Histamine induces postprandial tachycardia through a direct effect on cardiac H₂-receptors in pythons

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Running title: Cardiovascular action of histamine in snakes
ABSTRACT

The intrinsic heart rate of most vertebrates studied, including humans, is elevated during digestion, suggesting that a non-adrenergic-non-cholinergic factor contributes to the postprandial tachycardia. The regulating factor, however, remains elusive and difficult to identify. Pythons can ingest very large meals and digestion is associated with a marked rise in metabolism that is sustained for several days. The metabolic rise causes more than a doubling of heart rate and a four-fold rise in cardiac output. This makes the python an interesting model to investigate the postprandial tachycardia. We measured blood pressure and heart rate in fasting *Python regius*, and at 24 and 48h after ingestion of a meal amounting to 25% of body weight. Digestion caused heart rate to increase from 25 to 56 min⁻¹ while blood pressure was unchanged. The postprandial rise in heart rate was partially due to a doubling of intrinsic heart rate. The H₂-antagonist did not affect heart rate of fasting snakes, but decreased heart rate by 15-20 min⁻¹ at 24h into digestion, while it had no effects at 48h. Thus, the histaminergic tone on the heart rose from none to 30% at 24h, but vanished after 48h. In anesthetised snakes histamine caused a systemic vasodilatation and a marked increase in heart rate and cardiac output mediated through a direct effect on H₂-receptors. Our study strongly indicates that histamine regulates heart rate during the initial phase of digestion in pythons. This study describes a novel regulation of the vertebrate heart.

*Keywords: Reptile, python, digestion, histamine, heart rate, blood flow, blood pressure*
INTRODUCTION

The elevated metabolic rate during digestion and the increased need for intestinal absorption and subsequent nutrient transport must be met by an increased blood flow to the gastrointestinal organs. In humans, the postprandial dilation of the splanchnic vascular bed is attended by increased cardiac output accomplished through a rise in both heart rate and stroke volume, so that systemic blood pressure is maintained (11, 20, 43). Little is known, however, about the regulation of this response. In addition to the autonomic nervous systems (8, 15), the postprandial cardiovascular response is probably governed by the direct action of hormones such as endocrine regulatory peptides released from the gastrointestinal organs (3, 7, 12). In fact, patients with transplanted and fully denervated hearts exhibit a pronounced postprandial rise in cardiac output (19, 44, 45), suggesting that the cardiac stimulation is not reflex in nature, but, at least partially, mediated by humoral factor(s).

Humans and the common mammalian model species, such as mice and rats, are adapted to consuming small meals at frequent intervals. The magnitude of the digestive responses is, therefore, low and the underlying regulatory processes may be difficult to identify. Pythons, in contrast, ingest large whole preys at infrequent intervals with large changes in digestive performance, making these snakes an interesting and suitable model species for investigating cardiovascular and gastrointestinal regulatory physiology associated with digestion (33, 46). Thus, in pythons, digestion causes a ten-fold rise in metabolism that can be sustained for up to two weeks attended by a marked and rapid hypertrophy of visceral organs including a 40% fully reversible increase in ventricular muscle mass within 48h after feeding (2, 31, 32, 33). The large factorial scope and prolonged period of the postprandial response allows for a good resolution in
physiological studies on fundamental mechanisms of digestion that apply to all vertebrates.

The cardiovascular responses to digestion in pythons include a doubling of heart rate and a four-fold increase in cardiac output, as well as a dilation of the mesenteric vascular bed leading to intestinal hyperemia (34, 42). Furthermore, plasma levels of gastrointestinal regulatory peptides increases many-fold after feeding (35). In comparison to humans where the non-adrenergic-non-cholinergic (NANC) contribution to postprandial heart rate is modest, it contributes significantly in infrequently feeding snakes, such as pythons and boas, making them convenient animal models to study postprandial regulation of the heart (47).

The effects of histamine on cardiac function have been appreciated since the work of Dale and Laidlaw (10) showing that synthetic histamine, β-imidazolylethylamine, modify cardiac rhythm in the mammalian heart. In mammals, histamine exerts cardiovascular effects that resemble the postprandial cardiovascular changes including dilation of the systemic vasculature as well as a direct positive chronotropic and inotropic effect on the heart (6). Apart from the stimulation of heart rate, histamine also exerts cardiovascular effects in reptiles, and evidence for both a constrictive and dilatory vascular response exist (29). However, the effects have not been studied in detail and simultaneous in vivo measurements of hemodynamic variables have not been conducted. Here we investigate the hypothesis that the postprandial tachycardia is induced by histamine. In addition, we study the cardiovascular effects of exogenous administered histamine, as well as the underlying mechanism, in anaesthetised pythons (Python regius). Furthermore, we investigate the direct effect of histamine on cardiac
receptors through specific $H_1$- and $H_2$-receptor agonists and antagonists on sinus venosus-atrial preparation.

MATERIALS AND METHODS

Experimental animals

Experiments were undertaken on 34 pythons ($Python$ regius) of undetermined sex and age weighing between 0.14 kg and 0.80 kg (0.30 kg ± 0.02 kg; mean ± S.E.M.). The animals were obtained from a local animal supplier (Avifauna, Denmark) and kept in vivariums at 25-30°C with free access to water. The snakes were fasted no less than a week prior to experimentation. All animals appeared healthy and experiments were performed according to Danish Federal Regulations.

