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SHORT COMMUNICATION

Maintained barostatic regulation of heart rate in digesting snakes (*Boa constrictor*)

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ABSTRACT

When snakes digest large meals, heart rate is accelerated by withdrawal of vagal tone and an increased non-adrenergic-non-cholinergic tone that seems to stem from circulating blood-borne factors exerting positive chronotropic effects. To investigate whether this tonic elevation of heart rate impairs the ability for autonomic regulation of heart during digestion, we characterised heart rate responses to pharmacological manipulation of blood pressure in the snake *Boa constrictor* through serial injections of sodium nitroprusside and phenylephrine. Both fasting and digesting snakes responded with a robust tachycardia to hypotension induced by sodium nitroprusside, with digesting snakes attaining higher maximal heart rates than fasting snakes. Both fasting and digesting snakes exhibited small reductions of the cardiac chronotropic response to hypertension, induced by injection of phenylephrine. All heart rate changes were abolished by autonomic blockade with the combination of atropine and propranolol. The digesting snakes retained the capacity for compensatory heart rate responses to hypotension, despite their higher resting values, and the upward shift of the barostatic response curve enables snakes to maintain the cardiac limb of barostatic regulation for blood pressure regulation.

KEY WORDS: Reptile, Snake, Cardiovascular, Blood pressure, Baroreception, Phenylephrine, Nitroprusside, Autonomic control

INTRODUCTION

All vertebrates maintain mean arterial blood pressure (MAP) within relatively narrow limits by fast autonomic reflexes that modulate heart rate (f_H) and peripheral resistance (Bagshaw, 1985; Van Vliet and West, 1994; Wang, 2012). In reptiles, these autonomic reflexes are initiated by baroreceptors within the vessel wall of the truncus arteriosus and the systemic and pulmonary arteries, and the efferent regulation of f_H is provided by a coordinated balance between parasympathetic inhibition and sympathetic stimulation (Bagshaw, 1985; Berger, 1987; Lillywhite and Donald, 1994; Van Vliet and West, 1994; Wang, 2012).

Because of the diffuse and uncertain anatomical location of the baroreceptors (e.g. Berger et al., 1980), the cardiac limb of barostatic regulation is most often studied by measurement of the f_H responses to brief pharmacological manipulation of blood pressure upon injection of drugs that alter peripheral vascular resistance. This approach relies on the assumption that the vasoactive drugs do not exert direct effects on the heart or the baroreceptors, and effective results can be obtained by injection of the vasoconstricting α-adrenergic agonist phenylephrine (PE) and the nitric oxide (NO) donor sodium nitroprusside (SNP) that elicits vasodilatation. During the pharmacological manipulation of systemic pressure, the baroreceptors exert feedback control on blood pressure by inducing modulations of f_H. This 'closed-loop' approach is likely to underestimate the gain of the response compared with 'open-loop' studies where the pressure at the baroreceptor is manipulated independently of systemic pressure (Van Vliet and West, 1994), but allow for measurements to be performed after minimal instrumentation on conscious animals.

Snakes are renowned for their impressive ability to digest very large meals where metabolism increases 5- to 10-fold and remains elevated for many days (e.g. Wang and Rindom, 2021). The large metabolic responses to digestion in snakes are accompanied by a tachycardia that stems from withdrawal of parasympathetic tone and positive chronotropic effects of a non-cholinergic-non-adrenergic factor(s) that appear humoral in origin (Wang et al., 2001; Skovgaard et al., 2009; Enok et al., 2012). The humoral signals are unlikely to be able to participate in short-term responses to altered blood pressure and it remains unknown whether this tonic stimulation of f_H interferes with barostatic regulation of the heart in the postprandial period, when the high metabolic rate could be expected to require adequate blood pressure regulation.

In the present study, we describe the heart rate response to altered blood pressure in fasting and digesting snakes. The experiments were conducted on *Boa constrictor*. Like other constricting snakes, such as pythons, the boas exhibit large and prolonged metabolic responses to digestion (Andrade et al., 2004). The large specific dynamic action (SDA) response in snakes is primarily due to increased protein synthesis and virtually all organs – with the notable exception of the heart – typically enlarge during digestion (Wang and Rindom, 2021). We also chose to study boa because our previous studies show that the postprandial tachycardia of this species is due to withdrawal of vagal tone and the presence of a significant non-cholinergic-non-adrenergic factor stimulation of the heart (Wang et al., 2001).

