Autonomic control of heart rate during forced activity and digestion in the snake *Boa constrictor*

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Summary

Reptiles, particularly snakes, exhibit large and quantitatively similar increments in metabolic rate during muscular exercise and following a meal, when they are apparently inactive. The cardiovascular responses are similar during these two states, but the underlying autonomic control of the heart remains unknown. We describe both adrenergic and cholinergic tonus on the heart during rest, during enforced activity and during digestion (24–36 h after ingestion of 30 % of their body mass) in the snake *Boa constrictor*. The snakes were equipped with an arterial catheter for measurements of blood pressure and heart rate, and autonomic tonus was determined following infusion of the β-adrenergic antagonist propranolol ($3\,mg\,kg^{-1}$) and the muscarinic cholinoceptor antagonist atropine ($3\,mg\,kg^{-1}$).

The mean heart rate of fasting animals at rest was $26.4\pm1.4\,min^{-1}$, and this increased to $36.1\pm1.4\,min^{-1}$ (means ± S.E.M.; N=8) following double autonomic block (atropine and propranolol). The calculated cholinergic and adrenergic tones were $60.1\pm9.3\,\%$ and $19.8\pm2.2\,\%$, respectively. Heart rate increased to $61.4\pm1.5\,min^{-1}$ during enforced activity, and this response was significantly reduced by propranolol (maximum values of $35.8\pm1.6\,min^{-1}$), but unaffected by atropine. The cholinergic and adrenergic tones were 2.6 ± 2.2 and $41.3\pm1.9\,\%$ during activity, respectively. Double autonomic block virtually abolished tachycardia associated with enforced activity (heart rate increased significantly from 36.1 ± 1.4 to $37.6\pm1.3\,min^{-1}$), indicating that non-adrenergic, non-cholinergic effectors are not involved in regulating heart rate during activity. Blood pressure also increased during activity.

Digestion was accompanied by an increase in heart rate from 25.6 ± 1.3 to $47.7\pm2.2\,min^{-1}$ (N=8). In these animals, heart rate decreased to $44.2\pm2.7\,min^{-1}$ following propranolol infusion and increased to $53.9\pm1.8\,min^{-1}$ after infusion of atropine, resulting in small cholinergic and adrenergic tones (6.0 ± 3.5 and $11.1\pm1.1\,\%$, respectively). The heart rate of digesting snakes was $47.0\pm1.0\,min^{-1}$ after double autonomic blockade, which is significantly higher than the value of $36.1\pm1.4\,min^{-1}$ in double-blocked fasting animals at rest. Therefore, it appears that some other factor exerts a positive chronotropic effect during digestion, and we propose that this factor may be a circulating regulatory peptide, possibly liberated from the gastrointestinal system in response to the presence of food.

Introduction

The metabolic increments associated with digestion or exercise are similar in a number of snake species that ingest large meals at irregular intervals (Secor and Diamond, 1995; Andrade et al., 1997; Secor et al., 2000). Consequently, these two physiological states impose similar demands on the cardiorespiratory system to sustain adequate gas exchange and transport (Wang et al., 2001). However, two recent studies, comparing the cardiopulmonary responses to exercise and digestion in the snake *Python molurus* and the lizard *Varanus exanthematicus*, show some marked differences in the ventilatory responses between these states (Hicks et al., 2000; Secor et al., 2000): digestion is associated with a relative hypoventilation (i.e. ventilation does not increase in proportion to gas exchange) to counter the 'alkaline tide'

(Overgaard et al., 1999; Busk et al., 2000), whereas exercise is associated with hyperventilation [e.g. (Wang et al., 1997)]. In contrast, the cardiovascular response is directly related to the prevailing metabolic demand, irrespective of whether it is caused by muscular activity or digestive processes (Hicks et al., 2000; Secor et al., 2000).

These observations point to a tight link between metabolic rate and cardiac function, but it is not known whether the cardiac responses to exercise and to digestion are accomplished through similar mechanisms in terms of cardiac control. As in other vertebrates, the heart of reptiles is dually innervated: the sympathetic nervous system exerts an excitatory adrenergic innervation, while the cholinergic innervation from the

parasympathetic nervous system is inhibitory [for a review on snakes, see (Lillywhite and Donald, 1994)]. These nerves run in a mixed vagosympathetic nerve and appear to innervate the atria and the pacemaker region as well as the ventricle, e.g. (Hedberg and Nilsson, 1975). Increased heart rate could follow an increase in sympathetic activity or a withdrawal of an inhibitory vagal tone. In addition, the levels of circulating catecholamines increase during forced activity in snakes and have been suggested to exert an important chronotropic effect (Stinner and Ely, 1993). Studies on other ectothermic vertebrates also point to an important role for the sympathetic nervous system in causing tachycardia during exercise, e.g. (Wahlqvist and Campbell, 1988; Axelsson, 1988; Axelsson et al., 1989). However, because metabolic rate remains elevated for several days during digestion, the efferent autonomic control of the heart may be different from that exerted during exercise. Also, it is unlikely that circulating catecholamines contribute substantially to the tachycardia over the long time span of digestion.

