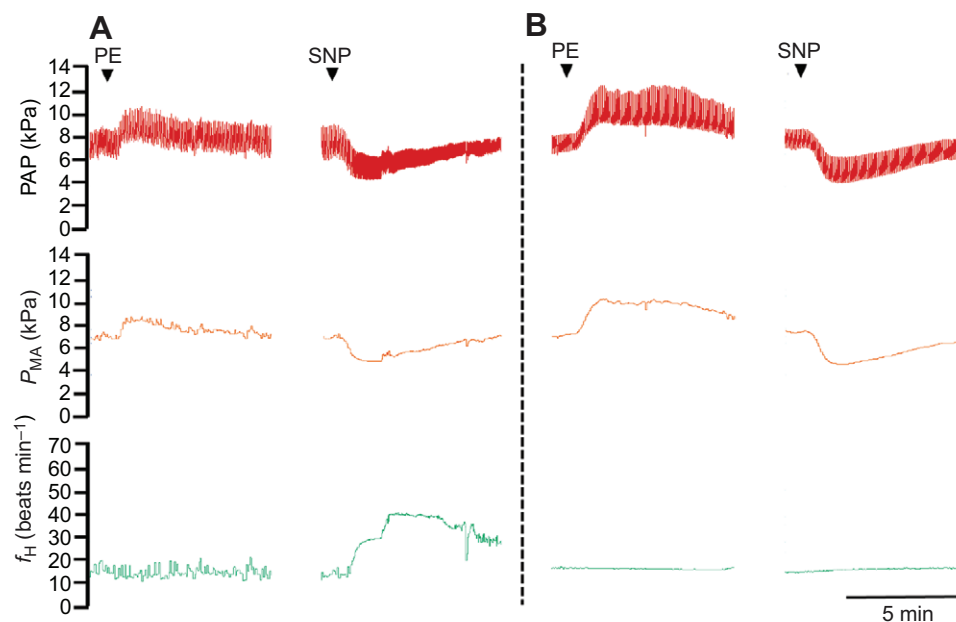


Fig. 1. Pulsatile arterial blood pressure (PAP), mean arterial blood pressure (P_{MA}) and heart rate (f_H) of original traces from a spring–summer-acclimated tegu, *Salvator merianae*. Intravenous injections of phenylephrine (PE; 25.0 μ g kg⁻¹) and sodium nitroprusside (SNP; 10.0 μ g kg⁻¹) before (A) and after (B) full autonomic blockade with atropine (1.5 mg kg⁻¹) and sotalolol (6.0 mg kg⁻¹). Arrowheads indicate the moment of injections.