MATERIALS AND METHODS

Animals, surgery and measurements of blood pressure and heart rate

The experiments were performed on 14 specimens of *Boa constrictor* Linneaus 1758 that had been bred at the Jacarezario at Universidade Estadual Paulista 'Julio de Mesquita Filho' (UNESP) in Rio Claro (SP, Brazil) and reared for approximately 2 years on rodents and chickens. The snakes were maintained at 28±5°C under a natural light cycle, appeared healthy and had attained a body mass of 1.5 to 2.6 kg. All animals fasted for 2 to 3 weeks before surgery. The procedure was approved by the UNESP ethical committee on animal experimentation (Comissão de Ética na Experimentação Animal).

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List of abbreviations

$f_{H,max}$	the maximal heart rate elicited by the baroreflex
$f_{H,min}$	the minimal heart rate elicited by the baroreflex
MAP_{50}	MAP at the midpoint in the heart rate range heart rate
n	the Hill coefficient of the barostatic response curve
G_{50}	Gain of the baroreflex
MAP	mean arterial blood pressure
NANC	non-adrenergic-non-cholinergic
PE	phenylephrine
SNP	sodium nitroprusside

Occlusive arterial catheters (PE90 containing heparinised saline) were inserted in the cranial branch of the vertebral artery or the caudal portion of the dorsal aorta under CO_2 anaesthesia (see Wang et al., 1993). In either case, the vessel was accessed through a 5 cm incision, subsequently closed by sutures, whilst the catheter was secured dorsally to the skin. The procedure lasted 20–30 min and all animals appeared to regain normal behaviour within an hour after surgery.

For measurements of blood pressure, the catheters were connected to PX600 Baxter Edward pressure transducers (Irvine, CA, USA) supplying in-house built preamplifiers. Signals were recorded at 50 s^{-1} using a Biopac MP100 unit (Biopac, Goleta, CA, USA), and heart rate (f_H) was derived from the pulsatile pressure signal. Transducers were calibrated daily against a static water column.

Experimental protocol and pharmacological manipulation of blood pressure

The snakes were allowed to recover from surgery for 24–48 h in 60×30×15 cm plastic boxes within a climatic chamber (Fanem, SP, Brazil) at 30°C. The cannulae were exteriorised and all measurements and drug infusions could be performed without disturbance. Blood pressure regulation was studied in 8 fasting snakes, while the remaining 6 snakes were studied 24–48 h after voluntary ingestion of rodents amounting to 8–25% of their body mass. This period corresponds to the maximal rise in metabolism due to SDA (Andrade et al., 2004). In each individual, we determined resting f_H and blood pressure of undisturbed individuals. Then, blood pressure was manipulated by vascular injections of sodium nitroprusside (SNP) or phenylephrine (PE) in dosages from 5 to 50 µg kg^{-1}. All injections were given as 0.1 ml kg^{-1} followed by 0.5 ml of heparinised saline to flush the catheter. Sham injections of the same volume of saline, performed in all individuals, elicited negligible changes in MAP and f_H.

Calculation of the baroreflex

The f_H and mean systemic arterial blood pressures (MAP) obtained in response to the various dosages of PE and SNP for each animal were fitted to a four-variable sigmoidal logistic function (Altimiras et al., 1998) using a Quasi-Newtonian iteration in Statistica:

$$f_H = f_{H,min} + (f_{H,max} - f_{H,min}) \frac{MAP_{50}^n}{MAP_{50}^n + MAP^n}, \quad (1)$$

where $f_{H,max}$ and $f_{H,min}$ are the maximal and minimal f_H elicited by the baroreflex, n is the Hill coefficient and the MAP_{50} is MAP at the midpoint in the f_H range. The gain of the baroreflex (G_{50}) was calculated as:

$$G_{50} = \frac{-n(f_{H,max} - f_{H,min})}{4MAP_{50}}. \quad (2)$$

Table 1. Mean arterial blood pressure (MAP) and heart rate (f_H) of undisturbed boas (*Boa constrictor*) during fasting and digestion

	Fasting	Digestion
MAP (kPa)	5.7±0.4	6.3±0.4
f_H (min^{-1})	29.6±1.4	45.9±3.1*
N	8	6

Values are means±s.e.m.; significant differences between fasting and digesting snakes are denoted with an asterisk.