The aim of the present study was to compare changes in heart rate and blood pressure during enforced activity and digestion and to describe their autonomic control in a snake. The experiments were conducted on the snake *Boa constrictor*, which is able to ingest large meals, and autonomic control was quantified using specific antagonists to the cholinergic and adrenergic effectors on the heart.

Materials and methods

Animals, surgery and measurements of blood pressure and heart rate

Nineteen specimens of *Boa constrictor* (L.) were bred and reared in captivity for approximately 2 years at the Jacarezario at UNESP Rio Claro, SP, Brazil. They were fed a mixed diet of rodents and chickens, were kept at $28\pm5\,^{\circ}C$ under a natural photoperiod and all appeared healthy. By the time of the experiments, they had attained body masses between 1.5 and 2.4 kg. All animals had been fasted for more than 3 weeks before the experiments were conducted.

The surgery was performed under CO_2 anaesthesia. The cranial branch of the vertebral artery was accessed through a 5 cm long lateral incision, immediately cranial to the heart, and was occlusively cannulated. The cannula (PE60 containing heparinised saline) was pushed forward into the right aortic arch. The incision was then closed, and the cannula was secured onto the back of the snake by three sutures. All snakes resumed breathing spontaneously and appeared to regain normal activity levels within an hour of surgery, which normally lasted 20–30 min [for the physiological effects of CO_2 anaesthesia, see (Wang et al., 1993)].

For measurements of blood pressure, the catheter was connected to a Statham blood pressure transducer that, in turn, was connected to a BIOPAC preamplifier. The transducer was calibrated daily against a static water column. The signal was recorded at 25 samples s^{-1} on a Twinhead PC using a BIOPAC MP100 unit, and heart rate (f_H) was derived from the pulsatile arterial pressure signal.

Experimental protocols

Each animal was allowed to recover for 24–48 h after surgery and was held in a 60 cm×30 cm×15 cm plastic box within a climatic chamber (Fanem, SP, Brazil) maintained at $30\,^{\circ}C$. The snakes were maintained at this temperature for a minimum of 12 h before measurements were taken. The catheter was passed through an opening in the box and out of the climatic chamber during measurements to minimise disturbance to the snake.

Resting heart rate and blood pressure were obtained from 16 undisturbed snakes when these variables had been stable over a period of approximately 30 min. The animals were then forced into activity by provoking them to strike and bite the masked hand of the investigator. The animals invariably hissed and made between two and five rapid strikes within the 2 min period. Having recorded these cardiovascular responses, the snakes were subsequently used in one of two experimental protocols.

Autonomic regulation during rest and forced activity

Following the bout of enforced activity, eight snakes were left undisturbed until blood pressure and heart rate had returned to the previously recorded resting value (this occurred within 60–150 min). Atropine or propranolol ($3\,\mathrm{mg\,kg^{-1}}$) was then injected through the arterial catheter and allowed to take its effects for 20 min, before activity was enforced for 2 min as above. Following a recovery period of 30 min, the other autonomic blocker was injected, and the activity protocol was repeated after an additional 20 min. In four animals, atropine was injected before propranolol; this order of administration was reversed in the other four animals. The behaviour of the snakes during enforced activity appeared to be completely unaffected by the autonomic blockade, and the differences observed following the drug infusions did not appear to be a secondary consequence of the drugs rendering the snakes lethargic or unresponsive.

Autonomic regulation during digestion

In this series, the other eight snakes were presented with freshly killed rats, which they struck and constricted before swallowing. The snakes normally ate two rats, and the meals constituted approximately 30 % (range 26–36 %) of body mass. All snakes ate voluntarily within a few minutes after presentation of the rats. At approximately 24–36 h after ingestion, propranolol and atropine were injected, with a 30 min interval between injections. In four animals, atropine was injected before propranolol; this order of administration was reversed in the other four animals. Two of the eight snakes regurgitated their meal the day after autonomic blockade, but the remaining six animals completed the digestion of their meal.

Control experiments

To verify that the changes observed following autonomic blockade in the studies described above were not due to progressive exhaustion, three snakes were exposed to repeated bouts of enforced activity, using the protocol described above, but without the infusion of drugs.

Table 1. *Heart rate and systemic blood pressure during undisturbed rest and during forced activity in the two groups of* Boa constrictor *used for the activity and feeding studies*

	Rest		Forced activity	
	f_H (min^{-1})	P_{sys} (kPa)	f_H (min^{-1})	P_{sys} (kPa)
Animals for activity studies (mean body mass 1.45±0.34 kg)	26.4±1.6	5.33±0.47	61.4±1.5*	8.47±0.24*
Animals for feeding studies (mean body mass 2.38±0.45 kg)	25.6±1.3	6.83±0.38‡	60.3±1.2*	10.22±0.50*,‡

Values are means ± S.E.M. (*N*=8 in each group). An asterisk indicates a significant difference between rest and forced activity within each group; a double dagger indicates a significant difference between the two groups of snakes.

f_H, heart rate; P_{sys}, systemic blood pressure.

