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RESEARCH ARTICLE

Snake constriction rapidly induces circulatory arrest in rats

Scott M. Boback[1,*], Katelyn J. McCann[1], Kevin A. Wood[1], Patrick M. McNeal[1], Emmett L. Blankenship[2] and Charles F. Zwemer[1]

ABSTRACT

As legless predators, snakes are unique in their ability to immobilize and kill their prey through the process of constriction, and yet how this pressure incapacitates and ultimately kills the prey remains unknown. In this study, we examined the cardiovascular function of anesthetized rats before, during and after being constricted by boas (*Boa constrictor*) to examine the effect of constriction on the prey's circulatory function. The results demonstrate that within 6 s of being constricted, peripheral arterial blood pressure (PBP) at the femoral artery dropped to 1/2 of baseline values while central venous pressure (CVP) increased 6-fold from baseline during the same time. Electrocardiographic recordings from the anesthetized rat's heart revealed profound bradycardia as heart rate (f_H) dropped to nearly half of baseline within 60 s of being constricted, and QRS duration nearly doubled over the same time period. By the end of constriction (mean 6.5±1 min), rat PBP dropped 2.9-fold, f_H dropped 3.9-fold, systemic perfusion pressure (SPP=PBP−CVP) dropped 5.7-fold, and 91% of rats (10 of 11) had evidence of cardiac electrical dysfunction. Blood drawn immediately after constriction revealed that, relative to baseline, rats were hyperkalemic (serum potassium levels nearly doubled) and acidotic (blood pH dropped from 7.4 to 7.0). These results are the first to document the physiological response of prey to constriction and support the hypothesis that snake constriction induces rapid prey death due to circulatory arrest.

KEY WORDS: *Boa constrictor*, Central venous pressure, Metabolic acidosis, Peripheral blood pressure, Prey

INTRODUCTION

Vertebrate predators have evolved numerous behaviors to efficiently subdue and kill dangerous prey while minimizing their own risk. Many of these tactics involve the use of limbs and associated weapons such as talons, claws and powerful appendicular musculature. As legless predators, snakes evolved other means to immobilize and kill their prey, including venom delivery systems and constriction. Snake constriction is a behavioral pattern in which prey are restrained by two or more points of pressure along the snake's body, typically in the form of one or more encircling loops or coils (Greene and Burghardt, 1978; Greene, 1983; Mehta and Burghardt, 2008). As a key innovation in the adaptive radiation of snakes, constriction behavior enabled snakes to efficiently subdue large prey species not accessible to their lizard ancestors (Greene and Burghardt, 1978; Greene, 1994). Despite its prominence in snake evolution, constriction behavior and physiology are poorly understood and the proximate cause of prey death remains unknown. Therefore, a more thorough understanding of the mechanisms by which constriction impacts prey physiology is needed to understand the development of constriction behavior and ultimately the evolution of this trait.

For years it had been widely accepted that constricting snakes kill their prey by suffocation (e.g. Ditmars, 1931; Parker and Grandison, 1977; Shine, 1991). McLees (1928) and Hardy (1994) argued otherwise and suggested that the cause of death in prey during constriction was circulatory arrest rather than suffocation. Hardy (1994) proposed that constriction pressure leads to an interruption of blood flow into the prey's heart; direct compression could also prevent expansion of atria and ventricles. An absence of systemic circulation, argued Hardy (1994), would ultimately lead to a failure of cellular respiration in the myocardium and other major organ systems, subsequently resulting in cardiac arrest and organ failure. Although these arguments were presented two decades ago, researchers have yet to test Hardy's ideas.

Boas (*Boa constrictor* Linnaeus 1758) are members of the early alethinophidian group Booidea (Scanlon and Lee, 2011) that employ a relatively fixed constriction behavior pattern on a wide variety of prey (Mehta and Burghardt, 2008). Boas strike the anterior portion of the prey (head and shoulders) and apply two or more fully encircling loops of their bodies (ventral and lateral sides against the prey) typically around the prey's thorax. Alignment of these loops over the prey's ribcage not only allows the snake to apply circumferential compression directly to vital organs but also may enable their monitoring of the heartbeat as a cue to meter their effort (Boback et al., 2012). Additionally, boas readily strike and constrict rodents (including deceased rats) in the laboratory. These features combine to make boas ideal candidates for testing hypotheses regarding the impacts of snake constriction on prey physiology.

As a first step in understanding the physiological effects of snake constriction, we examined the cardiovascular responses of anesthetized rats (*Rattus rattus*) constricted by *B. constrictor*. Based on the hypotheses of Hardy (1994) and McLees (1928), and our previous work measuring constriction pressures (Boback et al., 2012), we formulated a number of predictions regarding the impact of snake constriction on rat circulation. Circumferential thoracic constriction pressures exceeding 140 mmHg (Boback et al., 2012) should increase central venous pressure (CVP) and simultaneously elevate aortic root afterload pressures at the heart's great vessels. Once CVP exceeds 7 mmHg, right ventricular filling is greatly limited (Potts et al., 1998). Similarly, aortic root pressures exceeding arterial pressures of 120 mmHg would limit the opening of the aortic semilunar valve, contributing to systemic ischemia. Respectively, these constriction-induced changes should significantly reduce venous return to the right heart and consequently left ventricular cardiac output. Constriction-dependent reductions in thoracic volume and compliance would also quickly restrict ventilation and could result in combined arterial hypercapnia and hypoxia. Even if a marginal cardiac output were to be maintained, coronary perfusion with this blood would promote myocardial failure in a dose-dependent manner.

[1]Dickinson College, Department of Biology, Carlisle, PA 17013, USA. [2]All Pets Emergency and Referral Clinic, Alpharetta, GA 30004, USA.

*Author for correspondence (bobacks@dickinson.edu)

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Our objectives were, first, to document potential hemodynamic changes in rats while they were being constricted by snakes and, second, to examine hematologic parameters both before and after constriction. We monitored pressure exerted by snakes while simultaneously measuring peripheral blood pressure (PBP), CVP and cardiac electrical activity (electrocardiogram, ECG) in the rat. Arterial blood samples were collected from rats before (post-operative and prior to constriction) and immediately after the constriction bout for blood gas analysis [arterial O_2 and CO_2 partial pressure (Pa_{O_2}, Pa_{CO_2}) and pH] and plasma potassium concentration [K^+] measurement. Documenting hemodynamic changes in constricted prey as proposed will result in a clearer picture of the physiological effects of snake constriction and will clarify the importance of this behavior in facilitating the adaptive radiation of snakes.

RESULTS

Characterization of constriction behavior and baseline hemodynamic variables

Rats (N=15) averaged 398±7 g and 28±3% of snake body mass. After surgery and prior to being presented to snakes, rat heart rate (f_H) averaged 436±11 beats min^{-1}, PBP at the femoral artery was 82±2 mmHg [PBP was pulsatile in most (88%, 7 of 8) trials] and CVP at the jugular vein was −4.2±0.5 mmHg.