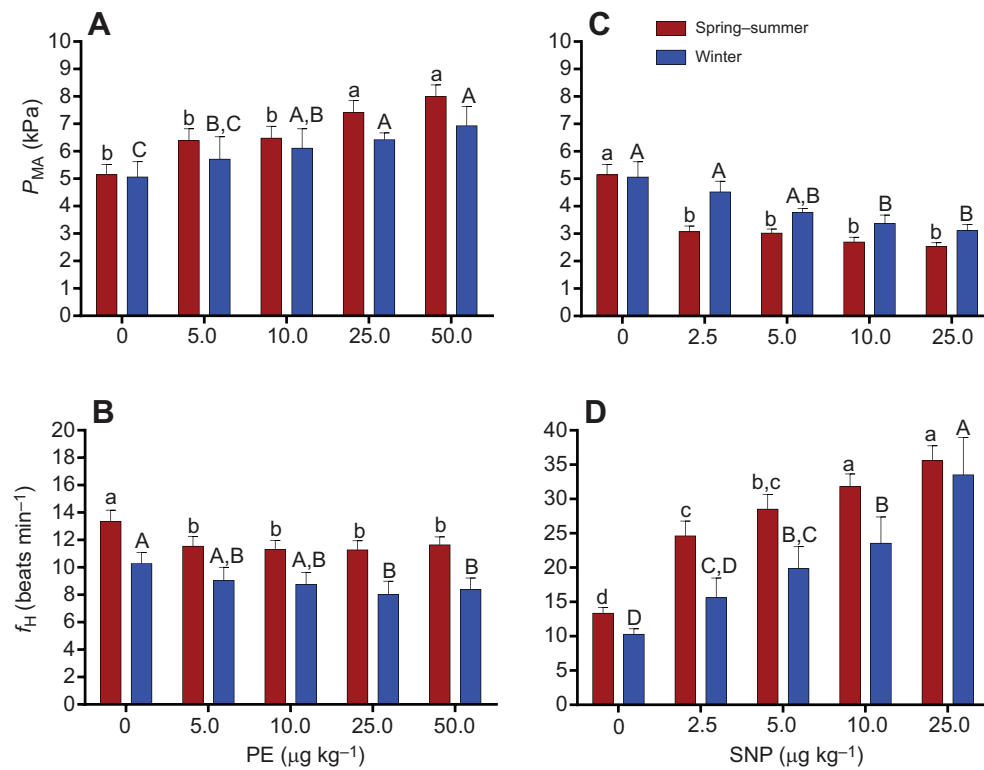


Fig. 2. Haemodynamic effects of pharmacological manipulation of P_{MA} in spring-summer- and winter-acclimated tegus, *S. merianae*, by injections of SNP and PE. Effect of different doses of (A,B) PE and (C,D) SNP on P_{MA} and f_H in winter ($N=5$; blue) and spring-summer tegus ($N=11$; red). Different uppercase (lowercase) letters indicate a significant effect of drugs in winter (spring-summer) tegus ($P<0.05$; Tukey's test). Data are means \pm s.e.m.

RESULTS

Mean resting cardiovascular parameters for spring-summer and winter tegus are shown in Table 1. No significant seasonal difference in P_{MA} was observed ($P=0.91$), whereas f_H was significantly lower during winter ($P=0.039$).

Fig. 1A depicts original traces of PAP of a tegu exhibiting a typical tachycardic reflex response to reduction in P_{MA} after injection of SNP ($10.0 \mu\text{g kg}^{-1}$), and an increase in P_{MA} after a bolus injection of PE ($25.0 \mu\text{g kg}^{-1}$). In contrast to a prominent tachycardia induced by hypotension, hypertension caused a very small bradycardia. In Fig. 1B, an abolition of reflex changes of f_H is shown after animals' pre-treatment with atropine and sotalol (full autonomic blockade).

PE caused significant increases in P_{MA} in both winter and spring-summer animals (effect of treatment: $P<0.001$; Fig. 2A). The

hypertensive effect of PE did not differ between groups ($P=0.38$). Additionally, PE caused a small reflex bradycardia for all doses tested in spring-summer ($P<0.001$; Fig. 2B), and for the two higher doses (10.0 and $25.0 \mu\text{g kg}^{-1}$) in winter animals ($P<0.05$; Fig. 2B); winter animals reached significantly lower values for reflex bradycardia compared with spring-summer ($P<0.01$).

SNP induced a significant decrease in P_{MA} at all doses tested in spring-summer animals (effect of treatment: $P<0.001$; Fig. 2C). During winter, only the two higher doses of SNP (10.0 and $25.0 \mu\text{g kg}^{-1}$) decreased P_{MA} ($P<0.001$). The hypotensive effects of SNP were always followed by tachycardia in both groups ($P<0.001$; Fig. 2D), although spring-summer animals showed higher values (effect of season: $P=0.026$).

Fig. 3 depicts the responses to changes in P_{MA} with PE and SNP in baroreflex- f_H curves at the two groups for each animal and the