In two fasting and two digesting snakes, we verified that the autonomic blockers successfully and completely abolished the effects of injected adrenaline and acetylcholine over the time course of an experiment. Injection of 0.2 ml of 10^{-6} mol l^{-1} acetylcholine caused a transient cardiac arrest, followed by slow beating. This response was completely blocked by prior injection of atropine. Injection of 0.2 ml of 10^{-6} mol l^{-1} adrenaline caused a transient cardiac acceleration (heart rate increased to approximately 50 min^{-1}) that was completely abolished by prior injection of propranolol.

Calculation of adrenergic and cholinergic tones

The adrenergic and cholinergic tones on the heart were calculated on the basis of R–R interval ($1/f_H$; in s) using the equations provided by Altimiras et al. (Altimiras et al., 1997). In this approach, the changes in the R–R interval induced by propranolol and atropine are expressed relative to the intrinsic R–R interval. The intrinsic R–R interval is the value obtained after complete autonomic block (i.e. after atropine and propranolol).

Data analysis and statistical analyses

The data were analysed using Acknowledge (3.0) data-analysis software (Biopac system, Inc. CA, USA). Within each of the two experimental groups, the effects of digestion and/or activity were identified using paired *t*-test or a one-way analysis of variance (ANOVA) for repeated measures followed by a Bonferroni *post-hoc* test to identify significant differences between mean values. Differences in the physiological variables between the animals studied during feeding and activity were assessed using a *t*-test. Differences between means were considered statistically significant when $P \leqslant 0.05$, and all results are presented as means ± 1 S.E.M.

Results

The mean heart rates during undisturbed rest and during forced activity were the same for the two experimental groups (Table 1). However, mean systemic blood pressure was significantly higher, both at rest and during forced activity, in the animals used for the feeding studies. This may correlate with the significantly larger mass of these animals (Table 1).

As depicted in the data trace from one of the animals, shown in Fig. 1A, heart rate and blood pressure increased immediately during enforced activity and reached maximum values within 40–90 s. The act of capturing, constricting and swallowing the prey elicited similar rapid increases in blood pressure and heart rate (Fig. 1B). Although not investigated in detail, because we did not want to disturb the snakes while eating, maximum blood pressures occurred during swallowing in the four snakes that were visually observed during the process. The trace presented in Fig. 1B also shows the large effect of hissing, where the fast inspirations are associated with large reductions in systemic blood pressure.

The mean blood pressures during the experimental protocols are presented in Fig. 2. The blood pressure of resting and undisturbed snakes was 5.33±0.47 kPa (Table 1) and was not affected by propranolol, whereas atropine caused a small but not statistically significant increase to 6.27±0.24 kPa. Following double autonomic block, blood pressure was 7.00±0.78 kPa (Fig. 2A). It is possible that this increase is due to incomplete recovery from the preceding period of enforced activity. Blood pressure increased significantly to 8.47±0.23 kPa during forced activity, and this response was not affected by autonomic blockade (Fig. 2B). The fasting blood pressure of the animals used for the feeding experiments was 6.83±0.38 kPa (Table 1), and this increased, albeit not statistically significantly, to 7.68±0.26 kPa during digestion (Fig. 2C). Blood pressure was not affected by autonomic blockade during digestion (Fig. 2C).