Upon striking the anesthetized rats, snakes applied loops of their bodies typically around the rat's thorax and abdomen [88% (15 of 17) of trails] but occasionally around the rat's neck [12% (2 of 17) of trials]. Data from the two trials in which snakes applied constriction loops around the neck were not used in analyses of rat blood pressures (PBP, CVP) but were used in other analyses (e.g. constriction duration, constriction pressure, f_H, ECG and arterial blood chemistry). Constriction duration (time from the snake strike to when it released its mouth from the rat; see Canjani et al., 2003) averaged 6.5±1 min and was characterized by a rapid increase in constriction pressure followed by a slow decay with infrequent coil adjustments (1.28±0.22 adjustments per constriction event; Table 1, Fig. 1).

Effect of constriction on rat intravascular pressures

Immediately upon applying body loops, constriction pressure (as measured in the subcutaneous pressure probe) rose dramatically and, on average, snakes achieved their maximum (peak) constriction pressures (156±15 mmHg) 5.9±0.8 s after the strike (Tables 1,2). At the same time that constriction pressure from the snake rapidly increased, CVP of the rat increased significantly (F=47.34, d.f.=11, P<0.0001, N=10; Fig. 1) achieving its maximum pressure (33±8 mmHg) at the same time as peak constriction pressure (within 6 s of the strike; Fig. 2B, Table 2). CVP remained significantly elevated above pre-constriction levels for the first 2 min of constriction (P-values, with Tukey–Kramer adjustment, from all differences of least squares means <0.0031), after which time CVP dropped to levels similar to those pre-constriction (Fig. 2B).

Table 1. Summary statistics for different components of constriction tests performed in this study

	N	Mean±s.e.m.
Snake mass (g)	9	1533±139
Rat mass (g)	15	398±7
Constriction duration (min)	13	6.49±1
Maximum constriction pressure (mmHg)	14	156±15
Total pressure (integral of pressure versus time; s×mmHg)	13	16,557±2370
Time to maximum (peak) pressure (s)	15	5.9±0.8
Number of adjustments per trial	18	1.28±0.22

During the same time of rapid increase in constriction pressure, PBP at the femoral artery of the rat dropped significantly (F=10.52, d.f.=11, P<0.001, N=6; Fig. 1). Within 6 s of the strike (at peak constriction pressure), rat PBP decreased to half that of pre-constriction levels (41.5±13 mmHg) and remained significantly depressed throughout the constriction event (P-values, with Tukey–Kramer adjustment, from all differences of least squares means <0.0001; Fig. 2A). In a single trial, PBP in the rat increased at the onset of constriction and remained elevated for the first 1.5 min but then decreased to half pre-constriction levels by 4.5 min.

We found that both snake mass and rat mass were significant covariates in some of our models. Rats constricted by larger snakes had greater PBP (F=8.67, d.f.=3, P=0.001, N=6) but did not vary in CVP values (F=0.51, d.f.=1, P=0.484, N=10). Relative to small rats, large rats had lower PBP values (F=32.54, d.f.=5, P<0.0001, N=6) and lower CVP values (F=95.86, d.f.=8, P<0.0001, N=10). In addition, the increase in CVP was greater in small rats relative to large rats (time×rat mass interaction: F=3.46, d.f.=80, P=0.014, N=10). There was no effect of snake mass on the rate of change in CVP values over time (time×snake mass interaction: F=0.12, d.f.=66, P=1.0, N=10).

Systemic perfusion pressure (SPP=PBP−CVP) dropped immediately with onset of constriction and was significantly depressed by 3 min into constriction (87±3 versus 17±20 mmHg, P<0.05, N=5; supplementary material Fig. S1). One rat in this cohort had a coil around the abdomen rather than the thorax, and when this trial was removed from the analysis, all post-constriction SPP were significantly lower (P<0.0001, N=4; supplementary material Fig. S1, inset) than baseline; the highest SPP was 12±19 mmHg.

Effect of constriction on f_H

In addition to the effects on vascular pressures, constriction by boas resulted in a significant decrease in rat f_H (F=36.07, d.f.=11, P<0.0001, N=16). Within 30 s of the snake strike and initiation of constriction, rat f_H had dropped significantly relative to pre-constriction levels and continued to decrease throughout the constriction bout (P-values, with Tukey–Kramer adjustment, from all differences of least squares means <0.05; Fig. 2C, Table 2). However, in 19% (3 of 16) of trials, f_H actually increased above pre-constriction levels for the first 30 s of constriction and then decreased dramatically 60 s into the constriction bout.

Constriction altered rat cardiac electrical activity as the QRS duration increased significantly through the first 4 min of constriction (F=86.89, d.f.=11, P<0.0001, N=15; Fig. 2D). Within 30 s of the strike, QRS duration increased significantly relative to pre-constriction levels and continued to increase throughout constriction [P-values, with Tukey–Kramer adjustment, from differences of least squares means were <0.02 for 30, 60 and 90 s into constriction – the remaining comparisons (2–5 min post-constriction) were not precisely estimated by the model; Fig. 2D, Table 2].

When we tested effects of constriction on cardiac performance, both snake mass and rat mass were significant covariates in some of our models. Relative to small snakes, large snakes had a greater impact on rat f_H (F=6.04, d.f.=8, P<0.0001, N=15) but there was no effect of snake mass on QRS duration (F=0.61, d.f.=8, P=0.767, N=15) or on the rate of change in f_H or QRS duration (all interactions P>0.6, N=15). Relative to small rats, large rats had significantly lower f_H (F=4.49, d.f.=13, P=0.009, N=15) and greater QRS

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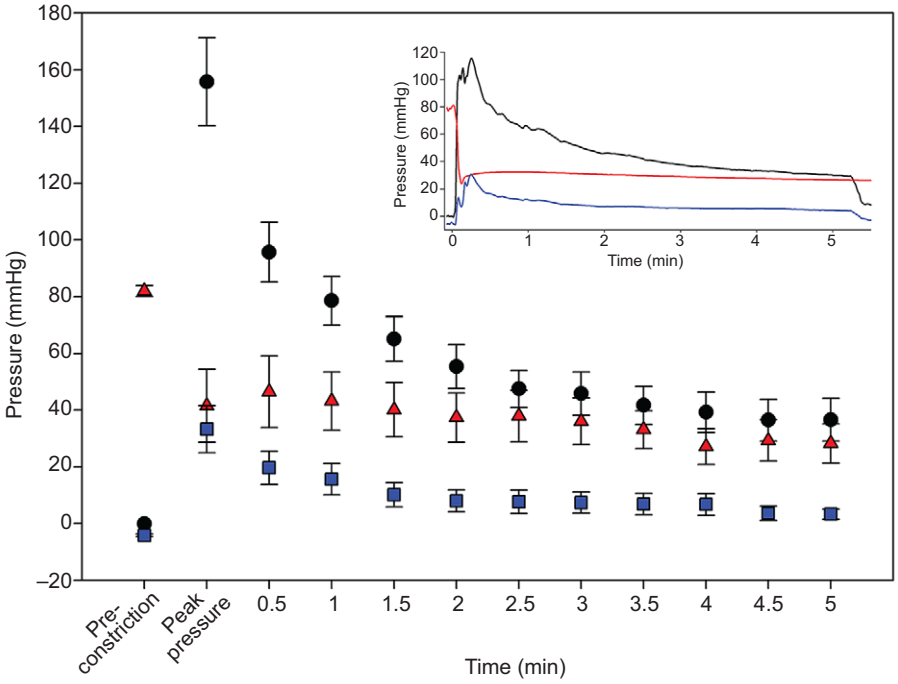

