Humoral regulation of heart rate during digestion in pythons (Python molurus and Python regius)

Sanne Enok, Lasse Stærdal Simonsen, Signe VesterSkov Pedersen, Tobias Wang, and Nini Skovgaard

Zoophysiology, Department of Bioscience, Aarhus University, Denmark

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Humoral regulation of heart rate during digestion in pythons (Python molurus and Python regius). Am J Physiol Regul Integr Comp Physiol 302: R1176–R1183, 2012. First published March 14, 2012; doi:10.1152/ajpregu.00661.2011.—Pythons exhibit a doubling of heart rate when metabolism increases several times during digestion. Pythons, therefore, represent a promising model organism to study autonomic cardiovascular regulation during the postprandial state, and previous studies show that the postprandial tachycardia is governed by a release of vagal tone as well as a pronounced stimulation from nonadrenergic, noncholinergic (NANC) factors. Here we show that infusion of plasma from digesting donor pythons elicits a marked tachycardia in fasting snakes, demonstrating that the NANC factor resides in the blood. Injections of the gastrin and cholecystokinin receptor antagonist proglumide had no effect on double-blocked heart rate or blood pressure. Histamine has been recognized as a NANC factor in the early postprandial period in pythons, but the mechanism of its release has not been identified. Mast cells represent the largest repository of histamine in vertebrates, and it has been speculated that mast cells release histamine during digestion. Treatment with the mast cell stabilizer cromolyn significantly reduced postprandial heart rate in pythons compared with an untreated group but did not affect double-blocked heart rate. While this study indicates that histamine induces postprandial tachycardia in pythons, its release during digestion is not stimulated by gastrin or cholecystokinin nor is its release from mast cells a stimulant of postprandial tachycardia.

Histamine; nonadrenergic, noncholinergic factor; ganglion blockade; gastrin; reptile

THE METABOLIC RATE of all animals increases during digestion, but the metabolic response to digestion is particularly pronounced in species, such as snakes, that ingest very large meals at irregular intervals (21). As an example, when pythons, a typical sit-and-wait predator, ingest meals of 20–50% of their own body mass, the prolonged digestive response is associated with a 3- to 10-fold rise in oxygen uptake (12, 13, 22, 29). This increased oxygen demand is met by a rise in cardiac output, which is accomplished through a rise in both heart rate and stroke volume (6, 22). The rise in heart rate is mediated by released parasympathetic tone on the heart (24, 28), but most of the tachycardia arises from a nonadrenergic, noncholinergic (NANC) factor (24, 28).

Histamine is a NANC factor that exerts a positive chronotropic effect on the heart during the first 24 h of digestion in pythons (24), but the source and mechanism of release has not been identified. Plasma concentrations of histamine do not increase during digestion in pythons (24) and circulating histamine, therefore, does not act directly on the heart. Digestion is accompanied by the release of several hormones and endocrine regulatory peptides such as neurotensin and cholecystokinin (CCK) (19). These may be involved in the postprandial cardiovascular responses and could act as the initiating hormonal factor on postprandial tachycardia. A major store of histamine in the vertebrates is mast cells (14, 15, 17), distributed throughout the body, including cardiac tissues. It is possible that histamine is released from mast cells due to a hormonal stimulation. Alternatively, histamine may be released from cardiac nerve endings. In mammals, it is known that norepinephrine and histamine are coreleased from nerve endings in the cardiac ganglion (8). Also, in invertebrates and nonmammalian vertebrates, both central and peripheral cholinergic neurons have been identified and might be the site of histamine release (8, 10). It has been suggested, therefore, that histamine is either coreleased from nerve endings or released from cardiac mast cells in response to hormonal stimulation (24). Here we investigate these hypotheses to understand the mechanism underlying histamine release and its role as a NANC factor on the heart.