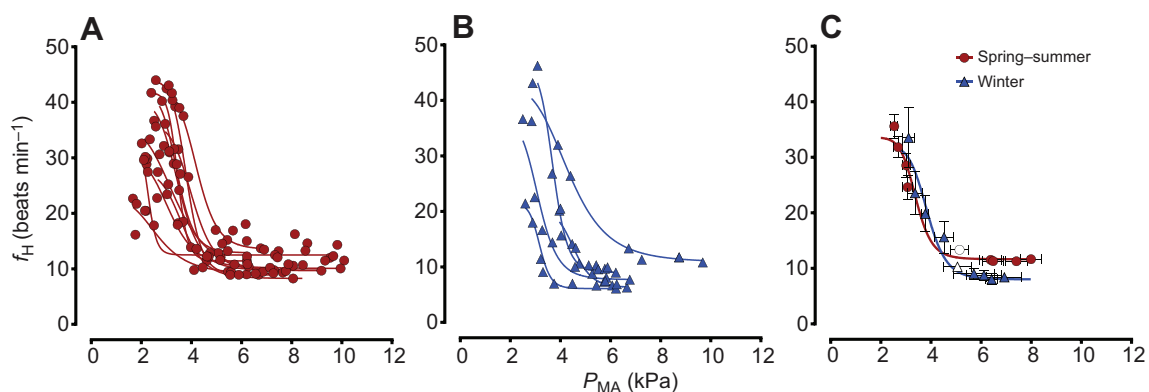


Fig. 3. Individual and mean baroreflex curves of spring-summer- and winter-acclimated tegus, *S. merianae*. Shown are curves of each animal for (A) spring-summer and (B) winter groups, and (C) mean (\pm s.e.m.) baroreflex curves for both groups at $\sim 25^\circ\text{C}$. Blue triangles and red circles represent winter ($N=5$) and spring-summer ($N=11$) groups, respectively. In C, the relationship between P_{MA} and f_H was generated from the mean values of the curve-fit parameters for each animal (see Table 1). Resting f_H and P_{MA} are represented by an open triangle (winter) and an open circle (spring-summer).

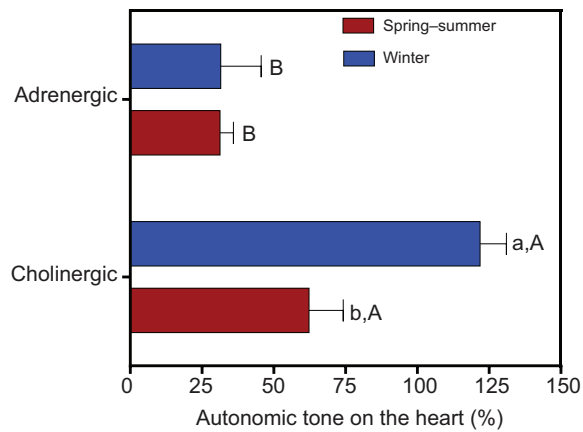


Fig. 4. Calculated autonomic tones on the heart of resting spring-summer- and winter-acclimated tegus, *S. merianae*. Regardless of acclimation, the cholinergic tone was always higher than the adrenergic tone; however, winter tegus ($N=3$) exhibited higher cholinergic tone than spring-summer tegus ($N=5$). Different uppercase letters indicate a significant difference between cholinergic and adrenergic tone, and different lowercase letters indicate a significant difference between winter and spring-summer groups ($P<0.05$; Tukey's test). Data are means \pm s.e.m.

mean P_{MA}/f_H relationships. The coefficients of determination (R^2) of the mean curves were 0.92 ± 0.02 (spring-summer) and 0.86 ± 0.06 (winter), indicating that 92% and 86% of the f_H variations in the respective seasons are explained by P_{MA} changes. In addition, resting f_H values were at the low end of the reflex curves. As for the baroreflex parameters derived from sigmoidal baroreflex- f_H curves, the maximal f_H , the slope of the linear part of the curve, the P_{MA} at the midpoint in the f_H range, the maximal gain and the normalized gain were not different between groups (Table 1). In contrast,

minimum f_H was significantly higher during spring-summer compared with winter tegus ($P=0.013$; Table 1).

Cholinergic and adrenergic tones on the heart in winter and spring-summer tegus are shown in Fig. 4. Resting and undisturbed tegus from both groups were characterized by a large cholinergic tone (winter: $122\pm 9.2\%$; spring-summer: $62.3\pm 11.9\%$) in comparison to a low adrenergic tone (winter: $31.5\pm 14.1\%$; spring-summer: $31.3\pm 4.6\%$; $P<0.001$). Winter animals had the highest parasympathetic modulation of f_H ($P=0.015$).

Pharmacological autonomic blockade attenuated the reflex bradycardia ($P<0.05$; Fig. 5B) and further increased P_{MA} ($P<0.01$; Fig. 5A) after PE injection. Reflex tachycardia induced by hypotension was completely inhibited in those animals pretreated with atropine plus sotalol ($P<0.001$; Fig. 5D); in contrast, there was no change in hypotensive effect evoked by SNP ($P=0.06$; Fig. 5C).