Fig. 1. Mean (±s.e.m.) pressure recordings obtained while anesthetized rats (*Rattus rattus*) were constricted by boas (*Boa constrictor*). Constriction pressure exerted by snakes (black circles) was measured via a pressure probe inserted into the subcutaneous space on the left lateral aspect of the rat. Peripheral blood pressure (PBP, red triangles) was recorded via a vascular catheter inserted into the left femoral artery. Central venous pressure (CVP, blue squares) was obtained through a vascular catheter inserted into the jugular vein and advanced approximately 2.5 cm to the confluence of right and left cranial vena cavae. Inset: a representative constriction trial illustrating the coordinated depression of PBP (red line) and increase in CVP (blue line) with increasing constriction pressure (black line). This constriction event had a duration of 5.5 min; peak constriction pressure (115 mmHg) was recorded 16 s after the strike (time 0). Immediately prior to the strike, PBP was 81.0 mmHg (and pulsatile) then dropped to 24.3 mmHg at the peak of constriction pressure; CVP began at −4.8 mmHg and rose to 30.5 mmHg at the peak of constriction pressure.

duration (F=11.69, d.f.=13, P=0.0001, N=15). Additionally, QRS duration in large rats increased at a greater rate than in small rats (time×rat interaction: F=3.70, d.f.=123, P=0.01, N=15) but there was no effect of rat mass on the rate of change in f_H (time×rat interaction: F=0.85, d.f.=128, P=0.686, N=15).

Simultaneously comparing recorded ECG and vascular pressures from one representative trial can summarize the effect on rat cardiac performance of these constriction-induced changes in PBP and CVP (Fig. 3). As circumferential pressure was applied to the rat's thoracic region, concomitant changes occurred to the rat's circulation and cardiac electrical activity. After surgery and before constriction began, rat PBP was pulsatile between 80 and 85 mmHg. After 30 s of constriction, PBP became non-pulsatile, decreased to below 26 mmHg, and remained low 60 s into constriction. Prior to constriction, CVP was −5 mmHg and increased to 4 mmHg 30 s into constriction, remaining above 3 mmHg at 60 s into constriction (Fig. 3). By the end of constriction, nearly all rats (91%, 10 of 11) demonstrated cardiac electrical dysfunction.

Effect of constriction on blood chemistry

Arterial blood gasses, blood pH and plasma [K^+] levels were all within normal range at the post-operative/pre-constriction time point. However, after the completion of the constriction bout (average of 6.49±1 min after initial strike), blood pH was significantly lower (7.0±0.03 versus 7.4±0.01; t=−13.44, d.f.=22, P<0.0001, N=16) and potassium levels were significantly higher (9.2±0.6 versus 4.8±0.2 mmol l^{-1}; t=7.52, d.f.=20, P<0.0001, N=17) than pre-constriction levels. Further, blood O_2 levels were significantly lower (89±16 versus 132±14 mmHg; t=−2.08, d.f.=30, P=0.05, N=16) and blood CO_2 levels were significantly elevated after constriction (86±6 versus 40±2 mmHg; t=7.38, d.f.=15, P<0.0001, N=14; Fig. 4).

DISCUSSION

These data provide the first evidence of the physiological effects of snake constriction on the cardiovascular system of their prey. Our measures of blood pressures, calculation of systemic perfusion pressures, ECGs and blood chemistry in rats being constricted by snakes demonstrate a direct effect of constriction on hemodynamic and electrical aspects of rat cardiac activity. In response to constriction, anesthetized rats averaged a 6-fold increase in CVP, a 2-fold decrease in PBP and a 3.9-fold decrease in f_H. These significant hemodynamic changes were accompanied by a 4.8-fold increase in QRS duration, indicating a significant disruption of normal cardiac rhythm, and, by the end of constriction bouts (mean 6.49±1 min), nearly all rats (91%) showed evidence of cardiac

Table 2. Comparison of cardiovascular parameters of rats (*Rattus rattus*) as they were constricted by boas (*Boa constrictor*)

				Post-strike		
Variable	*N*	Post-surgery/ Pre-constriction	Peak constriction pressure	1 min	3 min	5 min
Constriction pressure (mmHg)	14	0	156±15	78±8	46±8	37±8
PBP (mmHg)	6	82±2	42±13***	43±10***	36±8***	28±7***
CVP (mmHg)	10	−4±1	33±8***	20±6***	7±4^{NS}	3±2^{NS}
f_H (beats min^{-1})	16	436±11	399±12^{NS}	265±12**	155±15**	112±19***
QRS duration (ms)	15	19±11	19±12^{NS}	40±13***	77±16***	87±21***

Measurements were post-operative/pre-constriction, peak (maximum) constriction pressure, and 1 min, 3 min and 5 min post-strike.
PBP, peripheral blood pressure; CVP, central venous pressure; f_H, heart rate.
The level of statistical significance is indicated: ^{NS}P>0.05, **0.01>P>0.001, ***P<0.001, from comparisons of least squares means relative to pre-constriction levels using a Tukey–Kramer adjustment for multiple comparisons.

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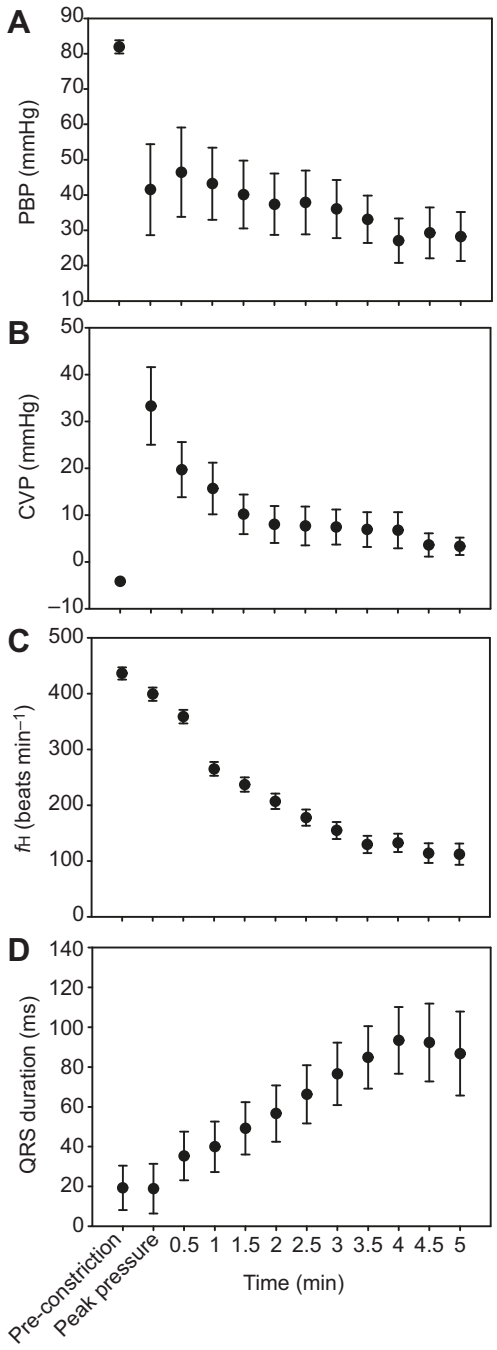