MATERIALS AND METHODS

Experimental Animals

Eighteen Python regius and 23 Python molurus with a body mass ranging from 150 to 1,600 g were purchased from a commercial supplier and kept at Aarhus University. A heating system ensured a temperature gradient between 25°C and 32°C within the vivaria. The animals were fed once a week and had free access to water but were fasted at least 1 wk before experimentation. All animals grew during captivity and appeared healthy. Experiments were performed according to Danish Federal Regulations.

Surgery and Instrumentation

Studies of anesthetized snakes (Python regius). Snakes were anesthetized by an intramuscular injection of pentobarbital sodium (25 mg/kg, Mebumal, Sygehus Apotekerne, Denmark) and tracheotomized for artificial ventilation at 10 breaths/min and 50 ml/kg using a Harvard Apparatus mechanical ventilator (Cambridge, MA) when reflexes had disappeared. A ventrolateral incision was made posterior to the kidney, and a polyethylene (PE)-50 catheter containing heparinized saline (50 IU/ml) was advanced into the aorta to measure systemic blood pressure. The left pulmonary artery, which perfuses the smaller left lung and carries less than a quarter of the total pulmonary blood flow (N. Skovgaard and T. Wang; unpublished observations), was occlusively cannulated with a PE-50 catheter for measurements of pulmonary blood pressure. Blood flows were measured using 1.5 R transit-time ultrasonic blood flow probes (Transonic System) placed around the left aortic arch and the right pulmonary artery. Acoustical gel was infused around the blood flow probes to enhance the signal.

To allow vagal stimulation of the heart, a ventral incision was made posterior to the head in two snakes and the vagus nerves were
exposed. The nerves were placed on two silver electrodes and stimulated using a Grass s48 Stimulator (Grass Instruments, Quincy, MA).

Studies on recovered snakes (Python regius and Python molurus). Anesthesia was induced by inhalation of ~5% isoflurane (Baxter, Denmark) until all reflexes disappeared. The snakes were then intubated for mechanical ventilation with 1–2% isoflurane during the remaining surgery. A 4-cm ventrolateral incision gained access to the vertebral artery or the dorsal aorta posterior to the kidney, which was occlusively cannulated with PE catheters (PE-50 or PE-76) filled with heparinized saline (50 IU/ml). The catheter was fastened with surgical silk (5-0) before exteriorized through the skin and secured with surgical silk (2/0). The incision was closed with surgical silk (2/0). All snakes were left to recover overnight at 30°C within a climatic chamber. We have previously shown that heart rate, blood pressure, and circulating levels of catecholamines return to resting values within this period (11).

Measurements of Heart Rate, Blood Pressure, and Blood Flow

Arterial catheters were connected to disposable pressure transducers (model PX600; Baxter Edwards, Irvine, CA) that were calibrated daily against a static water column, and the signal was amplified using an in-house built preamplifier. Flow probes were connected to a Transonic dual flowmeter (T206, Transonic Systems, Ithaca, NY). Signals from the pressure transducers and the blood flow meter were recorded with a Biopac MP100 data acquisition system (Biopac Systems, Goleta, CA) at 100 or 200 Hz.

Experimental Protocols

Experiments were conducted on anesthetized as well as fully recovered snakes. The recovered snakes were kept in boxes within a climatic chamber at 30°C during the entire experiment, where they were shielded from visual and auditory disturbances. Experiments on anesthetized snakes were conducted with the animals lying on a heating pad to keep the body temperature at 30°C. Python regius were unwilling to feed voluntarily and were force fed with freshly killed adult mice or preweaned rats. Python molurus were fed with live adult mice. Snakes had been fed a meal equivalent to 23.2 ± 0.8% (means ± SE) of their body mass (to be referred as “digesting snakes” throughout the paper). All measurements on digesting snakes were taken 24 h into the postprandial period. Resting values of blood pressure and heart rate of recovered snakes were obtained 1–2 h after the catheters were connected. All injections were given in aliquots of 1 ml/kg in the systemic arterial catheter and separated by 30–60 min. Thus antagonists were allowed at least 30 min to take effect. All chemicals were purchased from Sigma-Aldrich (Denmark).