The antagonism of α_1 -adrenergic receptors with prazosin alone ($0.2\ \mu\text{g}\ \text{kg}^{-1}$) did not decrease P_{MA} ($P=0.16$), but inhibited the hypertensive effect induced by PE ($25.0\ \mu\text{g}\ \text{kg}^{-1}$, $P<0.001$; Fig. 6A); prazosin did not change f_H ($P=0.56$; Fig. 6B). Antagonism of β -adrenergic receptors with sotalol had no effect on resting P_{MA} or f_H ($P=0.36$; Fig. 6C,D); however, it was able to inhibit the great β -adrenergic stimulation on the heart induced by isoproterenol ($P<0.001$; Fig. 6D).

DISCUSSION

The main findings of the present study are that resting winter tegu lizards, even exhibiting lower f_H and higher cardiac parasympathetic tone, maintain unchanged f_H baroreflex sensitivity in comparison with spring-summer animals at the same temperature ($\sim 25^\circ\text{C}$); moreover, both winter and spring-summer tegus have higher vagal over sympathetic tone on the heart, allowing them to mount a

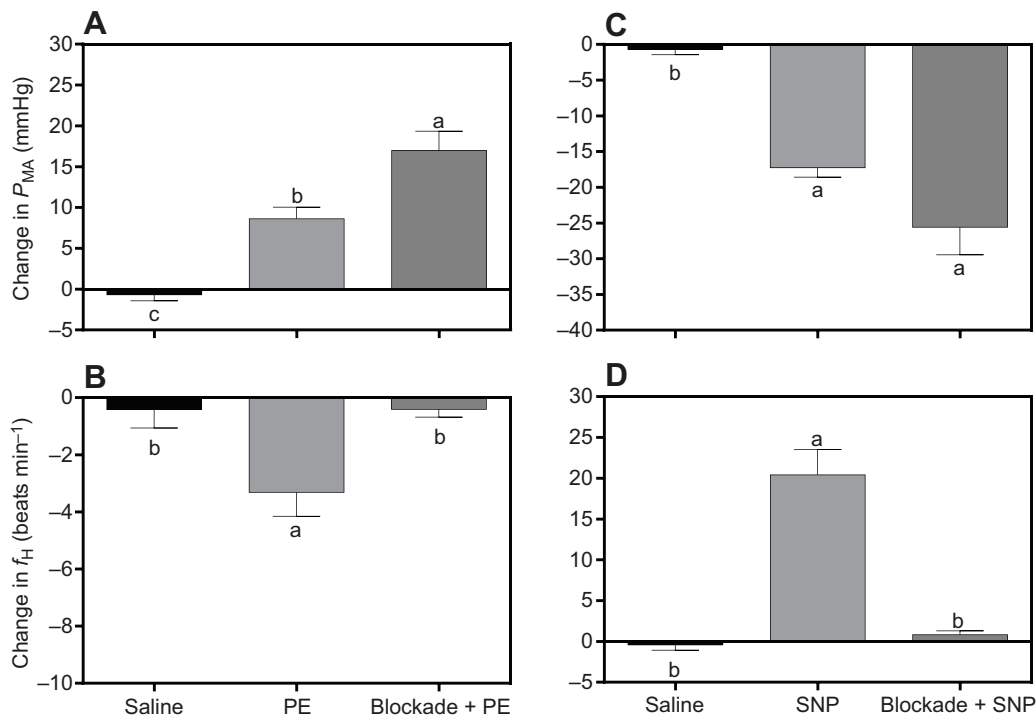


Fig. 5. Changes in P_{MA} and f_H of spring-summer-acclimated tegus, *Salvator merianae* before and after full autonomic blockade effect in hypotension and hypertension. Shown are changes in (A,C) P_{MA} and (B,D) f_H after i.v. injections of saline (vehicle), PE ($25.0\ \mu\text{g}\ \text{kg}^{-1}$) and SNP ($10.0\ \mu\text{g}\ \text{kg}^{-1}$) before and after full autonomic blockade with atropine ($1.5\ \text{mg}\ \text{kg}^{-1}$) and sotalol ($6.0\ \text{mg}\ \text{kg}^{-1}$) ($N=7$). Values indicated by different letters are significantly different from each other as determined by Tukey's test. Data are means \pm s.e.m.