Data analysis and statistics

Differences between fasting and digesting snakes were considered statistically significant when a *t*-test yielded P values lower than 0.05 and all results are presented as means±1 s.e.m.

RESULTS AND DISCUSSION

MAP and f_H of fasting and digesting boas (Table 1) resemble our earlier measurements on *Boa constrictor* (Wang et al., 2001) and the fasting values are consistent with other terrestrial snakes of similar body size (e.g. Lillywhite and Donald, 1994). The pronounced postprandial tachycardia in *Boa* (Table 1) is due to withdrawal of vagal tone and an increased non-adrenergic-non-cholinergic tone (Wang et al., 2001). This response has been characterised in more detail in pythons and shown to involve humoral factors that exert positive chronotropic effects on the heart (Skovgaard et al., 2009; Enok et al., 2012). As a result of this tonic humoral stimulation of the heart, both maximal and minimal f_H in response ($f_{H,max}$ and $f_{H,min}$) to altered MAP (presented as mean values in Table 2) were elevated during digestion. Thus, while both fasting and digesting snakes responded with a robust tachycardia to hypotension induced by SNP, the digesting boas attained maximal f_H of 63.0±3.9 min^{-1}, whereas fasting snakes only reached 50.9±2.6 min^{-1} (Fig. 1 and Table 2). The difference between resting and maximal f_H was remarkably similar in the two groups (21.3±2.4 and 17.1±2.1 min^{-1} in fasting and digestion, respectively) showing that digesting boas retain the capacity for compensatory heart rate responses to hypotension. We did not observe any obvious behavioural responses to the blood pressure manipulations, but we also tried to shield the snakes as much as possible, rendering visual observations rather difficult. In 4 snakes, we showed that the f_H responses to hypotension were abolished 20 min after complete autonomic blockade by the combined injection of atropine and propranolol (3 mg kg^{-1}). These dosages were effective in abolishing the effects of injected adrenaline and acetylcholine on f_H (Wang et al., 2001). Thus, the tachycardia in response to SNP is indeed an autonomic reflex.

MAP only increased by approximately 6 mmHg during digestion (Table 1), and this value is remarkably similar to the rise in MAP at

Table 2. Coefficients for the baroreflex curves fitted to heart rate in response to manipulation of blood pressures in fasting and digesting *Boa constrictor* fitted to the four-variable sigmoidal logistic function in Eqn 1

	Fasting	Digestion
$f_{H,min}$ (min^{-1})	30.2±1.4	40.0±2.0*
$f_{H,max}$ (min^{-1})	50.9±2.6	63.0±3.9*
n	14.8±1.7	5.6±1.1*
MAP_{50} (kPa)	4.6±0.3	5.2±0.6
G_{50} (min^{-1} kPa^{-1})	−17.6±4.6	−5.3±0.8*

Values are means±s.e.m. (*N*=8 and 6 for fasting and digesting snakes, respectively), and significant differences between fasting and digesting snakes are denoted with an asterisk.