Fig. 2. Hemodynamic and electrocardiographic data versus constriction time. (A) PBP (N=6), (B) CVP (N=10), (C) heart rate (f_H, N=16) and (D) QRS duration (N=15) of rats as a function of time of constriction by boas. Relative to pre-constriction levels, PBP significantly decreased while CVP significantly increased within 5.9 s of the strike and initiation of constriction. f_H became significantly reduced and QRS duration became significantly longer within 30 s of the strike and initiation of constriction.

electrical dysfunction. Further, constriction by snakes caused rats to become significantly acidotic and hyperkalemic but mildly hypoxic.

Effect of constriction on CVP

In this study, anesthetized rats experienced a dramatic increase in CVP that corresponded precisely to an increase in applied external pressure from the snake's constriction coils. Within seconds, CVP increased 6-fold to a level (33±8 mmHg) well above normal (~1 mmHg; Cui et al., 2008) and remained elevated above 10 mmHg for at least 2 min. These constriction-induced increases in CVP likely had significant effects on cardiac output. For instance, return of systemic blood to the mammalian heart from the peripheral veins is a function of the pressure difference between the peripheral and central venous compartments divided by the resistance to this flow. Hence, right ventricular filling is a direct consequence. CVP, or pressure of blood in the proximal caudal and cranial cavae in mammals, is normally amongst the lowest of hydraulic pressures in the circuit (Mohrman and Heller, 2010). As such, PVP is higher than CVP to ensure adequate delivery of blood back toward the heart. This pressure difference typically ranges between 5 and 6 mmHg in rats (Cui et al., 2008) and thus even small changes in intra-thoracic pressures of 1 mmHg, as can occur during normal ventilation, slightly affect right end-diastolic filling volumes by altering CVP. However, when evaluated in the context of thoracic compression pressure exceeding 150 mmHg as we observed, a CVP of 33 mmHg would massively exceed PVP by almost 7-fold. That this occurs within a few seconds suggests that venous return to the right heart is greatly minimized or even halted. Within a few beats of the heart under these conditions, it follows that cardiac output from the left heart would also be minimized as the right and left hearts are in series.

Circumferential compression on the outside of a prey animal during constriction should be transmitted to the thoracic compartment and ultimately the pericardial space (Kaplan et al., 1995). Many of the features of cardiac stress due to excessive external pressures seen in human and veterinary medicine therefore parallel some of the constriction-induced increases in CVP we report here. For instance, the pressure surrounding the heart is moderately increased during normal respiratory movements but can also reach dangerous levels with certain pathologies such as acute thoracic compression, constrictive pericarditis and pleural effusion; each of these can significantly impact vascular blood flow (Shamblin and McGoon, 1963; Goldstein, 2004). Cardiac tamponade, or obstructive compression of the heart, occurs by rapid increases in pericardial pressure (15–30 mmHg in humans) typically induced by hemorrhage (hemopericardium) or effusion (Bolling and Stewart, 1997; Shoemaker, 2000). During tamponade, pericardial pressure approaches diastolic pressures (Bolling and Stewart, 1997). As these pressures equilibrate, the ventricles no longer fill adequately and cardiac output will not meet metabolic demand; ultimately, death occurs. Studies support the idea that the proximal effect of pericardial tamponade is on venous return to the heart (Louie et al., 1995). Indeed, pericardial pressures as low as just 3 mmHg above left ventricular diastolic pressure were sufficient to induce complete diastolic collapse in dogs (Schwartz et al., 1993); pressures slightly higher (10–20 mmHg) resulted in compression of the right atrium and great veins, leading to elevated pressures in the right atrium (Fowler and Gabel, 1985, 1987). Thus, even modest pressures at the level of the pericardium can induce significant cardiovascular effects. That we found constriction pressures in excess of 150 mmHg suggests rats experienced a more substantial increase in pressure surrounding the heart, leading to the massive increase in CVP.

In our study, some of the snakes applied loops of their bodies around the abdomen of the rats and thus applied circumferential compression directly to the abdominal region. Positive pressure around the abdominal compartment, consistent with elements of the clinically relevant abdominal compartment syndrome (ACS), has a number of pathophysiological effects including a dramatic decrease

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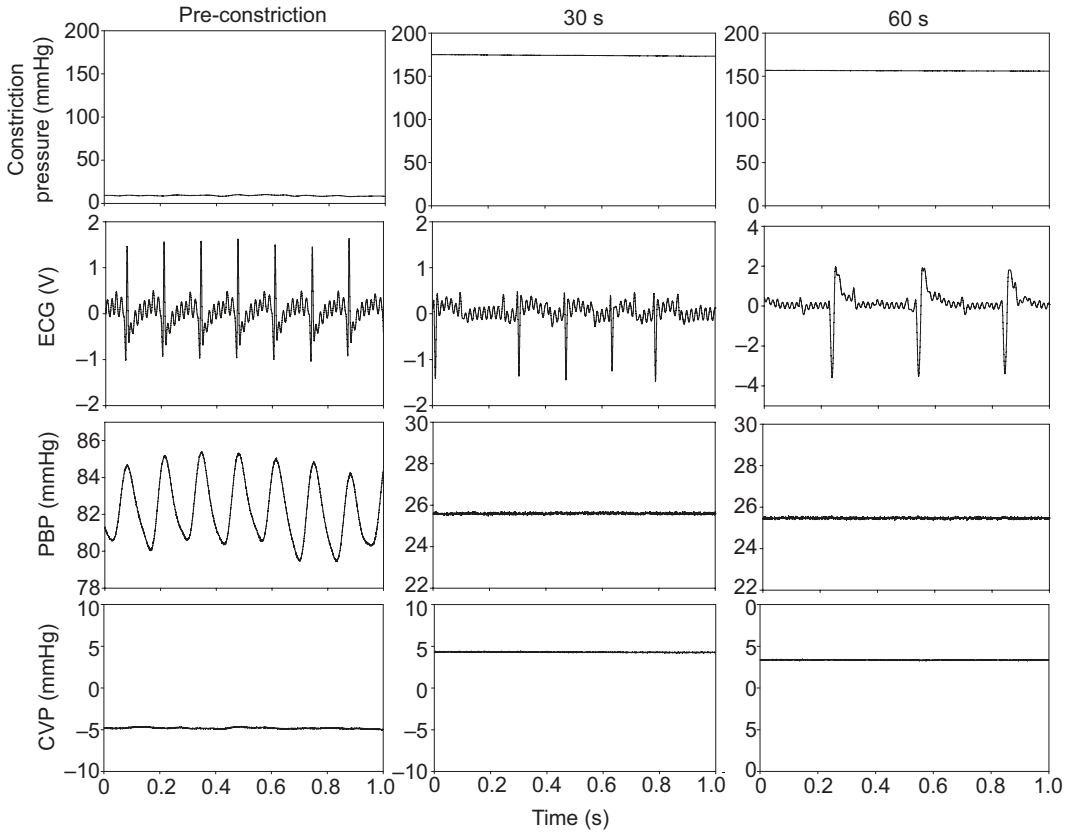