Surgery and instrumentation

Anaesthetised snakes. Five pythons were anaesthetised by an intramuscular injection of a sodium pentobarbital (Mebumal, Sygehusapotekerne, Denmark; 25 mg kg$^{-1}$). All reflexes disappeared within 30 min and the animals were then tracheotomised for artificial ventilation at 10 breaths min$^{-1}$ and a tidal volume of 50 ml kg$^{-1}$ using a Harvard Apparatus mechanical ventilator (Cambridge, MA). A 5 cm latroventral incision was made cranial to the heart, and a PE50 catheter, filled with heparinised saline (50 IU ml$^{-1}$), was advanced into the vertebral artery for measurements of systemic blood pressure ($P_{sys}$). The left pulmonary artery, which perfuses the smaller left lung and carries less than a quarter of the total pulmonary blood flow (unpublished observations), was occlusively cannulated with a PE50 catheter for measurements of pulmonary blood pressure ($P_{pul}$). For measurements of blood flows 1.5R transit-time ultrasonic blood flow
probes (Transonic System, Inc., NY) were placed around the left aortic arch and the right pulmonary artery. Acoustical gel was infused around the blood flow probes to enhance the signal.

**Sinus venosus-atrial preparations.** Pythons (N = 7) were anaesthetised by ventilation with 2-3% isofluran (Isofluran, Baxter, Denmark) and the heart exposed through a latroventral incision. The ascending part of the sinus venosus was ligated with 3-0 surgical silk and dissected free along with half of the right atrium to preserve the pacemaker region. To record isometric force development, the spontaneously beating preparations were mounted vertically using 3-0 surgical silk; the upper end of the preparation was connected to a force transducer (Statham UC 2, Oxnard, CA, USA), while the lower end was fastened to a fixed rod. The preparations were suspended in a water-jacketed organ bath containing 50 ml of Ringer solution at 30°C (mM): 95 NaCl, 25 NaHCO₃, 1.0 NaH₂PO₄, 2.5 KCl, 1.0 MgSO₄, 1.5 CaCl₂ and 5 glucose; gassed with 2% CO₂ and 98% O₂ (pH ~ 7.5) that was delivered by a gas mixing pump (Wösthoff, Bochum, Germany). The mounted preparations were left for 30 min to stabilise and then stretched by adjusting the length of the preparation with a micrometer screw to reach maximum force of contraction, followed by 30 min rest to stabilise contractions. Signals from the force transducer were recorded with Biopac MP100 data acquisition system (Biopac Systems, Inc., Goleta, CA) at 100 Hz.

**Recovered snakes.** Anaesthesia was induced through inhalation of approximately 5% isofluran (Isofluran, Baxter, Denmark). The snakes were then intubated and maintained at 1-2% isofluran during surgery using a Harvard Apparatus mechanical ventilator (10 breaths min⁻¹ and a tidal volume of 50 ml kg⁻¹). A 4 cm ventrolateral incision was made
posterior to the kidney and a PE50 catheter, filled with heparinised saline, was advanced into the aorta for measurements of systemic blood pressure. The catheter was externalised, the incision closed, and the snakes were allowed to recover from surgery within a climatic chamber at 30°C until the following day.

Measurements of blood pressures and flows
Catheters were connected to Baxter Edward (model PX600, Irvine, CA) disposable pressure transducers and the signals were amplified using an in-house built preamplifier. The pressure transducers were positioned at heart level of the snake and calibrated daily against a static water column. Flow probes were connected to a Transonic dual-channel blood flow meter (T206). Signals from the pressure transducers and the blood flow meter were recorded with a Biopac MP100 data acquisition system (Biopac Systems, Inc., Goleta, CA) at 100 Hz.

Experimental protocol
Anaesthetised snakes. After instrumentation, basal hemodynamic variables (systemic and pulmonary blood flow and pressure) were recorded for up to 45 min. To determine whether the vehicle for injections exerted hemodynamic effects, a 1ml kg⁻¹ injection of 0.9% (w/v) saline was given. All animals then received a series of bolus injections with increasing doses of histamine: 0.1, 0.3, 1, 3, 10, 30, 100, and 300 nmol kg⁻¹. Hemodynamic variables were allowed to return to baselines between each injection. All drugs were given in 1.0 ml kg⁻¹ aliquots and injected through the systemic catheter. To investigate the mechanism underlying the hemodynamic effects of histamine, bolus injections of histamine were given before and after a double autonomic blockade with
β-adrenergic and muscarinic receptor antagonists (propranolol and atropine, respectively, 3 mg kg$^{-1}$). The efficacy of the autonomic blockade was verified through injections of acetylcholine (5 µg kg$^{-1}$) and adrenaline (2 µg kg$^{-1}$) before and after the antagonists. Also, a bolus of histamine (100 nmol kg$^{-1}$) was given before and after double blockade and the histamine H$_2$-receptor antagonist ranitidine, 40 mg kg$^{-1}$. In initial experiments, a H$_1$-receptor antagonist (diphenhydramine or mepyramine, 40 mg kg$^{-1}$) was given. The antagonists were allowed 20 min to take affect before subsequent injections of histamine. Experiments were carried out at 30°C. After ended protocol animals were killed.