The mean heart rates are presented in Fig. 3. Infusion of propranolol in resting snakes caused a significant reduction from 26.4±1.4 to 23.9±0.6 min^{-1}, whereas infusion of atropine elicited an increase to 45.7±0.1 min^{-1} (Fig. 3A). Heart rate increased to a maximum of 61.4±1.5 min^{-1} during forced activity and, while this response was not affected by atropine (60.4±0.7 min^{-1}), it was greatly reduced by propranolol (35.8±1.6 min^{-1}). Heart rate increased significantly, but only modestly during forced activity, following double autonomic
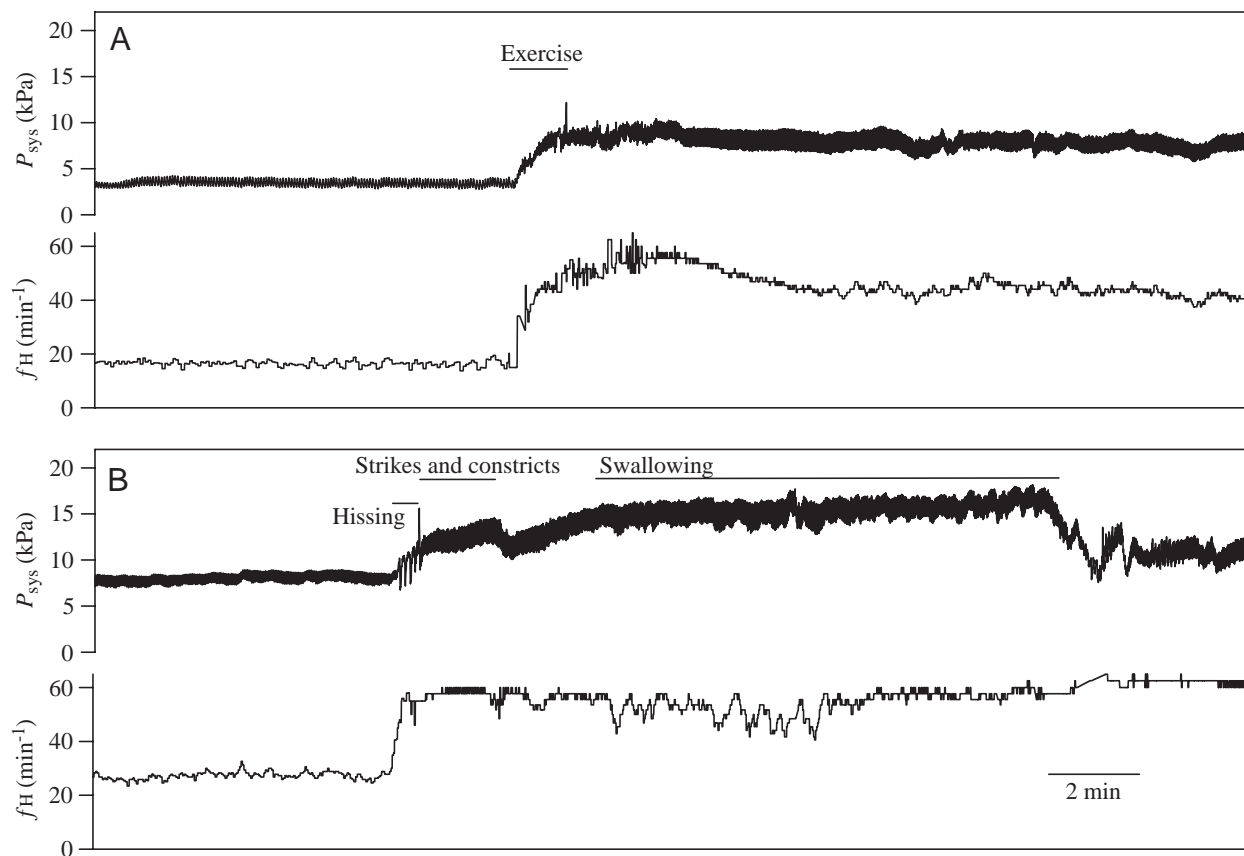
Fig. 1. Original recordings of heart rate (f_H) and systemic blood pressure (P_{sys}) in two different snakes used for the studies of enforced activity (A) and the effects of digestion (B).

blockade (from $36.1\pm1.4\,min^{-1}$ in double-blocked resting animals to $37.6\pm1.3\,min^{-1}$). Digestion was accompanied by an increase in heart rate from $25.6\pm1.3\,min^{-1}$ in resting and undisturbed fasting snakes to $47.7\pm2.2\,min^{-1}$ at 24–36 h following ingestion (Fig. 3C). The heart rate of digesting animals decreased to $44.2\pm2.7\,min^{-1}$ following propranolol infusion and increased to $53.9\pm1.8\,min^{-1}$ after infusion of atropine. The heart rate of double-blocked animals was $47.0\pm1.0\,min^{-1}$ in digesting animals, which is significantly higher than the value of $36.1\pm1.4\,min^{-1}$ in double-blocked, resting and fasting snakes.

That the changes observed after infusion of autonomic blockers, compared with the first bout of activity, were not caused by cumulative effects or progressive fatigue was shown by the heart rates in non-blocked animals subjected to the same activity protocol, which were 60.8 ± 1.1, 59.8 ± 2.1 and $60.3\pm1.4\,min^{-1}$ ($N=3$) during the first, second and third bouts of forced activity, respectively. These mean values were not significantly different.

The effects of the autonomic blockers allowed calculation of the cholinergic and adrenergic tones on the heart during the different situations studied (Fig. 4). Resting and undisturbed snakes were characterised by a large cholinergic tone of $60.1\pm9.3\,\%$ and an adrenergic tone of $19.8\pm2.2\,\%$. The cholinergic tone varied more than the adrenergic tone between

Table 2. *Cholinergic and adrenergic tones on the heart during undisturbed rest, during forced activity and during digestion in* Boa constrictor *grouped according to the sequence of autonomic blockade*

	Rest	Forced activity	Digestion
Cholinergic tone (%)			
Propranolol before atropine	53.5±9.5	6.9±3.2	12.9±5.1
Atropine before propranolol	66.6±18.3	−1.8±1.8	−0.9±2.7
Adrenergic tone (%)			
Propranolol before atropine	17.2±2.8	43.5±3.0	13.5±1.2
Atropine before propranolol	22.4±3.5	39.0±2.4	8.6±1.1*

Values are means ± S.E.M. ($N=4$ in each group), and the asterisk indicates a significant difference between the order of pharmacological manipulation.