Fig. 3. Example of constriction pressure, electrocardiogram (ECG), PBP at the femoral artery and CVP at the jugular vein recorded from an anesthetized rat constricted by a boa. This constriction bout was 1.9 min in duration and a maximum constriction pressure of 207.2 mmHg occurred 5.4 s after the strike. Note that the scaling of axes varies for different parameters between time points for clarity. PBP was pulsatile prior to constriction and became non-pulsatile within seconds of the snake applying force to the rat; this pattern characterized 7 of 8 constriction trials.

in cardiac output (Burch et al., 1996). While we did not directly measure intra-abdominal or post-caval pressures, it is possible that the abdominal constriction coils contributed to significant venous insufficiency. Investigations using animal models have isolated the cause of the decrease in cardiac output during ACS to be reduced venous return, increased peripheral resistance, or both (Burch et al., 1996). For instance intra-abdominal pressures of 40 mmHg in dogs were sufficient to induce a 1.6-fold increase in right atrial pressure and a 3.3-fold increase in venous resistance (Kashtan et al., 1981). These studies support the idea that direct pressure on either the heart (via thoracic constriction loops) or the abdominal compartment (via abdominal constriction loops) could induce the 6-fold increase in CVP we observed in rats during constriction, ultimately leading to impairment of circulation.

Effect of constriction on PBP

At the same time that rat CVP reached its maximum, rat PBP fell to its minimum. PBP at the level of the femoral artery in the rat dropped 2-fold in the first 6 s of the strike and remained depressed throughout the constriction bout. Like the spike in CVP, the precipitous drop in rat PBP precisely matched the sharp increase in constriction pressure from the snake. As the catheter measuring arterial pressure was placed at the femoral artery, the pressures measured were distal to the thoracic or abdominal compartment and imply constriction has immediate consequences on the peripheral blood flow.

The sharp drop in PBP was consistent with our predictions and mimics the effects of cardiogenic shock induced by excessive pericardial pressure. Pericardial pressure of just 3 mmHg generated by cardiac tamponade leads to significant and rapid decreases in ventricular filling and, as mentioned previously, may have the most direct effect on venous return (Schwartz et al., 1993). However, regardless of the proximate effect, excessive pressure on the heart leads to a decrease in cardiac output and ultimately a dramatic and significant decrease in systemic blood pressure (Louie et al., 1995). Thus, the precise match of increased constriction pressure (as measured with our external pressure probe) with both increased CVP and decreased PBP (as measured with internal vascular catheters) is consistent with elevated intrapericardial and/or pleural pressures in our rat model (Fowler and Gabel, 1987; Kaplan et al., 1995). The combined effect of high CVP and low PBP resulted in systemic perfusion pressures below 25 mmHg and strongly suggests an outcome of systemic ischemia. The lack of pulsatility in PBP we observed immediately following constriction is consistent with the idea that tissue ischemia follows snake constriction (Fig. 3). Other studies would also support ischemia as a consequence of constriction. For instance, in studies on dogs, pleural pressures of 60 mmHg were sufficient to induce myocardial ischemia through reduction in coronary artery perfusion (Fessler et al., 1990).

Additionally, if arterial pressures in the carotid and basilar arteries feeding the brain match those in the femoral artery seen here, rats could become unconscious within seconds (Rossen et al., 1943). The possibility that thoracic compression leads to brain ischemia is supported by previous research (Schurr et al., 1995). For instance, to study how the brain responds to extended periods of hypoxia, neuroscientists frequently induce cerebral ischemia in rats using

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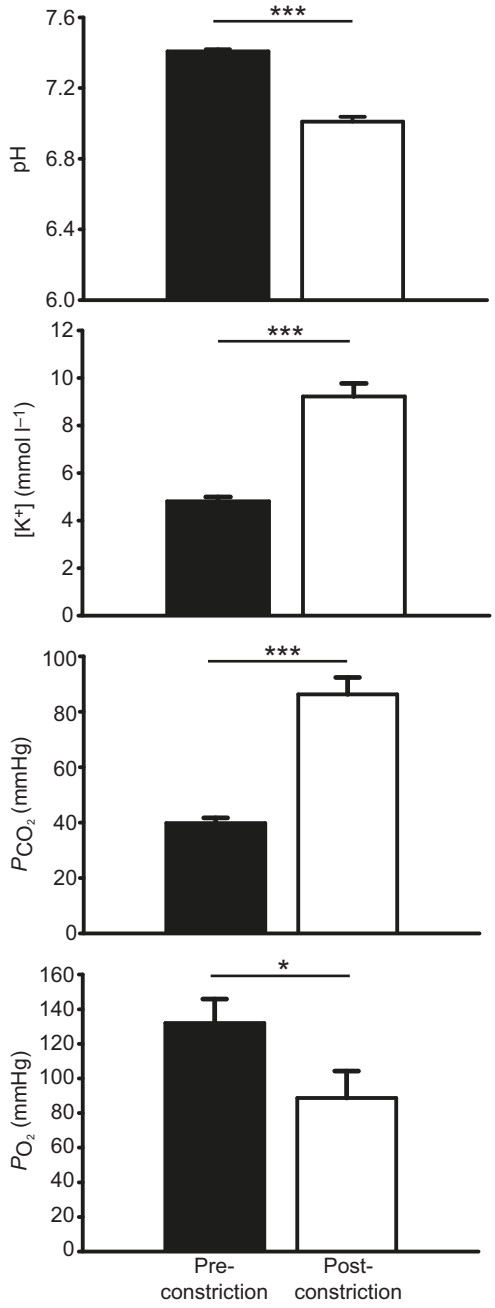