*Sinus venosus-atrial preparations.* Spontaneously developed isometric twitch force was recorded in sinus venosus-atrial preparations subjected to a progressive increase in histamine bath concentration as follows: 10$^{-8}$M, 10$^{-7}$M, 10$^{-6}$M and 10$^{-5}$M. The chamber was washed twice with Ringer solution and the preparations left for another 30 min to stabilise. Preparations were then subjected to the following protocol: H$_1$-agonist (2-((3-Trifluoromethyl)phenyl)histamine dimaleate, 10$^{-5}$M), histamine (10$^{-6}$M) and H$_2$-agonist (Amthamine dihydrobromide, 10$^{-5}$M). The chamber was washed twice, the cardiac strips were incubated with H$_2$-antagonist ranitidine (10$^{-3}$M) for 30 min and the protocol was repeated. At end, the sinus venosus-atrial preparations were incubated with the H$_1$-antagonist diphenhydramine (10$^{-3}$M).

*Recovered snakes.* The 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. Resting values of blood pressure and heart rate of all snakes were obtained
one to two hours after having connected the catheters and a control blood sample was collected. The snakes were then randomly assigned to one of three experimental groups: fasting snakes (N=7), snakes digesting 10% (10.5% ± 0.3%; N=7) and 25% (25.7% ± 0.3%; N=8) of body weight. The digesting snakes were force-fed with freshly killed adult mice or pre-weaned rats. All three groups were left for 24h before measurements and blood sampling; however, four snakes digesting 25% were not measured until 48h after feeding. Previous studies on pythons have shown that cardiovascular and metabolic responses to digestion are maximal at 24-48 h after ingestion (31).

At 24h or 48h after feeding, drugs were administered according to the following protocol in all 22 snakes: β-adrenergic antagonist propranolol and cholinergic antagonist atropine (3 mg kg⁻¹ each; the order of injection of propranolol and atropine was alternated), histamine (10 nmol kg⁻¹), histamine H₂-receptor antagonist ranitidine (40 mg kg⁻¹), histamine (10 nmol kg⁻¹). The efficacy of the autonomic blockade was verified in both fasting and digesting snakes through injections of acetylcholine (5 µg kg⁻¹) and adrenaline (2 µg kg⁻¹) before and after the antagonists. The antagonists were allowed 20 min to take effect before subsequent injections. All chemicals were purchased from Sigma-Aldrich, Denmark.

**Blood samples and analysis of histamine plasma levels**

Blood samples for determination of plasma histamine concentrations were taken from all recovered snakes at 24h after instrumentation and immediately prior to the injection of drugs. All blood samples (200 µL) were taken in microvettes coated with EDTA (Microvette®300, Sarstedt, Germany), spun down at 5000 rpm for 2 min (Sigma-3MK, Germany), and the plasma stored at -80°C for later analysis. Plasma concentration of
histamine was measured using a standard commercial histamine ELISA kit (SPI Bio, France).

**Data analysis and statistics**

*Calculations of blood flows, stroke volume and vascular conductance in anaesthetised pythons.* Because the left pulmonary artery was occlusively cannulated, blood flow measurements in the right pulmonary artery represent total pulmonary blood flow ($Q_{pul}$). In anaesthetised pythons, total systemic blood flow ($Q_{sys}$) can be estimated as 2.5 times left aortic blood flow ($Q_{LAo}$) (37). Total cardiac output ($Q_{tot}$) 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 $Q_{tot}/f_{H}$. Pulmonary and systemic conductance ($G_{pul}$ and $G_{sys}$, respectively) were calculated from mean blood flow and mean blood pressure ($G_{pul} = Q_{pul}/P_{pul}$ and $G_{sys} = Q_{sys}/P_{sys}$) assuming that central venous blood pressures are negligible.

*Twitch force of sinus venosus-atrial preparations.* Twitch force was measured as the peak force during a contraction, reported as the average twitch force produced during five consecutive contractions and expressed relative to resting twitch force before treatment.

*Calculations of adrenergic, cholinergic and histaminergic tones on the heart in recovered snakes.* Heart rate ($f_{H}$) was calculated from the instantaneous blood pressure trace. The adrenergic and cholinergic tones on the heart were calculated on basis of the R–R interval ($1/f_{H}$) using the equations provided by Altimiras et al. (1). In this approach, the changes in the R–R interval induced by propranolol and atropine are
expressed relative to the R–R interval after double autonomic blockade (i.e. after atropine and propranolol). The histaminergic tone was calculated as the changes in R-R interval induced by the histamine receptor blockade relative to the triple block (i.e. after double autonomic block and histamine H2-receptor blockade).

All data recordings were analysed using AcqKnowledge data analysis software (version 3.7.2., Biopac, Goleta, CA). Data were evaluated using paired t-test, one-way ANOVA for repeated measures followed by a Dunnett’s post hoc test or one-way and two-way ANOVAs followed by a Tukey post hoc test. Differences were considered statistically significant at a 95% level of confidence (P < 0.05). All data are presented as mean ± S.E.M.