the individual animals. There was a good correlation between resting heart rate and cholinergic tone, whereas such a relationship was not present between heart rate and adrenergic
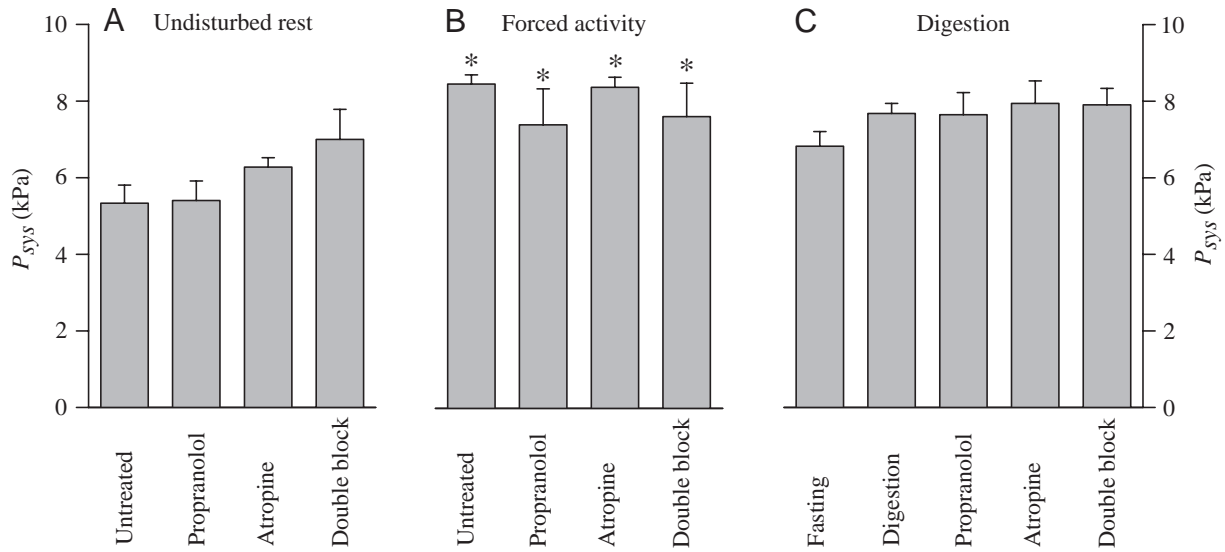
Fig. 2. Systemic blood pressure (P_{sys}) for *Boa constrictor* during resting undisturbed conditions (A), during forced activity (B) and during digestion (C). The figure depicts the untreated control condition (N=8) and the values obtained following autonomic blockade with the β-adrenergic antagonist propranolol ($3\,mg\,kg^{-1}$) and the muscarinic cholinoceptor antagonist atropine ($3\,mg\,kg^{-1}$; N=4 for both) and following double autonomic blockade (N=8). The fasting blood pressure is also shown for the snakes studied during digestion (C). Values are means + S.E.M. An asterisk indicates a significant difference from the resting value.
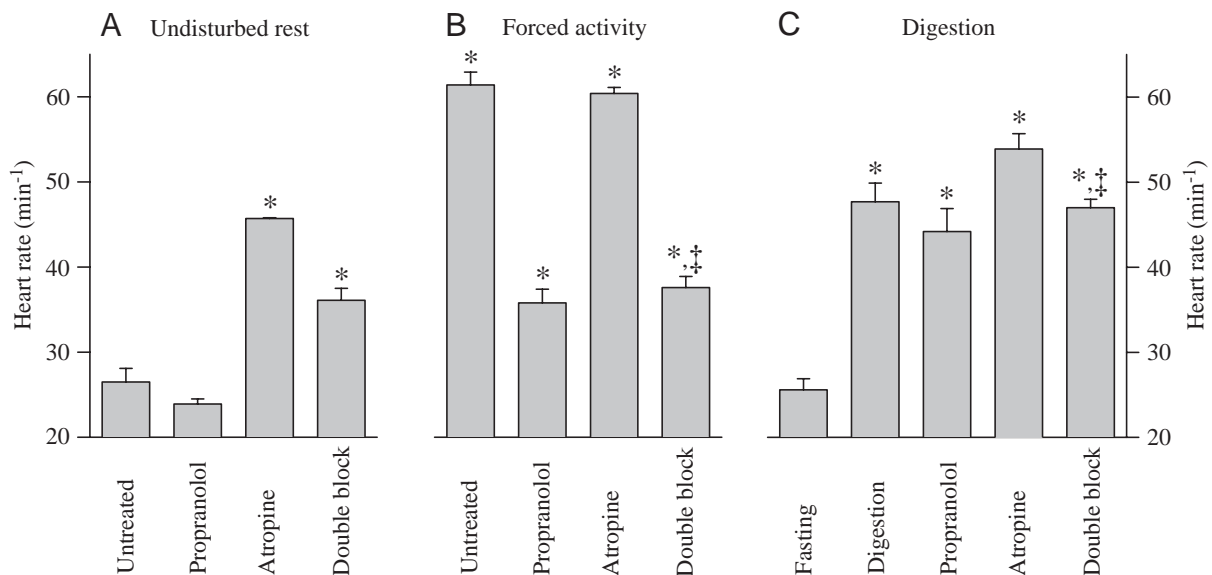