Fig. 4. Blood pH (*N*=16), [K^+] (*N*=17), P_{CO_2} (*N*=16) and P_{O_2} (*N*=14) levels of rats post-operative (pre-constriction) and immediately after being constricted by boas (post-constriction). *t*-tests comparing pre- and post-constriction means revealed significant differences among the variables: **P*=0.05, ****P*<0.0001.

hydraulic chest compression; absence of blood flow to the brain is then confirmed by abolition of cerebral electrical activity (e.g. Edmonds et al., 1989). Thus, we believe that a rapid induction of syncope via brain ischemia may be a key mechanism of snake constriction. Through this process, prey could be immobilized and any retaliation would be swiftly eliminated. Counter-attacks by prey, which often possess claws and teeth capable of inflicting fatal wounds (Frye, 1992) and can exceed the snake's own body mass, is a valid concern given the proximity of the struggling prey. In contrast, venomous viperid snakes attack in relative safety: in one swift motion they strike out from ambush locations, inject their venom and retract their heads, thus allowing their prey to struggle and die a safe distance away (Kardong and Bels, 1998). Additional experiments to record blood flow in carotid arteries and electroencephalography data of rats being constricted by snakes would help to clarify the importance of these ideas.

While we did not measure aortic root/arch pressure it is also likely that thoracic constriction pressure generated larger than normal increases in cardiac afterload, therefore resulting in the decreased PBP we observed. Indeed, intrapericardial pressures of 10 mmHg caused a significant decrease in aortic root pressure in dogs (Fowler and Gabel, 1987). Combined with a greatly reduced ventricular filling that reduces cardiac output, increases in afterload would make the chances of generating enough ventricular pressure to open the aortic semilunar valves far more difficult. We note that these constriction-induced afterload pressure increases would not need to be very great, as ventricular filling volume and therefore ventricular systolic pressure would be quite low because of a compression-induced reduction in sarcomere length (i.e. the Frank–Starling mechanism; Mohrman and Heller, 2010). Thus, the dramatic decrease in rat PBP we observed could have been caused by a rapid onset of supra-systolic blood pressure in the aortic root/arch; this would make opening the aortic semilunar valves impossible, resulting in systemic and cardiac ischemia. Future investigations should include aortic root/arch pressure monitoring during constriction to confirm this notion.

Effect of constriction on blood chemistry

Arterial blood chemistry in the left ventricle was profoundly altered following thoracic constriction. While pre-constriction values for Pa_{O_2}, Pa_{CO_2}, pH and [K^+] were well within normal range for our rats, Pa_{CO_2}, pH and [K^+] were each squarely within pathological range post-constriction. The elevations of Pa_{CO_2} and [K^+] and depression of pH are typical of those routinely observed during resuscitation from cardiac arrest (Makino et al., 2005; Le Guen et al., 2011). Additionally, during constriction, Pa_{O_2} fell significantly. However, the value of Pa_{O_2} post-constriction of 89 mmHg equates to ~95% peripheral capillary oxygen saturation (Sp_{O_2}; Goodgame et al., 2012). Thus, although Pa_{O_2} significantly decreased after constriction, the post-constriction Pa_{O_2} level suggests that systemic hypoxemia is not a significant factor in death due to snake constriction. This does not rule out the possibility that a depletion of blood oxygen worked in concert with other pathologies, such as decreased perfusion, to exacerbate tissue ischemia.

Our post-constriction arterial chemistry values are consistent with metabolic acidosis and may be derived from under-perfusion of systemic tissue and/or progressive metabolic failure of the ventricular endocardium. Regardless of the source, we would expect each to be consistent with an increase in lactic acid production from anaerobic metabolism. The addition of non-volatile acid (e.g. lactic acid)-donating hydronium ions to the blood would drive the equilibrium reaction (hydration of carbon dioxide) to the left and thus drive CO_2 out of solution. This would explain the dramatic increase in Pa_{CO_2} we observed in rats post-constriction. As we did not sample arterial blood for lactate, it is impossible to confirm lactate as the cause of these changes and we suggest future experiments measure blood lactate levels. Indeed, rats became significantly acidotic after being constricted by snakes. Remarkably, pH levels observed in rats post-constriction meet or exceed the drop in pH seen in rats after 3 h+ of hemorrhagic hypotension (Torres et al., 2004).

Increases in free potassium post-constriction are also consistent with cell energy failure and could originate from either

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endocardium/subendocardium ischemia or blood returning to the heart from distant ischemic tissues, or both. Although the source of the excess blood potassium is unclear to us, evidence from experiments inducing shock in rats suggests this could be attributed to hepatic ischemia. During hemorrhagic shock the liver experiences the most severe reduction in $\dot{V}_{O_2}$ (Ba et al., 2000), as well as a significant reduction in blood flow (Schlichtig et al., 1991). Relative to other organs, the liver therefore appears to be most susceptible to regional ischemia (Ba et al., 2000). Regardless of the source, hyperkalemia levels of 9 mmol l^{-1} of K^+ in our rats post-constriction are similar to those found at death in other studies (Van der Meer et al., 1986; Johnson et al., 1996). If this amount of K^+ is allowed to perfuse tissues (i.e. cardiac myocardium from coronary arteries), it would begin to depolarize excitable membranes in cardiac muscle tissue.

While these K^+ levels may be lethal and most certainly would cause cardiac dysrhythmia (Van der Meer et al., 1986), we do not believe hyperkalemia was the proximate cause of cardiac failure in the constricted rats. We measured blood K^+ levels at only two time points: before and after the constriction bout. Therefore the level of K^+ at other time points is unknown. If the K^+ is a result of crush injury and/or tissue ischemia, the flood of K^+ may have occurred after cardiac dysrhythmia was initiated. In contrast, the swift increase in CVP and decrease in PBP occurred within seconds of the snakes' strike and initiation of coil formation; we believe these to have a more proximate role in causing death.

Effect of constriction on cardiac electrical function

The effect of snake constriction was clearly manifest in the rat ECG as f_H significantly decreased (a 3.9-fold decrease; bradycardia) within 30 s of the strike and continued to decrease throughout the constriction bout. However, the heart not only slowed but also showed evidence of an abnormal rhythm. At the same time that f_H decreased, QRS duration increased rapidly (it was significantly higher than pre-constriction levels within 30 s of the strike) and continued to increase throughout constriction. These changes in f_H and QRS are not trivial and indicate a rapid and devastating impact on cardiac function within 30 s of the start of constriction.