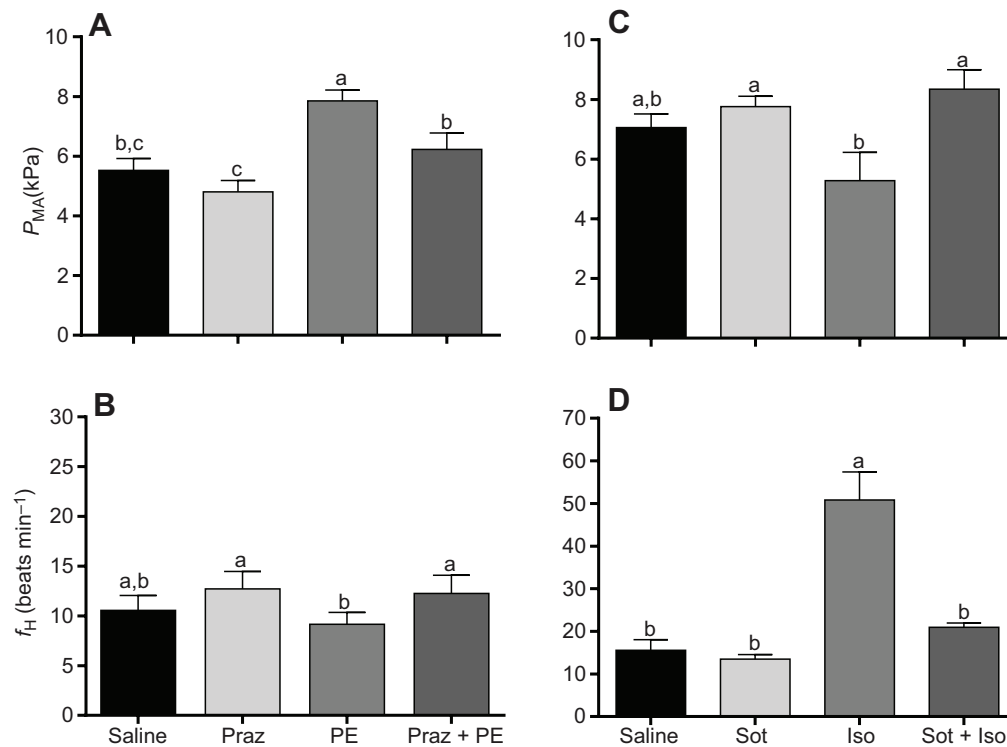


Fig. 6. Influence of α - and β -adrenergic receptors on P_{MA} and f_H of spring–summer-acclimated tegus, *S. merianae*. (A,B) Intravenous injections of saline (vehicle), prazosin (Praz; 0.2 mg kg^{-1}), PE ($25 \mu\text{g kg}^{-1}$) or Praz+PE ($N=7$) on P_{MA} and f_H . (C,D) Intravenous injections of saline (vehicle), sotalol (Sot; 6.0 mg kg^{-1}), isoproterenol (Iso; $2.5 \mu\text{g kg}^{-1}$) or Sot+Iso ($N=4-7$) on P_{MA} and f_H . Values indicated by different letters are significantly different from each other as determined by Tukey's test. Data are means \pm s.e.m.

pronounced reflex tachycardia against hypotension over a negligible bradycardia against hypertension.

Resting cardiovascular variables and seasonal variation

In winter tegus, f_H was $\sim 23\%$ lower than in spring–summer tegus at the same temperature (Table 1), which is in agreement with a higher parasympathetic drive on the heart (Fig. 4). Interestingly, it was recently reported that tegus implanted with a telemetry ECG device and kept under semi-natural conditions anticipate the winter season through a gradual monthly decrease in f_H at constant body temperatures (Sanders et al., 2015). Collectively, these results indicate a certain degree of metabolic depression, which is supported by observations in reptiles, including tegu lizards, of a linear correlation between f_H and metabolic rate under steady-state conditions (Butler et al., 2002; Piercy et al., 2015). Therefore, gas exchange and f_H are seasonally adjusted to match the differing steady-state metabolic demands (Andrade et al., 2004; Clark et al., 2005; Piercy et al., 2015). In fact, hibernation in *S. merianae* is characterized by an active metabolic reduction during the winter season (Abe, 1983, 1995; Andrade and Abe, 1999; Lopes and Abe, 1999; de Souza et al., 2004; Sanders et al., 2015), which, in southeastern Brazil, is synchronized with the driest phase of the year. The rate of oxygen consumption reported for winter tegus can be 32% lower than values reported for resting animals during the active season at the same temperature (Abe, 1995).