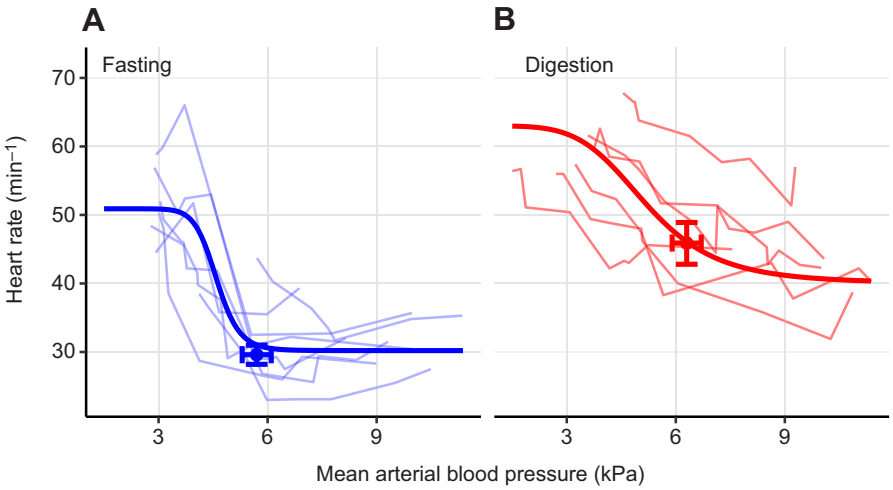

Fig. 1. Baroflex response of fasting and digesting boas (*Boa constrictor*). Heart rate (f_H) is depicted as a function of mean arterial blood pressure (MAP) for 8 fasting (A) and 6 digesting (B) snakes. The fitted line in each panel is based on the average parameters of the sigmoidal fits to the relationship between f_H and MAP in each individual snake (see Table 2), and responses of all individual snakes are shown in lighter colours. The resting f_H and MAP for both fasting and digesting snakes (Table 1) are shown by dots (mean±s.e.m.).

the midpoint of the f_H response (C in Table 2). Thus, as total peripheral resistance decreases in response to an extensive vasodilatation within the gastrointestinal organs during digestion (Starck and Wimmer, 2005; Secor and White, 2010), the relationship between f_H and MAP, i.e. the response curve for barostatic heart rate regulation, shifts upwards allowing for a maintenance of the cardiac limb in barostatic regulation (Fig. 1). Nevertheless, the gain of the barostatic response of all individuals varied inversely with resting f_H, and digesting snakes had significantly lower G_{50} than fasting animals (Table 2).

The upwards shift of the barostatic response curve in digesting snakes is akin to the resetting of the arterial baroreflex in humans and other mammals during exercise, which serves to maintain functional compensatory responses to blood pressure fluctuations, although both f_H and MAP increase (Raven et al., 2006). In mammals, this resetting is achieved by a feed-forward mechanism arising as central command from higher brain centres, as well as afferent information from skeletal muscles (the exercise pressor reflex) (Raven et al., 2006). It would be of considerable interest to further investigate the apparent resetting of the barostatic response in digesting snakes and to address the putative roles of afferent input from the visceral organs and feed-forward regulation in this mechanism.

It is noteworthy that f_H in *Boa constrictor* showed virtually no response to increased MAP, revealing that the cardiac limb of the barostatic response is primarily sensitive to hypotension. A similar response was described in the broad-nosed caiman (Hagensen et al., 2010). Freshwater turtles, saltwater crocodiles and the lizard *Trachydosaurus* also respond more vigorously to decreased pressure compared with hypertension (Millard and Moalli, 1980; Berger et al., 1980; Altimiras et al., 1998). These findings are not consistent with the view that amphibians and reptiles primarily rely on barostatic responses to prevent pulmonary oedema as their undivided heart entails similar pressure increases in the pulmonary and systemic circulations during hypertension (Van Vliet and West, 1994). In fact, it seems that the barostatic response primarily acts to secure tissue perfusion in response to lowered blood pressure.

In conclusion, ingestion of a meal caused a pronounced tachycardia in *Boa constrictor*. Both fasting and digesting snakes responded with a robust tachycardia to hypotension induced by SNP. Although the digesting snakes had higher resting and maximal values of f_H than the unfed animals, the difference between resting and maximal values was remarkably similar in the two groups, showing that digesting snakes retain the capacity for compensatory heart rate responses to hypotension. An upward shift of the barostatic response curve seems to accompany the decrease in total peripheral resistance concomitant with extensive vasodilatation within the gastrointestinal organs during digestion. It may be speculated that the tonic stimulation of f_H during digestion by a circulating NANC factor provides for a tachycardia to accommodate for the postprandial rise in venous return (Enok et al., 2016; Joyce and Wang, 2020), but maintain the capacity for autonomic regulation to buffer rapid changes in blood pressure changes, and hence enable cardiovascular homeostasis during the prolonged digestive process.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: T.W.; Methodology: T.W., A.P., D.V.A.; Software: E.W.T.; Formal analysis: T.W., A.P.; Writing - original draft: T.W., A.P., E.W.T.; Writing - review & editing: T.W., A.S.A., D.V.A., E.W.T.; Visualization: E.W.T.; Project administration: T.W.; Funding acquisition: T.W., A.S.A.

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