RESULTS

Anaesthetised snakes. The effects of a 100 nmol kg⁻¹ bolus intraarterial injection of histamine in a single animal are depicted in Figure 1. Histamine caused a transient reduction in Psys attended by a rise in QLAo. Also, there was a prolonged increase in fH reaching maximum values after the peak hypotensive response. The increase in fH was accompanied by an increase in Ppul and Qpul. The effects of increasing doses of histamine on maximum changes in hemodynamic parameters are presented in Figures 2 and 3. Bolus injections of histamine produced a dose-dependent and immediate systemic vasodilatation at doses above 3 nmol kg⁻¹, which was associated with a decrease in Psys and a rise in Qsys causing Gsys to increase (Fig 2A-C). There were no effects on Gpul, but Qpul and Ppul increased concurrently with a rise in Qtot (Figs 2D-F and 3A). There was a large increase in fH (3B), however, the heart rate response was delayed
relative to the systemic dilation reaching a maximum change of $15.7 \pm 1.4 \text{ min}^{-1}$ at 1000 nmol kg$^{-1}$ (data not shown) after approximately 3 minutes.

The effects of a bolus injection of histamine (100 nmol kg$^{-1}$) in untreated animals, after autonomic double block and after H$_2$-receptor block are shown in Figures 4 and 5. Double autonomic block did not abolish the hemodynamic effects of histamine. An increase in VS$_{tot}$ was revealed in these experiments (Fig. 5C). Effects of adrenaline and acetylcholine were abolished upon administration of propranolol and atropine, respectively, verifying successful autonomic blockade (data not shown). The H$_2$-antagonist abolished both the systemic vasodilatation and the increase in $f_{II}$ and VS$_{tot}$. Furthermore, there was a small pressor effect of histamine after H$_2$-receptor blockade (Fig. 5A). Administration of either of the H$_1$-antagonists caused immediate cardiac arrest in the pythons and the antagonist was left out of subsequent experiments.

**Sinus venosus-atrial preparations.** Histamine caused a dose-dependent increase in both frequency and twitch-force in spontaneously beating sinus venosus-atrial preparations (Fig. 6A-B). The H$_1$-agonist had no effects on frequency or twitch force before the H$_2$-antagonist (Fig. 6C), however, after H$_2$-blockade there was a decrease in frequency ($P = 0.014$). The H$_2$-agonist caused an increase in both frequency and twitch force similar to the effects of histamine (Fig. 6C-D). Incubation with the H$_2$-antagonist abolished the effect of histamine. However, the effect of the H$_2$-agonist on frequency persisted after H$_2$-block and there was a doubling in twitch-force. Incubation with the H$_1$-antagonist stopped the spontaneous frequency of the preparation.
Recovered snakes. Mean blood pressures and heart rates during the experimental protocol are depicted in Figure 7. Blood pressure was not affected by digestion and the effects of the autonomic antagonists were generally small and only after infusion of atropine in fasting snakes did blood pressure increase significantly (Fig. 7A-C). Fasting heart rates were similar in the three experimental groups and digestion elicited a rise in heart rate dependent of meal size from $25.2 \pm 2.4 \text{ min}^{-1}$ in fasting animals to $55.5 \pm 4.3 \text{ min}^{-1}$ after 24h in snakes digesting 25%. The postprandial rise in heart rate was partially due to a doubling of the intrinsic heart rate from $27.5 \pm 2.2 \text{ min}^{-1}$ to $55.0 \pm 3.6 \text{ min}^{-1}$ (Fig. 7D-F). Effects of adrenaline and acetylcholine were abolished upon administration of propranolol and atropine, respectively verifying successful autonomic blockade (data not shown).

Injection of the histamine H$_2$-receptor antagonist ranitidine after double autonomic blockade did not affect heart rate of fasting animals, but decreased heart rate in animals digesting 25% (24h) from $57.0 \pm 3.0 \text{ min}^{-1}$ to $39.8 \pm 2.34 \text{ min}^{-1}$ (Fig. 8). This value was, however, still elevated above the fasting value of $27.7 \pm 1.9 \text{ min}^{-1}$. Bolus injections of histamine (10 nmol kg$^{-1}$) exerted a positive chronotropic effect after double autonomic blockade (Fig. 9). This effect was attenuated in digesting animals and was completely abolished after H$_2$-receptor block.

Fasting snakes were characterized by a large inhibitory cholinergic tone, a low stimulatory adrenergic tone and an absence of a histaminergic tone (Fig. 10). Digestion (25%, 24h) was associated with a large reduction in cholinergic tone from $53.4 \pm 11.4\%$ to $8.9 \pm 3.9\%$. Also, there was a tendency towards a decrease in adrenergic tone and a large increase in histaminergic tone from $-0.1 \pm 4.3\%$ to $30.2 \pm 1.3\%$. Figure 11 shows double blocked heart rate and histaminergic tones in fasting and digesting (25%) snakes.
24h and 48h after feeding. After 48h into digestion double blocked heart rate was still elevated above fasting values whereas histaminergic tones had vanished. Table 1 shows histamine plasma concentrations in fasting and digesting snakes 24h and 48h after feeding, which revealed no significant changes in plasma levels after feeding.

**DISCUSSION**

This study shows there is a large histaminergic tone on the heart during the initial phase of digestion in pythons suggesting that histamine regulates the postprandial tachycardia. Furthermore, histamine dilates the systemic circulation and increases heart rate and force of contraction through stimulation of H2-receptors. These responses resemble the cardiovascular changes during digestion and in concert with the postprandial increase in cardiac histaminergic tone, it seems reasonable to propose that histamine partake in the regulation of the cardiovascular responses to digestion.