Besides significant decrease in f_H in winter tegus, RPP (Table 1), as a measure of myocardial activity (Glass et al., 1997; da Silva et al., 2008), remained unchanged between spring–summer and winter groups. Juvenile tegu lizards undergo cardiac hypertrophy in anticipation of winter hibernation (da Silveira et al., 2013), which could be a compensatory mechanism to maintain blood pressure at low f_H by sustaining stroke volume; however, the maintenance of blood pressure during dormancy in tegu lizards does not exclude a possible contribution from increased peripheral vascular resistance. It is interesting to note that hibernating golden-

mantled ground squirrels (*Callospermophilus lateralis*) undergo an $\sim 30\%$ increase in the left ventricular chamber mass maintaining extremely low f_H , while stroke volume is significantly increased (Nelson and Rourke, 2013). Heart hypertrophy has also been reported in the Andean lizard, *Liolaemus nigroviridis*, during hibernation (Naya et al., 2009). Therefore, adjustments in cardiac function that allow lower f_H and sustained blood pressure in winter tegus remain to be explored in the context of heart hypertrophy in anticipation of the winter season. Furthermore, a reduction in f_H without changes in blood pressure is also observed in aestivating toads and lungfish at constant body temperature (Glass et al., 1997; Bicego-Nahas et al., 2001; da Silva et al., 2008), which might indicate a pattern of cardiovascular regulation in ectotherms that face seasonal metabolic reductions such as hibernation and aestivation.

Baroreflex control of f_H and seasonal variation

The reflex responses of f_H against changes in blood pressure in tegus were accessed by pharmacological manipulation of arterial pressure, which is a common method of studying baroreflex in vertebrates (Bagshaw, 1985; Altimiras et al., 1998; Crossley et al., 2003; Hagensen et al., 2010). Although this 'closed-loop study' tends to overestimate the reflex sensitivity, it offers the advantages of both being performed with minimal instrumentation on unanaesthetized animals and providing an integrated response caused by activation of all relevant receptors (Altimiras et al., 1998; Crossley et al., 2003; Hagensen et al., 2010). This was an appropriate study to be performed in tegus because the baroreceptor locations as well as their characterization have not previously been demonstrated in these animals.

Even at reduced metabolic rate, suggested by smaller resting f_H in our winter tegus, f_H baroreflex sensitivity appeared to be preserved. In semi-natural conditions, the abandonment of thermoregulatory behaviour and the commitment to dormancy during winter in tegus allow body temperature to decline to an ambient temperature of

approximately 17°C (Abe, 1995; Andrade et al., 2004; Sanders et al., 2015). At that point, lower body temperatures could affect the central nervous system's capacity for regulating the cardiovascular system, thereby preventing tegus from responding to hypotension, as is seen in other ectotherms such as caimans (Hagensen et al., 2010), turtles (Crossley et al., 2015) and toads (Zena et al., 2015), where low temperature decreases f_H baroreflex sensitivity. Overall, these data indicate that seasonal variations in baroreflex sensitivity of *S. merianae* in the field, should they exist, are likely explained by differences in body temperature during the different seasons rather than an endogenous seasonal cycle of cardiovascular control.

Salvator merianae showed a clear tachycardic response to decreases in blood pressure with SNP, whereas PE-induced hypertension caused a negligible bradycardia in both winter and spring–summer tegus. The observed low resting f_H positioned at the low end of the baroreflex curves is compatible with the presence of low sympathetic and high parasympathetic drives on the heart. This is supported by a great increase in f_H induced by sympathetic activation in response to hypotension, which was confirmed by abolition of the reflex tachycardia after β -adrenergic blockade. Accordingly, the minimal bradycardia induced by PE hypertension is compatible with an already high parasympathetic drive operating on the heart (Fig. 4) that prevents these animals from further f_H reductions in response to increases in blood pressure. In fact, many species of undisturbed and resting reptiles are normally characterized by high vagal tone on the heart (Taylor et al., 2014).