Effects of ganglionic blockade by hexamethonium on postprandial heart rate. To examine the possibility that histamine is coreleased from postganglionic autonomic nerves innervating the heart, eight Python regius were treated with the ganglion blocker hexamethonium bromide. Resting heart rate and systemic blood pressure were measured in recovered fasting snakes and 24 h into the postprandial period. Atropine (4 mg/kg), propranolol (4 mg/kg), and hexamethonium bromide (10 mg/kg) were administered in alternating orders. Four snakes were injected with atropine followed by propranolol and then hexamethonium bromide. In another four snakes, hexamethonium bromide was injected before atropine and propranolol.

To verify the autonomic ganglionic blockade by hexamethonium bromide, the vagus nerves were stimulated with 20 V at 4 Hz in two anesthetized Python regius. Hexamethonium bromide (10 mg/kg) was then administered and after 20 min the vagus nerves were stimulated with 20 V at 4, 10, and 100 Hz.

Effects of donor plasma on systemic blood pressure and heart rate in fasting recovered snakes. To investigate whether the putative NANC factor is a circulating substance, donor plasma from fasting and fed Python molurus was injected as a 3% of body mass bolus, thus ~50% of total blood volume, in fasting Python molurus treated with a combination of the β-adrenergic antagonist propranolol (4 mg/kg) and the cholinergic antagonist atropine (4 mg/kg). Double-blocked heart rate was recorded after the double autonomic blockade with propranolol and atropine. Hereafter, the snakes were infused over 5 min with donor plasma from a fasting python followed by an infusion of donor plasma from a digesting python. Donor plasma from fed snakes was taken 24 h into digestion of a meal equivalent to 26% of body mass. The two infusions of donor plasma were separated by 2 h. Heart rate and systemic blood pressure were analyzed 10 min after infusion of each plasma sample.

Effects of histamine in digesting snakes. The role of histamine in regulating heart rate was investigated in three groups of recovered Python molurus. One group served as control and another group was treated with the mast cell stabilizer cromolyn (25 mg/kg; injected every 6 h over 27 h). Heart rate and systemic blood pressure were recorded in the same animals during fasting and digestion. Double-blocked heart rate was obtained after double autonomic blockade (atropine and propranolol; 4 mg/kg) followed by administration of the histamine H2-receptor antagonist ranitidine (40 mg/kg). The effect of ranitidine was verified by injections of histamine (250 nmol/kg) before and after blockade. In initial experiments, the H1-receptor antagonist diphenhydramine (20 mg/kg) was injected after ranitidine. In a third group of recovered Python molurus, histamine (250 nmol/kg) was injected in fasting and digesting snakes after double autonomic blockade.

Effects of pentagastrin and the gastrin and cholecystokinin receptor antagonist proglumide. After instrumentation of anesthetized Python regius, basal hemodynamic variables (systemic and pulmonary blood flow and pressure) were recorded for up to 45 min. The peptide was dissolved in 0.15% (wt/vol) acetic acid, and aliquots were

![Fig. 1. Effects of double autonomic blockade with the β-adrenergic antagonist propranolol (Prop; 4 mg/kg) and the muscarinic antagonist atropine (Atro; 4 mg/kg) in recovered fasting Python molurus (A and B; n = 10) and in digesting snakes (C and D; n = 8)]. Values are means ± SE. *Significant difference from fasting values. †Significant difference from fasting double-blocked animals. Differences were evaluated with a one-way ANOVA for repeated measures followed by a Holm-Sidak post hoc test (P < 0.05).
stored at −20°C. The peptide was diluted to the desired concentration in 0.9% (wt/vol) saline containing 0.5% (wt/vol) bovine serum albumin immediately before use. To determine whether the vehicle for injections exerted hemodynamic effects, a 1 ml/kg injection of 0.9% saline containing 0.5% bovine serum albumin and 0.15% acetone was given. A 30 nmol/kg pentagastrin bolus intraarterial injection was given in untreated and in double-blocked (atropine and propranolol; 3 mg/kg) animals. Pentagastrin injections were separated by 1 h.