*Cardiac effects of histamine*

The marked increase in heart rate and stroke volume after infusion of histamine in both anaesthetised and awake fasting pythons persisted after blockade of β-adrenergic and muscarinic receptors indicating that it was not an indirect effect mediated through stimulation of the adrenergic system or withdrawal of the vagal tone. However, the effects were abolished after the H2-receptor selective antagonist ranitidine, showing that the increase in frequency and force of contraction were mediated through stimulation of histamine H2-receptors directly in the cardiac tissue. Histamine also exerted marked positive chronotropic and inotropic effects in the isolated sinus/right atrial preparation, and these effects were also abolished upon H2-receptor blockade. The spontaneous
frequency of the sinus venosus-atrial preparations was not different from the double blocked intrinsic heart rate in the fasting snakes (33.4 ± 2.2 and 27.5 ± 2.2 respectively, P=0.087, N=6) showing that extrapolation from the in vitro to the in vivo situation is applicable. Ranitidine did not, however, abolish the effects of the H$_2$-agonist on frequency in the sinus/right atrial preparation and twitch force was doubled. These differences may be explained by the ten-fold higher concentration of the H$_2$-receptor agonist in the bath, which may compromise the competitive binding of the H$_2$-receptor selective antagonist. The increase in twitch force, however, may be explained by a combination of the above mentioned and the preceding lack of effect of histamine leaving a greater span in twitch force for increase in response to the H$_2$-agonist. Histamine also stimulates heart rate in mammals (6), where three of the four identified histamine receptor types have been localized on the heart; receptor H$_1$ and H$_2$ are located in the cardiac tissue whereas H$_3$ is a pre-junctional synaptic receptor (17, 18). The primary and direct effect of histamine in the mammalian heart includes an increase in sinus rate and an increase in force of contraction (49). The increase in heart rate is induced through a H$_2$-receptor mediated enhancement of slow inward Ca$^{2+}$ current and subsequent acceleration in the pacemaker potential (49). The positive inotropic effect of histamine is on the other hand a result of increased amounts of cAMP (14), which occur through H$_1$- and/or H$_2$-receptor activation. Previous studies on reptiles indicate that histaminergic effects on the heart vary among species, and reports that the positive inotropic effect is mediated through either H$_1$- or H$_2$-receptors (9, 21, 27). The chronotropic effect is often weak or even absent although histamine causes a marked increase in frequency of the spontaneous beating right atrium of the rainbow lizard (21, 27, 30).
We studied the effects of the H\textsubscript{1}-antagonists, diphenhydramine and mepyramine, in preliminary \textit{in vivo} and \textit{in vitro} studies, but both H\textsubscript{1}-antagonists caused immediate cardiac arrest in the pythons. In mammals, H\textsubscript{1}-antagonists are notorious for their cardiotoxic effects (22). Nevertheless, the H\textsubscript{1}-agonist decreased the frequency in the isolated sinus/right atrial preparation, which was intensified after the H\textsubscript{2}-receptor blockade, indicating that H\textsubscript{1}-receptors are in fact present in the heart mediating a negative chronotropic effect.

\textit{Vascular effects of histamine in anaesthetised animals}

Hemodynamic variables of the anaesthetised pythons studied here were similar to previous reports (37, 48), and the higher heart rate, compared to recovered and awake pythons (34), is caused by depression of autonomic and barostatic functions during anaesthesia. This makes anaesthetised animals suitable for studies on local regulatory mechanism because it is easier to discern the direct effect of the pharmacological manipulation.

Histamine dilated the systemic circulation, and this effect persisted after β-adrenergic and muscarinic blockade, but the dilation was abolished by histamine H\textsubscript{2}-receptor blockade. The response is likely, therefore, to be caused by stimulation of H\textsubscript{2}-receptors in the vasculature, and while there was no direct evidence for vascular H\textsubscript{1}-receptors in pythons, the depressor effect of histamine was reversed to a slight pressor response after H\textsubscript{2}-receptor blockade. \textit{In vitro} studies on other reptiles have revealed a dominant inhibitory effect of histamine causing vasodilation through H\textsubscript{2}-receptors and a stimulatory effect causing vasoconstriction mediated through H\textsubscript{1}-receptors (4, 29). In
mammals, histamine dilates the systemic circulation through both H1-receptors in the endothelium and H2-receptors directly on the vascular smooth muscle cells (5, 10).

There were virtually no effects of histamine on the pulmonary vasculature and the large increase in both pulmonary flow and pressure was merely the consequence of the rise in heart rate and cardiac output. In mammals, histamine constricts the pulmonary vasculature through histamine H1-receptors (10, 28). The lack of effects of histamine in the python lung is consistent with the very small effects of various regulatory peptides and NO in the pulmonary circulation of most reptiles studied so far (e.g. 38).