The reduced ability of tegus to decrease f_H in response to high pressures raises the question of why some ectotherms defend hypotension more than hypertension. Studies on anurans have recently demonstrated the presence of a specialized lymphatic system that has the ability to return excessive transcapillary fluids back to the venous system and thereby maintain blood volume homeostasis; these animals are capable of lymph fluid formation 10 times higher than mammals (Hedrick et al., 2007; Hillman et al., 2010). Therefore, the importance of baroreceptors for defending against increases in blood pressure seems not to be the case for anurans (Hedrick et al., 2015; Zena et al., 2015). Like anurans, tegus have cardiac ventricles that are anatomically and functionally undivided, and hence there is no pressure separation within the ventricle (Jensen et al., 2014). At least in the turtle *Chrysemys picta*, which also has a three-chambered heart, the net loss of plasma through the lung capillaries accounts for 10 to 20 times greater flow than in mammals because of a high pulmonary arterial blood pressure, a variable pulmonary blood flow and/or low plasma colloid osmotic pressure (Zweifach and Intaglietta, 1971; Burggren, 1982). In tegus, the absence of a meaningful bradycardic response against hypertension may expose their pulmonary capillaries to a relatively higher pressure, increasing the rate of fluid formation, which might be avoided by a protective mechanism, such as the lymphatic system. Among ectothermic reptiles, besides the presence of lymphatic vessels and lymph heart structure, which have been identified in some snakes, lizards, turtles and crocodylians, there is not sufficient information available regarding the function of this system in fluid volume homeostasis (for a review, see Hedrick et al., 2013). More studies are necessary to clarify the effect of lymph mobilization capacity on blood pressure homeostasis in reptiles. Besides this mechanism, one cannot rule out the possibility of vagal activity increasing pulmonary vascular resistance (Burggren, 1977; Milsom et al., 1977; Taylor et al., 2009), which could shunt blood away from the lungs during hypertensive episodes.

The normalized baroreflex gain in tegus (spring–summer: 94% kPa⁻¹; winter: 139% kPa⁻¹; Table 1) is similar to that of

another squamate, the lizard *T. rugosa* (98% kPa⁻¹; Berger et al., 1980), at the same temperature (~25°C), but is higher than that observed in anurans (ranging from 33.6 to 46% kPa⁻¹; temperature range: 21–25°C; Hedrick et al., 2015; Zena et al., 2015). Thus, in comparison with the anurans, the higher f_H baroreflex sensitivity of these lizards may be related to their higher P_{MA} and lower f_H (Crossley et al., 2003).

The presence of $\alpha 1$ -adrenoreceptors in the vasculature of tegus with a vasoconstrictor function as described for mammals and other reptiles (Overgaard et al., 2002; Breno et al., 2007) was revealed by the blockade of the hypertensive effect of PE by the $\alpha 1$ -adrenergic antagonist prazosin (0.2 mg kg⁻¹ i.v.). We also tested two other doses of prazosin (0.5 and 1 mg kg⁻¹; data not shown) in a few animals, and similar results on P_{MA} were observed, in addition to a long-lasting inhibition of the PE effect up to 4 h. Thus, resting *S. merianae* seems not to have a net $\alpha 1$ -adrenergic tone in its vasculature, at least under our experimental conditions. Regarding β -adrenergic receptors, the pharmacological stimulation with isoproterenol increased f_H more than threefold from saline values (~227% increase), which was almost completely blunted by previous injection of sotalol, a response mediated by β -adrenergic receptors on the heart. In resting tegus with low f_H , β -adrenergic blockade did not further reduce it; a result similar to that observed in awake and fully recovered rattlesnakes, which have a high parasympathetic tone on the heart (Campbell et al., 2006).

In summary, contrary to our initial hypothesis, the South American tegu *S. merianae*, known to have an annual cycle of metabolic change, seems to maintain relatively unchanged f_H baroreflex sensitivity at a temperature-independent low metabolic state. Even with lower resting f_H and higher vagal tone on the heart, the preserved baroreflex response of f_H in winter tegus may be related to a cardiac hypertrophy (da Silveira et al., 2013) and, thus, the maintenance of cardiac activity. Independent of acclimation, the predominant tachycardic response against hypotension, similar to what happens in anurans (Zena et al., 2015), crocodylians (Altimiras et al., 1998; Hagensen et al., 2010) and mammals (Crestani et al., 2010), indicates a possible pattern of baroreflex regulation in tetrapods. Finally, the absence of a meaningful bradycardic response to hypertension might account for the presence of an efficient lymphatic system that pulls back transcapillary fluid loss into the venous system, avoiding lung oedema, a mechanism that remains to be explored in reptiles.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

V.D., L.A.Z. and K.C.B. conceived and designed the experiments; V.D. performed the experiments; V.D., L.A.Z. and K.C.B. analyzed and interpreted the data; L.A.Z., K.C.B., V.D., D.V.A., L.H.G. and A.S.A. wrote the manuscript.

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