After we obtained values for heart rate and systemic blood pressure in recovered fasting and then fed *Python molurus*, atropine and propranolol (4 mg/kg) were administered and double-blocked heart rate was recorded. To investigate the role of gastrin and CCK in regulating heart rate during digestion, the gastrin and CCK receptor antagonist proglumide (20 mg/kg) was injected in fed *Python molurus* (N = 4).

**Data Analysis and Statistics**

Calculations of blood flows, stroke volume, and vascular resistance in anesthetized snakes. Because the left pulmonary artery was occlusively cannulated, blood flow measurements in the right pulmonary artery represent total pulmonary blood flow (Q_{pul}). In anesthetized *Python regius*, total systemic blood flow (Q_{sys}) can be estimated as 2.5 times left aortic blood flow (Q_{lAo}) (23a). Total cardiac output (Q_{co}) was calculated as Q_{sys} + Q_{pul}. Heart rate (f_{h}) was calculated from the instantaneous blood flow trace from the left aortic arch, and total stroke volume (VS_{tot}; pulmonary + systemic) was calculated as Q_{co}f_{h}. Pulmonary and systemic resistances (R_{pul} and R_{sys}, respectively) were calculated from mean blood pressure and mean blood flow (R_{pul} = P_{pul}/Q_{pul} and R_{sys} = P_{sys}/Q_{sys}) assuming that central venous blood pressures are negligible.

All data recordings were analyzed using AcqKnowledge data analysis software (version 3.9.1; Biopac, Goleta, CA). Data were evaluated using paired t-test and one-way or two-way ANOVA for repeated measures followed by a Holm-Sidak post hoc test. Differences were considered statistically significant at a 95% level of confidence (P < 0.05). All data are presented as means ± SE.

**RESULTS**

**NANC Factor**

Digestion caused a large increase in heart rate in *Python molurus* from 19.0 ± 1.2 beats/min to 46.2 ± 2.6 beats/min (Fig. 1). Double-autonomic blockade with atropine and propranolol resulted in a small but significant reduction in heart rate. However, the postprandial tachycardia was largely due to an increase in double-blocked heart rate from 22.4 ± 1.1 beats/min in fasting snakes to 40.2 ± 1.5 beats/min in digesting snakes. There were no effects of digestion or double autonomic blockade on systemic blood pressure.

**Ganglion Blockade With Hexamethonium Bromide**

The effects of ganglion blockade by hexamethonium in fully recovered and digesting *Python regius* are shown in Fig. 2. Heart rate increased significantly during digestion, but systemic blood pressure was not affected. After double autonomic blockade, hexamethonium had no effect on heart rate or blood pressure (Fig. 2, A and B). Hexamethonium, administered before double autonomic blockade, had no effect on heart rate or blood pressure, and the effects of subsequent injections of atropine and propranolol were abolished (Fig. 2, C and D). Stimulation of the vagus nerve in anesthetized snakes caused a decrease in heart rate accompanied by a decline in systemic blood pressure and left aortic blood flow (Fig. 3). Injection of hexamethonium abolished the effects of vagal stimulation verifying the ganglion blockade.

Fig. 2. Effects of the ganglion blocker hexamethonium on heart rate and systemic blood pressure in recovered *Python regius*. Hexamethonium bromide (Hexa; 10 mg/kg) was administered in digesting snakes after (A and B; n = 4) or before (C and D; n = 4) double autonomic blockade with Prop and Atro (4 mg/kg). Values are means ± SE. *Significant difference from fasting snakes. †Statistical significant difference from digesting snakes evaluated by a one-way ANOVA for repeated measures followed by a Holm-Šidák post hoc test (P < 0.05). NS, nonsignificant.
Donor Plasma

As shown in a representative trace in Fig. 4, infusion of plasma from a digesting donor python into fasting double-blocked snakes elicited a large increase in heart rate that persisted for more than 40 min. Infusion of donor plasma from a digesting snake caused a 57.7 ± 10.0% rise in heart rate, whereas plasma from a fasting donor python had a much smaller effect increasing heart rate with only 18.1 ± 5.6% (Fig. 4, C and E). There was no difference in the rise in blood pressure seen after infusion of fasting and digesting donor plasma (83.1 ± 19% and 88.4 ± 20.2%, respectively).