The role of histamine during digestion in pythons
Consistent with previous studies on snakes (34, 47), digestion caused a large and prolonged rise in heart rate of pythons. The magnitude of this cardiovascular response increased with meal size and a meal corresponding to a quarter of the snake’s body mass caused heart rate to double and to remain elevated for several days. Sympathetic tone on the heart did not increase during digestion, and the tachycardia was caused by a combination of withdrawal of vagal tone and a doubling of the intrinsic heart rate, revealed upon pharmacological blockade of both sympathetic and parasympathetic receptors. This implies that other factors than catecholamines or reduced cholinergic tone exert a pronounced positive chronotropic action during digestion. This non-adrenergic-non-cholinergic (NANC) stimulation of the heart could arise from increased circulating levels of a hormone that either acts directly on the heart or pre-synaptically on cardiac neurons causing release of chronotropic agents.

Our study shows that there is no histaminergic tone on the heart rate in fasting snakes, but that a large histaminergic tone develops by 24h into the postprandial period
and coincided with an attenuated heart rate response to histamine infusion. During digestion, most vertebrates release histamine in the stomach stimulating parietal cells to secrete acid (25, 30). In pythons, acid secretion lowers gastric pH from the fasting level of approximately 7.5 to about 2.0 during digestion (36). Histamine is released from specialised ECL cells within the gastric mucosa and reaches receptors on the acid secreting parietal cells, also situated in the gastric mucosa, either through diffusion or capillary transport (25). While an overflow of histamine from the gastric mucosa may increase circulating levels and stimulate cardiac histamine receptors, plasma concentration of histamine did not increase during digestion. Thus, the histaminergic stimulation of the heart is unlikely to stem from gastric release of histamine.

In mammals, histamine and noradrenalin are stored and coreleased from sympathetic nerve endings in cardiac ganglions (23). In snakes as well as humans, however, digestion is associated with a decrease or no changes in cardiac sympathetic tone (8, 47), making it unlikely that histamine is co-released from sympathetic cardiac neurons during digestion. However, as in mammals, central and peripheral histaminergic neurons have been identified in both invertebrates and non-mammalian vertebrates (16, 23, 26), which may release histamine during digestion.

A major store of histamine in vertebrates is mast cells that are distributed throughout the body including cardiac tissue (39, 49). In digesting mammals, gastrin is released from the pyloric antrum of the stomach from specialised cells, released into the bloodstream and carried to the ECL cells in the gastric mucosa where it stimulates the release of histamine through cholecystokinin 2 (CCK2) receptors (25). Moreover, pentagastrin, stimulates intrinsic heart rate in guinea pigs through a release of histamine from cardiac mast cells (41). Therefore, it is possible that during digestion gastrin released from the
stomach stimulates cardiac mast cells to release histamine, which then targets receptors within the heart. The whole body content of histamine is high in mammals, birds and reptiles and low in amphibians and most fish, which can be ascribed to variation in the histamine content of mast cells (30). Interestingly, the evolution of mast cells as storage site for histamine seems to parallel the evolution of strong vascular actions of histamine. This may provide a phylogenetically founded argument for the source of histamine regulating the postprandial cardiovascular effect being mast cells.

The histaminergic tone had subsided by 48h into digestion, while intrinsic heart rate remained elevated. Thus, while increased histaminergic tone appears to explain most of the tachycardia during the initial period of digestion, other NANC factors seem to be involved in regulating the postprandial heart rate, in particularly during the subsequent phases of digestion. Other hormones released from the duodenal-pancreatic region have been suggested to be involved in the regulation of the postprandial increase in cardiac output and heart rate. Insulin has been considered because of its cardiovascular effects including a positive chronotropic and ionotropic effect (3). However, the increase in cardiac output is not only observed after carbohydrate meals but also after ingestion of fat and protein where not much insulin is expected to be released. Secretin increases heart rate and cardiac output during digestion in dogs and humans (12, 13) and the intestinal hormone oxyntomodulin, which is released during digestion, increases intrinsic heart rate in mice when administered peripherally (40). It is tempting to speculate, that as the ingested food is being processed and moves along in the gastrointestinal system this progressively stimulates the release of various hormones and regulatory peptides, which then directly or indirectly induce the responses to digestion including the postprandial increase in cardiac output and heart rate.
In conclusion, our study shows that there is a large histaminergic cardiac tone during the initial phase of digestion in pythons suggesting that histamine regulates the postprandial tachycardia. The positive chronotropic and inotropic effects of histamine is mediated through a direct effect on cardiac H2-receptors, but the stomach is an unlikely source of the histamine. It seems, therefore, that other regulatory peptides or hormones causes histamine release from cardiac mast cells during the initial phase of digestion, whereas other signal molecules are important later in digestion. Histamine also dilates the systemic vasculature through histamine H2-receptors and mimics the postprandial hemodynamic changes in pythons.

**Perspectives and significance**

The presence of a pronounced cardiac stimulation by histamine and other NANC factors highlight that the autonomic regulation of the heart differ between digestion and exercise, even though both states elicit similar metabolic increments. The marked cardiac stimulation by NANC factors may imply that digestive organs stimulate the heart directly without involvement of the normal autonomic regulation, perhaps to secure a high and continuous perfusion. In any event, the NANC stimulation can explain that maximal heart rate is higher in postprandial versus digesting snakes (34). Thus, whether the NANC stimulation during the postprandial period allows for an intact autonomic regulation, despite the higher heart rate, or whether the NANC stimulation entirely overrides the normal autonomic regulation during digestion remains to be investigated. Moreover, apart from identifying the NANC factors that stimulate the heart later in the digestive process, when the histaminergic tone has subsided, it would
be of interest to evaluate whether the contribution of the NANC factors are directly correlated with the magnitude of the SDA response.