Fig. 3. Heart rate for *Boa constrictor* during resting and undisturbed conditions (A), during forced activity (B) and during digestion (C). The figure depicts the untreated control condition (N=8) and the values obtained following autonomic blockade with the β-adrenergic antagonist propranolol ($3\,mg\,kg^{-1}$) and the muscarinic cholinoceptor antagonist atropine ($3\,mg\,kg^{-1}$; N=4 for both) and following double autonomic blockade (N=8). The fasting heart rate is also shown for the snakes studied during digestion (C). Values are means + S.E.M. An asterisk indicates a significant difference from the value of untreated and resting animals; a double dagger indicates a significant difference from the value of double-blocked and resting animals.

tone (Fig. 5). The cholinergic tone virtually disappeared (2.6±2.2 %) during enforced activity, whereas the adrenergic tone doubled to a value of 41.3±1.9 % (Fig. 4B). Both the cholinergic and adrenergic tones were small during digestion (6.0±3.5 and 11.1±1.1 %, respectively).

The order of pharmacological manipulation did not affect the calculated autonomic tones of resting animals (Table 2). However, during digestion, the calculated adrenergic tones was significantly lower whenever atropine was applied before propranolol (Table 2). Nevertheless, these effects were relatively small compared with the differences in cardiac regulation between activity and digestion.

Fig. 4. The calculated autonomic tones on the heart for *Boa constrictor* during undisturbed rest (A), during forced activity (B) and during digestion (C). Values are means + S.E.M. An asterisk indicates a significant difference from the value of untreated and resting animals; a double dagger indicates a significant difference between forced activity and digestion. Cho, cholinergic; Adr, adrenergic.
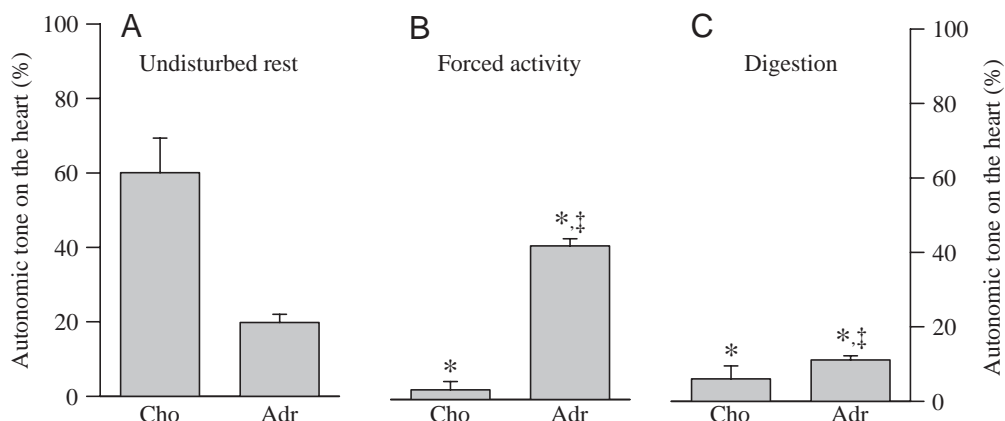
Discussion

Critique of the physiological states studied

The present study describes the effects of digestion and enforced activity on the autonomic control of the heart in *Boa constrictor*. We demonstrate some marked differences between these two states, although both situations are characterised by similar changes in heart rate compared with resting and undisturbed animals. We did not measure oxygen uptakes during these two states, but previous studies on the lizard *Varanus exanthematicus* (Hicks et al., 2000) showed that the heart rate response to increased metabolic rate during treadmill exercise or digestion is stereotypical (i.e. there were proportional changes in heart rate relative to changes in rates of oxygen consumption).

The digestive state is rather well defined as being the conditions measured 24–36 h following ingestion, and the measurements could be performed without disturbing the animals. In contrast, the state of enforced activity is less easily defined, and it is virtually impossible to distinguish the direct effects of increased metabolic rate associated with muscular work from the effects associated with agitation and stress imposed by the investigator. This is a general problem for exercise studies on reptiles since these animals generally do not perform without active encouragement by the investigators (e.g. animals on treadmills have to be pinched to maintain belt speed) (Wang et al., 1997). In *Bufo marinus*, Wahlqvist and Campbell (Wahlqvist and Campbell, 1988) noted that the initial cardiovascular responses to treadmill exercise became progressively dampened as the toads became used to the exercise protocol, and it was suggested that these responses were predominantly psychogenic. This was not the case in the present study, in which the snakes behaved consistently throughout the repeated bouts of forced activity and the cardiovascular responses of the non-treated animals were identical during the three successive bouts of activity. However, because striking is an aggressive and active behaviour that is different from locomotion, it seems very probable that psychogenic effects contributed significantly to the responses observed.