Histamine

The effects of a bolus injection of histamine in double-blocked fasting and digesting snakes are shown in Fig. 5. Histamine increased double-blocked heart rate by 47.7 ± 10.2% in fasting snakes, whereas digesting snakes showed a significantly lower rise in heart rate (10.3 ± 4.4%). There were no changes in systemic blood pressure in either fasting or digesting snakes.

Mast Cell Stabilizer (Cromolyn)

The postprandial increase in heart rate was significantly reduced in the cromolyn-treated group compared with the control group (Fig. 6). Atropine and propranolol decreased postprandial heart rate significantly in the control group but had no effect in the animals treated with cromolyn. The histamine H2-receptor antagonist ranitidine had no effect on double-blocked heart rate. There were no effects on systemic blood pressure.

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In anesthetized Python regius, pentagastrin bolus injections caused an increase in heart rate and cardiac output accompanied by a rise in systemic and pulmonary blood flows and
pressures (Table 1). Pentagastrin had no effects on vascular resistances. The effects of pentagastrin on heart rate and blood flows were abolished after treatment with atropine and propranolol, whereas the increase in blood pressures persisted.

Digestion more than doubled heart rate in recovered *Python molurus* (Fig. 7). Double autonomic blockade led to a small but significant decrease in heart rate, while subsequent injection of the CCK and gastrin antagonist proglumide had no effect on double-blocked heart rate but decreased systemic blood pressure slightly.

**DISCUSSION**

Our study confirms the presence of a NANC factor during digestion in pythons and shows that the majority of the tachycardia is caused by a factor in the plasma that is not released from nerve endings. While the nature of this substance remains unknown, our study indicates that the regulatory peptide gastrin does not directly stimulate heart rate. We show that *Python molurus* resemble *Python regius* by having a large histaminergic tone in the initial phase of digestion. Thus it seems reasonable to propose that an unknown factor, possibly one or more hormones or regulatory peptides, released to the blood stream during digestion, stimulates the sinusatrial node and induces the postprandial tachycardia through the action of histamine as a NANC factor.

**Is NANC Factor Released From Nerve Endings?**

In mammals, histamine and norepinephrine are stored and coreleased from sympathetic nerve endings in cardiac ganglia (8). In snakes, however, digestion is associated with decreased or unaltered cardiac sympathetic tone (28), making it unlikely that histamine is coreleased from sympathetic cardiac neurons during digestion. However, as in mammals, central and peripheral histaminergic neurons have been identified in both invertebrates and nonmammalian vertebrates (5, 8, 10), which may release histamine during digestion. The autonomic ganglion blocker hexamethonium effectively blocked the heart rate response to electrical stimulation of the vagosympathetic trunk in anesthetized snakes but did not reduce the postprandial heart rate response in recovered animals. It is unlikely, therefore, that a NANC factor is coreleased from nerve endings.
Is NANC Factor a Circulating Substance?