The profound bradycardia we observed could have been induced by a number of conditions including but not limited to hyperkalemia or myocardial ischemia. However, the swift appearance of the bradycardia (halving of resting f_H by 1.5 min of constriction) suggests a more rapid cause. We propose that bradycardia in constricted rats may have been due to the immediate response of the arterial baroreceptor reflex (Mohrman and Heller, 2010). When mean arterial blood pressure is elevated beyond an accepted limit, parasympathetic outflow from baroreceptors in the aortic arch and carotid bodies quickly reduces f_H (bradycardia) while central suppression of vascular sympathetic outflow drives arterial vasodilatation. If constriction loops located directly over the heart transmitted external pressures to the thoracic cavity and ultimately to the aorta, this increased pressure could have stimulated the aortic arch baroreceptors to induce bradycardia. Interestingly, our results are consistent with recent work in rats showing that the baroreceptors found in the aortic arch play a dominant role in controlling the cardiac (f_H) component of the baroreflex while the carotid baroreceptors tend to drive inhibition of sympathetic outflow to the vasculature (Pickering et al., 2008). Although our results are limited in this regard, two of the three rats that responded with an increased f_H (tachycardia rather than bradycardia) were constricted around the neck, not the thorax. With circumferential compression applied to the neck, baroreceptors in the aortic arch may have been relatively unaffected by direct compression and instead responded to a depression in systemic arterial pressure, resulting in an initial increase in f_H. The degree to which each baroreceptor bed was stimulated was not explicitly evaluated in our study and thus the actual proximate cause of the bradycardia remains unresolved. Regardless, sustained bradycardia without offsetting increases in contractility or adequate ventricular filling (as described above) suggest a profoundly reduced cardiac output in the rat in the early phases of thoracic constriction. Coupled with high aortic pressures due to constriction, it is unlikely that the minimized cardiac output would be enough to perfuse the coronary arteries. Given the high mass-specific metabolic rate of rat cardiac muscle, this ischemia would rapidly begin to cause heart cell failure, further weakening contractility and altering normal electrical activity.

The QRS complex is the most conspicuous part of an ECG and represents ventricle depolarization. Abnormal widening of the QRS interval (increased duration) indicates a slowing of the depolarization impulse and a progressive loss of right to left ventricle depolarization synchrony, and suggests either a malfunctioning of the conducting fibers themselves (often referred to as a bundle branch block) due to injury or altered blood electrolyte concentrations specifically elevated K^+ (hyperkalemia). Hence, the cause of QRS widening in constricted rats could be the elevated blood K^+ (regardless of its source) that was detected in our rats at the end of constriction. However, our data (ECG panels of Fig. 3) are also in line with recent work by Bao et al. (2011), who demonstrated that low flow ischemia in rat hearts (flow decreased from 10 to 2 ml min^{-1}) significantly prolonged QRS duration by activation of K_{ATP} channels. [Yet, in contrast with Bao et al. (2011), the rate of QRS widening was more rapid in our experiments and implies a more severe depression in coronary flow in constricted rats.] We also recognize the possibility of a synergetic influence of myocardial ischemia and hyperkalemia on the heart electrical activity during constriction.

Conclusion

Our results suggest that boas are subduing and killing their prey during constriction by significantly affecting the prey's cardiovascular system. The snakes accomplish this by encircling their prey with loops of their bodies; pressure is then generated when this coil is reduced in diameter (Greene and Burghardt, 1978; Mehta and Burghardt, 2008). We believe that this circumferential compression generated by snake constriction is especially effective because of the efficiency of energy transfer from this geometric shape change (Halperin et al., 1993). When significant constriction pressure was placed on the rat's thoracic or abdominal region, CVP increased and PAP decreased almost immediately, presumably due to increased thoracic and/or abdominal pressures. Concurrently, constriction led to significant hyperkalemia and acidosis in the rats. Cardiac electrical dysfunction, as evidenced by bradycardia and widening of the QRS interval, followed these cardiovascular changes and supports the idea that snake constriction reduces the perfusion of vital organs. Organs such as the liver, brain and heart would be the first to succumb to rapid tissue hypoxia because of their high metabolic rates; the rapid widening of the QRS interval provides direct evidence that the cardiac muscle is indeed affected by such ischemia within the first 30 s of constriction. That our rats were under the influence of barbiturate anesthetic drugs (therefore effectively reducing the metabolic demand of the tissues), yet were still significantly impacted by constriction strongly suggests that these effects on non-anesthetized rats would be even more rapid. However, we do note that Tucker et al. (1982), using a different strain of rats (Munich–Wistar), reported that the use of the anesthetic thiobutabarbital (Inactin) correlated with a higher than

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normal systemic adrenergic activity. If our anesthetized rats were similarly affected, non-anesthetized rats may respond more similarly to anesthetized rats as non-anesthetized rats should have a strong adrenergic response to being bitten and then crushed by loops of a snake's body.

In contrast, the tissues of ectothermic prey species should be less affected by constriction as they possess lower vascular pressures and a greater resistance to hypoxia (Pough, 1983; Hardy, 1994). Dispatching ectothermic prey may require constriction bouts of greater duration, for which there is some evidence (e.g. Pope, 1961; Reed et al., 2006). Regardless, the greater reliance on anaerobic respiration causes ectotherms to fatigue more quickly (Pough, 1983) and therefore, as Hardy (1994) suggested, constriction may function to simply immobilize rather than kill ectothermic prey prior to being consumed. Interestingly, Wang et al. (2001) showed that snake blood pressure increased during a constriction event. A potentially fruitful future study could involve simultaneously measuring both predator (snake) and prey (both endothermic and ectothermic) cardiovascular function during snake constriction.

These results are also consistent with our previous work demonstrating that boas are capable of detecting the prey's heartbeat during constriction and meter their constriction effort based on this (Boback et al., 2012). However snakes in the current study released some rats prior to achieving asystole in the ECG. As our past experiments demonstrated that snakes are responding to the mechanical movement rather than the electrical activity of the heart (Boback et al., 2012), the current results suggest that snakes may actually be inducing pulseless electrical activity and therefore release prey precisely when they have achieved irreversible cardiac failure. Indeed, electromechanical dissociation of the rat heart has been demonstrated in prior studies as mechanical asystole was achieved 244±22 s after asphyxiation was induced while the electrical activity of the heart continued for at least 10 min (Hendrickx et al., 1984). Our results also suggest that even if prey are successful in escaping while being constricted, an excessive flood of blood K^+ as we observed could result in lethal hyperkalemia (Van der Meer et al., 1986; Blaisdell, 2002). While the precise mechanisms of death remain obscure, it is clear that circumferential pressure on the prey's thorax and/or abdomen has severe impacts on cardiovascular function. Our results are in complete agreement with the ideas of McLees (1928) and Hardy (1994): rather than suffocation, circulatory arrest may be the most proximate cause of death during snake constriction.

MATERIALS AND METHODS

Animals

Experiments were performed on 24 male Wistar rats (350–475 g) purchased from Charles River Laboratories Inc. (Wilmington, MA, USA) and maintained on an *ad libitum* diet of commercial rodent chow (Mazuri Rodent Diet) and tap water in standard rat tubs (17×10.5×7 in, ~43×27×18 cm) with wire lids. Prior to surgical procedures, food, but not water, was withheld from rats for 12 h. On the morning of surgery, rats were weighed on an electronic balance and were matched with snakes so that rats were the same relative size (25% of snake's mass) for all constriction tests. Boas (*B. constrictor*) were wild-caught in Belize, Central America, and maintained in a reptile facility (28±2°C, 60% relative humidity) on a diet of dead rats and chicks. We tested both wild-caught and captive-born (F1 generation) snakes in these tests. To ensure animals had similar readiness to feed, all snakes were a minimum of 14 days post-fed prior to being used in constriction tests.