The order of pharmacological manipulation showed a tendency to affect the calculated autonomic tones during both

activity and digestion (Table 2). This suggests an interaction between the autonomic systems, where the pharmacological blockade of the cholinergic tone is countered by a compensatory change in the adrenergic tone and *vice versa* (Rosenbluth and Simeone, 1934; Altimiras et al., 1997). However, these effects were small compared with the differences in cardiac regulation between activity and digestion and do not alter the conclusions drawn in the present study.

Comparison of the responses with previous studies

The heart rate of resting and undisturbed *Boa constrictor* was similar to the resting heart rate of 25 min^{-1} reported for similarly sized *Python molurus* at 30°C (Secor et al., 2000), and the blood pressure of resting and undisturbed *Boa constrictor* was consistent with the values of 5–8 kPa that
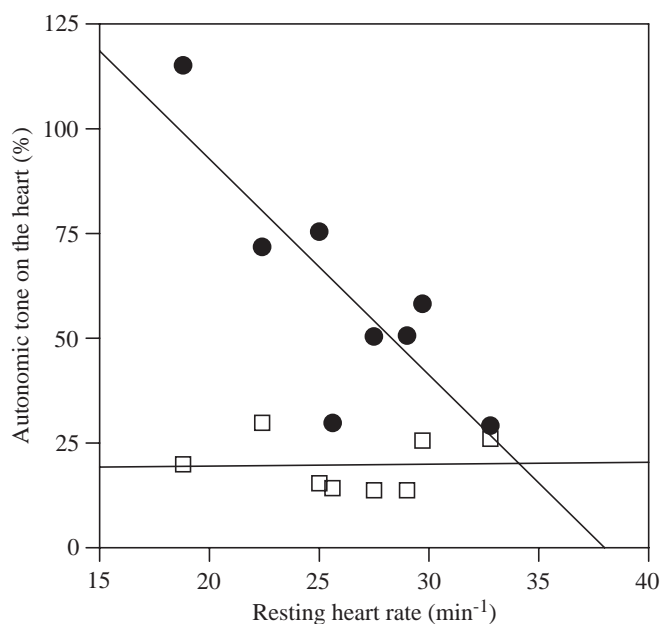

Fig. 5. Relationship between the heart rate of fasting, undisturbed and resting snakes and the cholinergic tone (filled circles) and the adrenergic tone (open squares) in *Boa constrictor*. The lines are linear regressions (cholinergic tone, r^2=0.663, P=0.01; adrenergic tone, r^2<0.01, P=0.94).

normally characterise terrestrial snakes (Lillywhite and Donald, 1994). Increased blood pressure and heart rate during forced activity at 30 °C have been reported for other snakes, such as *Coluber constrictor* and *Python molurus*, in which heart rate increases to 94 and 57 min⁻¹, respectively (Stinner and Ely, 1993; Secor et al., 2000) see also (Lillywhite et al., 1999). As in our study on *Boa constrictor*, blood pressure during killing and ingestion of prey in *Coluber constrictor* exceeded the values during activity (Stinner and Ely, 1993). In *Boa constrictor*, blood pressure attained maximum values during swallowing of the prey, and it is possible that part of this effect is caused by the muscular contractions associated with prey handling. Moon (Moon, 2000) showed that the undulatory movements associated with swallowing increase the pressure around the prey by up to 5 kPa.

Autonomic control of the heart during rest, enforced activity and digestion

The present study shows that resting animals are characterised by a large vagal cholinergic tone on the heart; the individual heart rate variability correlated well with that of the relative cardiac vagal tone (Fig. 5). A similar relationship has been found for fish (Altimiras et al., 1997). The relative adrenergic tone did not vary in a consistent pattern, suggesting that the parasympathetic nervous system exercises the dominant role in determining heart rate in resting animals.

The tachycardia induced by enforced activity is caused both by withdrawal of the vagal tone and by an increased adrenergic tone (Fig. 4). The adrenergic tone was quantified by pharmacological blockade of the β-receptors using injected propranolol, so that it is not possible to distinguish between the influences of adrenergic innervation and the effects of circulating catecholamines. In *Coluber constrictor*, the levels of circulating catecholamines increase 20-fold during forced activity (Stinner and Ely, 1993), and it appears likely that a large proportion of the increased sympathetic tone on the heart during activity in *Boa constrictor* can be explained by release of catecholamines from the adrenal gland. Injection of bretylium, which prevents postsynaptic release of catecholamines, has revealed an important role for circulating catecholamines in amphibians (Wahlqvist and Campbell, 1988; Axelsson et al., 1989). These studies also showed a reduced vagal tone during activity. Since the response of heart rate to activity was virtually abolished by double autonomic block (i.e. following infusion of both propranolol and atropine), other neurotransmitters seem to be of little importance in regulating the heart during exercise in *Boa constrictor*. Although not quantified, there was also no heart rate response to enforced activity in the digesting snakes following double autonomic block (*N*=3).