Infusion of plasma from a digesting donor python caused a marked and prolonged increase in double-blocked heart rate in fasting recipient pythons, indicating that the NANC factor is a circulating substance (Fig. 4). Plasma concentrations of many hormones and regulatory peptides increase manifolds during digestion in pythons and are involved in cardiovascular control (19, 23). The concentration of the gastrointestinal hormone gastrin peaks during the initial phase of digestion (19), and we have earlier hypothesized that postprandial NANC stimulation of the heart could arise from increased circulating levels of this hormone, which either acts directly on the heart or through the release of chronotropic agents such as histamine (24). However, injection of the gastrin/CCK antagonist proglumide had no effect on double-blocked heart rate in digesting snakes and, therefore, the involvement of either gastrin or CCK in the NANC stimulation is doubtful. In addition, pentagastrin, a synthetic form of gastrin, did not have a direct effect on the heart in anesthetized Python regius, but the increases in heart rate and cardiac output were mediated through either muscarinic or β-adrenergic receptors. Thus it is unlikely that gastrin stimulates release of the NANC factor. Other hormones released from the gastrointestinal system have been suggested to be involved in the regulation of the postprandial increase in cardiac output and heart rate. In dogs and humans, secretin increases heart rate and cardiac output during digestion (3, 4), and the intestinal hormone oxyntomodulin, which is released during digestion, increases intrinsic heart rate in mice when administered peripherally (26). Neither of these hormones was investigated in this study.

Role of Histamine in Inducing Postprandial Tachycardia

Injection of histamine in recovered Python molurus had a much greater effect on heart rate in double-blocked fasting snakes compared with double-blocked digesting snakes, indicating a histaminergic tone within digesting animals. The abolished effect of histamine during digestion could also be because the maximum cardiac capacity has been reached. This seems unlikely, however, since more pronounced increases in heart rate have been demonstrated in previous experiments (20, 22, 28).

Injection of the histamine H₂ receptor antagonist ranitidine did not change double-blocked heart rate in fed animals,
showing that cardiac H₂-receptors are not involved in the stimulation of postprandial tachycardia in *Python molurus*. Contrary to this, histamine stimulates postprandial tachycardia by the direct effect on cardiac H₂-receptors in *Python regius* (24). To determine the possible involvement of other histamine receptors, experiments were carried out with histamine H₁-receptor antagonists citirizine and diphenhydramine (data not shown). Both antagonists had severe cardioxic effects and experiments were stopped, and the involvement of H₁-receptors remains inconclusive. The cardiotoxic effect of histamine H₁-receptors antagonist has previously been seen in other animal species (9).

**Mast Cells**

The postprandial rise in heart rate was attenuated in snakes treated with cromolyn, which stabilizes mast cells indicating that mast cells are involved in the postprandial tachycardia. The result is not clear, however, since the difference between cromolyn-treated and control group did not persist in the double-blocked groups. Plasma concentrations of histamine are unchanged during digestion in *Python regius* (24) and histamine is, therefore, not transported within the circulation during digestion. Mast cells are distributed in most body compartments in vertebrates (16), including cardiac tissues in snakes (25). Along with ECL cells in the gastric mucosa, mast cells are the primary site for histamine storage site in most vertebrates (16). Histamine released from cardiac mast cells induces a chronotropic cardiac response in mammals, including humans (2, 27). Our results, however, indicate that degranulation of mast cells is associated with the autonomic regulation of the heart during digestion and possibly not the NANC response.

**Perspectives and Significance**

Because of the pronounced morphological and functional responses to digestion (1, 7, 11, 18, 24), pythons represent a suitable animal model to study the cardiovascular responses to increased metabolism. Our demonstration that heart rate is greatly influenced by a circulating factor present during digestion and possibly not the NANC response. Because of the pronounced morphological and functional responses to digestion (1, 7, 11, 18, 24), pythons represent a suitable animal model to study the cardiovascular responses to increased metabolism. Our demonstration that heart rate is greatly influenced by a circulating factor present during digestion and possibly not the NANC response. Our results indicate that the postprandial response of pythons is an example of an integrated physiological response where organs signal directly to each other.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: S.E., L.S.S., S.V.P., T.W., and N.S. conception and design of research; S.E., L.S.S., S.V.P., and N.S. performed experiments; S.E., L.S.S., S.V.P., and N.S. interpreted results of experiments; S.E., L.S.S., S.V.P., and N.S. prepared figures; S.E. drafted manuscript; S.E., T.W., and N.S. edited and revised manuscript; S.E., L.S.S., S.V.P., T.W., and N.S. approved final version of manuscript.

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