In line with the “August Krogh principle”, dictating that for many physiological problems, there will be an animal of choice on which it can be most conveniently studied, the pronounced and long-lasting effects of digestion on cardiovascular control in pythons may reveal fundamental mechanisms of digestion that apply to all vertebrates. Moreover, the python heart may have potential as a model to further explore the signalling and mechanical mechanisms that underlie human heart diseases. Postprandial angina pectoris in humans has been known for more than two centuries, still the disease is not well understood and conventional animal models have not revealed the underlying mechanism. However, coronary artery spasm plays an important role in the pathogenesis of ischemic heart diseases particularly variant angina or spontaneous angina pectoris (24). Furthermore, recent years research indicates that coronary vasospasm may be driven partially by inflammatory pathways involving histamine and mast cells (24). Therefore, the present study suggests that attention should be further extended to include studies on the possible role of histamine in postprandial angina.

Acknowledgements. The authors wish to thank Dorte Olsson for technical assistance. This study was supported by the Danish Research Council.
REFERENCES


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FIGURE LEGENDS

**Figure 1.** Original recording from one anaesthetised python showing the effects of a bolus injection of histamine. (A) \( P_{\text{sys}} \), systemic blood pressure; (B) \( Q_{\text{LAo}} \), left aortic blood flow; (C) \( P_{\text{pul}} \), pulmonary blood pressure; (D) \( Q_{\text{pul}} \), pulmonary blood flow; (E) \( f_H \), heart rate.

**Figure 2.** Effects of bolus intraarterial injections of histamine in anaesthetised pythons on the maximum changes in hemodynamic parameters. (A and D) \( P_{\text{sys}} \) and \( P_{\text{pul}} \), systemic and pulmonary blood pressure; (B and E) \( Q_{\text{sys}} \) and \( Q_{\text{pul}} \), systemic and pulmonary blood flow; and (C and F) \( G_{\text{sys}} \) and \( G_{\text{pul}} \), systemic and pulmonary vascular conductance. Data are mean ± S.E.M. (\( N = 5 \)). An asterisk indicates a significant difference from pre-injection values (\( P < 0.05 \)) evaluated by a one-way ANOVA for repeated measurements followed by a Dunnett’s *post hoc* test.

**Figure 3.** Effects of bolus intraarterial injections of histamine in anaesthetised pythons on the maximum changes in hemodynamic parameters. (A) \( Q_{\text{tot}} \), total cardiac output; (B) \( f_H \), heart rate; and (C) \( V_{\text{S}_{\text{tot}}} \), total stroke volume. Data are mean ± S.E.M. (\( N = 5 \)). An asterisk indicates a significant difference from pre-injection values (\( P < 0.05 \)) evaluated by a one-way ANOVA for repeated measurements followed by a Dunnett’s *post hoc* test.

**Figure 4.** The vascular effects of histamine are mediated directly through histamine H2-receptors. Effects of a bolus intraarterial injection of histamine (100 nmol kg\(^{-1}\)) in anaesthetised pythons: in untreated animals, after double block (atropine and
propranolol, 3 mg kg\(^{-1}\)), and after triple block including the H\(_2\)-receptor antagonist ranitidine (40 mg kg\(^{-1}\)). (A and D) \(P_{\text{sys}}\) and \(P_{\text{pul}}\), systemic and pulmonary blood pressure; (B and E) \(Q_{\text{sys}}\) and \(Q_{\text{pul}}\), systemic and pulmonary blood flow; and (C and F) \(G_{\text{sys}}\) and \(G_{\text{pul}}\), systemic and pulmonary vascular conductance. Pre-injection values are black bars and maximum responses are open bars. Data are mean ± S.E.M. (N = 5). An asterisk indicates a significant difference from pre-injection values (P < 0.05) evaluated by a paired t-test.

**Figure 5.** The cardiac effects of histamine are mediated directly through histamine H\(_2\)-receptors. Effects of a bolus intraarterial injection of histamine (100 nmol kg\(^{-1}\)) in anaesthetised pythons: in untreated animals, after double block (atropine and propranolol, 3 mg kg\(^{-1}\)), and after triple block including the H\(_2\)-receptor antagonist ranitidine (40 mg kg\(^{-1}\)). (A) \(Q_{\text{tot}}\), total cardiac output; (B) \(f_H\), heart rate; and (C) \(V_{\text{S tot}}\), total stroke volume. Pre-injection values are black bars and maximum responses are open bars. Data are mean ± S.E.M. (N = 5). An asterisk indicates a significant difference from pre-injection values (P < 0.05) evaluated by a paired t-test.

**Figure 6.** (A and B) Effects of cumulative increase in histamine bath concentration on frequency and relative change in twitch force in spontaneously beating sinus venosus-atrial preparations. (C and D) Effects of histamine agonists and H\(_2\)-receptor blockade on spontaneously beating sinus venosus-atrial preparations. Black bars show the effects of histamine agonists before, and open bars after, blockade of H\(_2\)-receptors. The grey bars show the effect of the H\(_2\)-receptor blockade. Data are mean ± S.E.M. An asterisk denotes a significant difference from control values evaluated by a one-way ANOVA.
for repeated measures followed by a Dunnett’s *post hoc* test, N = 6 (A-B) or a two-way ANOVA followed by a Tukey *post hoc* test (N = 5) (C-D).