Anesthesia

Anesthesia was induced in rats with inhaled isoflurane and then maintained with Inactin (thiobutabarbital sodium salt hydrate solubilized in saline immediately prior to each use) injected intraperitoneally (i.p.) with an initial dose of 120 mg kg^{-1}. Inactin was selected as the optimal anesthetic for our purposes because it is a long-acting (3–4 h; Vasthare et al., 1988; Phillipson et al., 2008) injectable barbiturate, with a minimal effect on the cardiovascular system (Polakowski et al., 2004). Because anesthesia inhibits voluntary skeletal muscle, we could eliminate muscular movement and struggling of the rats as a potential confounding variable on constriction behavior (Moon, 2000). After injection with Inactin, rats were monitored and maintained at Stage III, Plane 1 of surgical anesthesia as judged by loss of palpebral reflex, insensitivity to deep periosteal pain, and continued and obvious spontaneous ventilation. When necessary, additional quarter doses of Inactin were administered i.p. to maintain this level of anesthesia.

ECG lead placement

Once under anesthesia, rats were moved to a surgical table and placed on a heating blanket to maintain normal body temperature (38°C; Suckow et al., 2006). Rats were shaved with clippers at all operative sites. Three electrodes [Grass Ag (silver cup), Grass Technologies, West Warwick, RI, USA] were placed subcutaneously via stab wounds oriented in an equilateral triangle on the ventral thorax to continually record ECG. Electrode cups were affixed with suture and wounds were sutured closed using 4-0 silk. Leads were externalized via trochar to a common exit wound located in the right upper abdominal quadrant and were attached to an ECG cable. ECG was recorded using AcqKnowledge software (v 3.9, Biopac Systems Inc., Goleta, CA, USA) at a rate of 2000 Hz.

Constriction pressure

A hydraulic pressure probe measuring constriction pressure was constructed of Tygon tubing [20 cm in length, 1/4 in o.d.×1/16 in i.d. (6.4×1.6 mm); Sarns Inc., 3M, Ann Arbor, MI, USA) capped with a 3-way stopcock on one end and a plastic plug on the other. The probe was filled with water and connected to a pressure transducer (Gould/Statham P23XI). Following a four-point calibration and drift test (mercury manometer), the probe was placed subcutaneously on the left dorsolateral side perpendicular to the diaphragm. Constriction pressure was recorded using AcqKnowledge software (v 3.9, Biopac Systems Inc.).

Vascular catheters

Intravascular pressures (PBP and CVP) were recorded continuously via femoral artery and jugular vein catheterization, respectively. The jugular vein was catheterized with PE50 affixed to surgical Tygon tubing and advanced approximately 2.5 cm to the confluence of right and left cranial vena cavae. The left femoral artery was catheterized with a 24-guage IV catheter and needle (Angiocath, Abbott Laboratories, Abbott Park, IL, USA) inserted proximal to the popliteal artery bifurcation and distal to the epigastric branch. Catheters were filled with saline and connected to multipoint-calibrated pressure transducers (Gould/Statham P23Xl). Pressure was recorded using AcqKnowledge software (v 3.9, Biopac Systems Inc.).

Arterial blood chemistry

Following ECG lead placement, pressure probe insertion and vascular catheterizations, a pre-constriction arterial blood sample (~0.25 cc) was obtained from the femoral artery and immediately analyzed using an IRMA blood gas analyzer (2000 series, Edison, NJ, USA). After removal of the blood sample, an infusion plug (intermittent infusion plug 0.1 ml, Tyco Healthcare, Covidien, Mansfield, MA, USA) was secured to the femoral artery catheter. A 19-guage catheter needle and IV tubing (winged infusion set with 30 cm tubing, Terumo Surflo, Somerset, NJ, USA) was inserted through the infusion plug septum and secured with suture. This technique enabled a pre-surgical blood draw and pressure monitoring from a single catheterization. Immediately after the constriction bout, a post-constriction blood sample was taken from the rat's left ventricle or the aortic arch for blood gas analysis.

Constriction

Instrumented rats were presented to snakes head first, as snakes typically strike the anterior region of their prey (Kardong, 1992; Mehta and Burghardt, 2008). Once a strike occurred, the snakes applied loops of their bodies around the rats and proceeded to constrict. Rats remained with the

snakes until the snake disengaged its mouth and began to release its coil, at which point the rat was removed. In a pilot study, we found that constriction pressure decreased sharply just prior to snakes removing their mouths (S.M.B., Allison E. Hall, K.J.M., Amanda W. Hayes, Jeffrey S. Forrester and C.F.Z., unpublished data) and coincided with the start of prey inspection behavior (Canjani et al., 2003). A post-mortem necropsy confirmed catheter and ECG placements. Snakes were then fed a second non-instrumented, intact rat. All constriction experiments were conducted in a constant temperature room of 28±2°C with 50% relative humidity.

Data analysis

Arterial and venous pressures were measured at 30 s time intervals throughout the constriction event and were analyzed using mixed linear models in SAS 9.3 (SAS Institute Inc., Cary, NC, USA). For our mixed procedures, PBP and CVP were our dependent variables, time was a fixed effect, and individual snake and rat were treated as random effects (in separate models) to account for repeated measurements on individuals. We examined the influence of snake and rat body mass on our dependent variables by testing these as covariates. When we found a significant effect of time, we tested for differences within this effect using the LSMEANS statement with a Tukey–Kramer adjustment for multiple comparisons. Specifically, to examine how PBP and CVP changed throughout the constriction event, we compared values with pre-constriction (i.e. baseline) levels using the DIFF=CONTROL option in the LSMEANS statement.

At 30 s time intervals, average f_H was calculated over 5 s time intervals. For these same time intervals, we calculated QRS duration as the average of five QRS waves. These post-test analyses were managed using AcqKnowledge software (v 3.9, Biopac Systems Inc.). Changes in mean f_H and QRS durations during constriction were determined using mixed models described above but with f_H and QRS duration as dependent variables, time as a fixed effect, and individual snake and rat as random effects to account for repeated measurements on individuals.

Pre- and post-constriction arterial blood chemistry values were compared using paired *t*-tests. When variance around means was significantly different, we used the Satterthwaite test for unequal variances. We analyzed a total of 18 constriction trials using nine individual snakes and we report means±s.e.m. as our measure of central tendency.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

S.M.B., K.J.M., E.L.B.and C.F.Z. conceptualized and designed the study. S.M.B., K.J.M., K.A.W., P.M.M., E.L.B. and C.F.Z. performed experiments. S.M.B., K.J.M. and C.F.Z. analyzed the data and developed the manuscript. S.M.B. prepared the figures and tables.

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Supplementary material

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