The increased heart rate during digestion was caused partially by withdrawal of vagal tone. However, in contrast to the response to enforced activity, there was also a significant reduction in adrenergic tone. Further, heart rate following double autonomic block was significantly higher in the digesting snakes than in fasting, undisturbed animals (47.0±1.0

versus 36.1±1.4 min⁻¹). Some amphibians possess α-receptors on the heart (Stene-Larsen and Helle, 1979) and, to rule out the possibility that such receptors could be selectively recruited during digestion, we infused phentolamine (3.0 mg kg⁻¹) in three double-blocked, digesting *Boa constrictor* and observed no effects on heart rate, although blood pressure decreased. Collectively, our data indicate that some other factor exerts a positive chronotropic effect during digestion. Our experiments cannot identify this other factor, but there are at least three, not mutually exclusive, possibilities: (i) a mechanical effect on the heart in which the increased venous return during digestion stimulates atrial stretch receptors and causes acceleration of the heart (the non-neural component of the Bainbridge effect; this mechanism may not contribute significantly because the *in situ* perfused heart of *Python molurus* shows only a very small heart rate response to increased atrial filling; T. Wang, J. Altimiras and M. Axelsson, unpublished data); (ii) a release of a non-adrenergic, non-cholinergic (NANC) factor, i.e. a regulatory peptide, from nerve endings innervating the heart; and (iii) stimulation of the heart by a circulating NANC factor released from elsewhere in the body.

In a number of vertebrates, NANC effectors are co-released with acetylcholine and catecholamines from nerve endings in the heart (Lillywhite and Donald, 1994; Lillywhite et al., 1999; Morris and Nilsson, 1994). In snakes, somatostatin appears to be co-released with acetylcholine but, although it exerts negative inotropic and chronotropic effects on isolated heart strips *in vitro*, these effects are blocked after cholinergic and adrenergic blockade (Donald et al., 1990). Donald et al. also demonstrated the presence of substance P, calcitonin gene-related peptide and neuropeptide Y (NPY) immunoreactivity in the sinus venosus, atria and ventricle of snakes. However, it seems unlikely that the tachycardia during digestion is caused by a NANC factor released from autonomic nerves on the heart since the tachycardia observed during activity was greatly reduced following double autonomic block. This explanation would require that these nerve terminals release the NANC factor only during digestion and not during activity.

Digestion is associated with the release of a number of regulatory peptides that take part in the control of the digestive processes, and many of these have cardiovascular effects (Rigel, 1988; Morris, 1989; Morris and Nilsson, 1994). Therefore, it is a possibility that the NANC factor(s) that appear(s) to exert a positive chronotropic effect during digestion is a circulating regulatory peptide, possibly released from the gastrointestinal system in response to feeding. Species-specific substance P does not increase heart rate in *Python regius* (Wang et al., 2000) and, because neuropeptide Y appears to be co-released with catecholamines from sympathetic nerves, we expect neuropeptide Y to be more important during exercise than during digestion. Recently, we have shown that bradykinin, which circulates in increased levels during digestion in mammals [e.g. (Rothschild et al., 1996)], greatly stimulates heart rate in anaesthetised *Python regius* (Wang et al., 2000). However, injection of bradykinin elicited a large increase in the levels of circulating catecholamines, and the

effects on heart rate could be fully abolished by injection of the β-blocker sotalol (Wang et al., 2000). Thus, bradykinin is unlikely to exert the responses observed in our study.

Our finding of an additional mechanism pacing the heart during digestion is consistent with data reported by Secor et al. (Secor et al., 2000). They showed that f_H during a combination of exercise and digestion exceeds that during exercise alone, but concluded that this response was due to the increased metabolic demand during the combination of digestion and exercise. However, in the light of the present data, it is equally possible that the presence of a circulating NANC factor during digestion generates a higher heart rate that, in turn, sustains a higher rate of oxygen uptake during the combination of digestion and exercise.

In conclusion, our study shows that cardiac control differs markedly between activity and digestion, although both these physiological states are associated with similar increases in heart rate. In both cases, part of the tachycardia is caused by a reduction in vagal tone, but the adrenergic tone increases during activity, whereas it decreases during digestion. Heart rate following double autonomic blockade is much higher during digestion, suggesting a role for a circulating regulatory peptide. Clearly, future studies should characterise the cardiac effects of different regulatory peptides, following their identification in reptiles, that exhibit large metabolic and cardiovascular responses to feeding.

The physiological nature of activity and digestion are very different, although both states elicit similar metabolic increments in reptiles (Wang et al., 2001): activity predominantly involves an increased metabolic rate of skeletal muscle, whereas digestion is associated with an increased metabolic rate of the gastrointestinal system. Activity is to a large extent fuelled by anaerobic metabolism and is associated with acidosis (Ruben, 1976), whereas digestion is aerobic and characterised by an alkalosis (Overgaard et al., 1999; Busk et al., 2000). Activity can only be endured for short periods (maximum 1 h), whereas digestion lasts for days. Our study adds an additional difference to this list, showing that activity is associated with increased sympathetic activity, whereas heart rate during digestion is regulated by the parasympathetic nervous system and possibly by NANC regulatory peptides.

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