**Figure 7.** Effects of autonomic double blockade on systemic blood pressure and heart rate in fasting and digesting recovered pythons. (A-C) Psys, systemic blood pressure and (D-F) \(f_H\), heart rate. Propranolol and atropine, 3 mg kg\(^{-1}\). Values are mean ± S.E.M. (N-values are given in parentheses). An asterisk denotes values significantly different from untreated animals within the group evaluated by a paired t-test (P<0.05). ‡ denotes a significant difference from fasting animals within groups evaluated by a paired t-test. † denotes a significant difference from fasting double blocked animals evaluated by a one-way ANOVA followed by a Tukey *post hoc* test.

**Figure 8.** Blockade of histamine H\(_2\)-receptors decreases intrinsic heart rate in digesting snakes. Effects of the histamine H\(_2\)-receptor antagonists ranitidine on the double blocked heart rate (\(f_H\)) in fasting (N=6), digesting 10% (N=7) and digesting 25% (N=4) pythons. Black bars are double blocked heart rate and open bars are heart rate after histamine H\(_2\)-receptor blockade (40 mg kg\(^{-1}\)). Values are mean ± S.E.M. Different letters indicates significantly different values evaluated by a two-way ANOVA followed by a Tukey *post hoc* test (P<0.05).

**Figure 9.** Histamine induces a positive chronotropic response through histamine H\(_2\)-receptors in fasting snakes, which is attenuated in digesting snakes. Effects of bolus injection of histamine (10 nmol kg\(^{-1}\)) on double blocked heart rate (\(f_H\)) before (black bars) and after (open bars) H\(_2\)-receptor antagonists ranitidine (40 mg kg\(^{-1}\)) in fasting
(N=6), digesting 10% (N=7) and digesting 25% (N=4) pythons. Values are mean ± S.E.M. Different letters indicates significant differences between double blocked heart rates before histamine H2-receptor block and an asterisk denotes a significant difference in values before and after histamine H2-receptor block evaluated by a two-way ANOVA followed by a Tukey post hoc test (P<0.05).

**Figure 10.** Effects of digestive state on cholinergic, adrenergic and histaminergic cardiac tones. Values are mean ± S.E.M. (fasting (N=7), digesting 10% (N=7) and digesting 25% (N=4)). Different letters indicates significantly different values within each of the three groups of cardiac tones evaluated by a one-way ANOVA followed by a Tukey post hoc test (P<0.05).

**Figure 11.** Effects of time on double blocked heart rate and histaminergic cardiac tone in pythons digesting 25% of body weight. (A) $f_{HB}$, double blocked heart rate; (B) histaminergic cardiac tone. Values are mean ± S.E.M. (N=7, fasting and N=4, digesting). Different letters indicates significant differences evaluated by a one-way ANOVA followed by a Tukey post hoc test (P<0.05).
Table 1. Histamine plasma concentrations in fasting and digesting pythons (*Python regius*) at 30°C.

<table>
<thead>
<tr>
<th>Histamine (nM)</th>
<th>Fasting</th>
<th>Digesting (25%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24h</td>
<td>48h</td>
</tr>
<tr>
<td>Control</td>
<td>13.3 ± 2.0</td>
<td>7.5 ± 3.0</td>
</tr>
<tr>
<td>Fasting</td>
<td>10.1 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Postprandial (24h)</td>
<td>5.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Postprandial (48h)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N-values: 6 4 3

Values are mean ± S.E.M. Differences from control values were evaluated with a paired t-test (P<0.05)
His 100 nmol kg$^{-1}$
Maximum change $P_{sys}$ (kPa)

-2.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5

Maximum change $P_{pul}$ (kPa)

-0.2
0.0
0.2
0.4
0.6
0.8
1.0

Maximum change $Q_{sys}$ (ml min$^{-1}$ kg$^{-1}$)

0
5
10
15
20

Maximum change $Q_{pul}$ (ml min$^{-1}$ kg$^{-1}$)

0
2
4
6
8

Maximum change $G_{sys}$ (ml kPa$^{-1}$ min$^{-1}$ kg$^{-1}$)

-2
0
2
4
6
8
10

Maximum change $G_{pul}$ (ml kPa$^{-1}$ min$^{-1}$ kg$^{-1}$)

-7
-5
-3
-1
0
2
4
6
8
10

Histamine (nmol kg$^{-1}$)
**A**

Frequency (min⁻¹)

**B**

Relative change in twitch force (%)

**C**

Maximum change in frequency (min⁻¹)

**D**

- **H₁-agonist (10⁻⁵ M)**
- **Histamine (10⁻⁶ M)**
- **H₂-agonist (10⁻⁵ M)**
- **H₂-block (10⁻³ M)**

**Legend:**
- Histamine (10⁻⁶ M)
- H₁-agonist (10⁻⁵ M)
- H₂-agonist (10⁻⁵ M)
- H₂-block (10⁻³ M)

* indicates significance level.
Max changes in double blocked $f_n$ (min$^{-1}$)

- Fasting
- Digesting 10%
- Digesting 25%

* indicates significant difference from fasting.
Cardiac tones (%)

Fasting
Digesting 10%
Digesting 25%

Cholinergic Adrenergic Histaminergic