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

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Submitted 31 May 2008; accepted in final form 13 December 2008

Histamine induces postprandial tachycardia through a direct effect on cardiac H₂-receptors in pythons. Am J Physiol Regul Integr Comp Physiol 296: R774–R785, 2009. First published December 17, 2008; doi:10.1152/ajpregu.90466.2008.—The intrinsic heart rate of most vertebrates studied, including humans, is elevated during digestion, suggesting that a nonadrenergic-noncholinergic 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 fourfold 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 48 h after ingestion of a meal amounting to 25% of body wt. Digestion caused heart rate to increase from 25 to 56 min, whereas 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 at 24 h into digestion, whereas it had no effects at 48 h. Thus, the histaminergic tone on the heart rose from none to 30% at 24 h but vanished after 48 h. In anesthetized 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.

reptile; digestion; heart rate; blood flow; blood pressure

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 10-fold rise in metabolism that can be sustained for up to 2 wk attended by a marked and rapid hypertrophy of visceral organs including a 40% fully reversible increase in ventricular muscle mass within 48 h 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 fourfold increase in cardiac output, as well as a dilatation of the mesenteric vascular bed leading to intestinal hyperemia (34, 42). Furthermore, plasma levels of gastrointestinal regulatory peptides increases many-fold after feeding (35). Compared with humans, where the nonadrenergic-noncholinergic (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), which showed that synthetic histamine, β-imidazolylethylamine, modifies cardiac rhythm in the mammalian heart. In mammals, histamine exerts cardiovascular effects that resemble the postprandial cardiovascular changes including dilatation 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 exogenously administered histamine, as well as the underlying mechanism, in anesthetized pythons (Python regius). Furthermore, we investigate the direct effect of histamine on cardiac receptors through specific H₁- and H₂-receptor agonists and antagonists on sinus venous-atrial preparation.

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MATERIALS AND METHODS

Experimental Animals

Experiments were undertaken on 34 pythons (Python regius) of undetermined sex and age weighing between 0.14 and 0.80 kg (0.30 ± 0.02 kg; mean ± SE). 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 the experimental protocol was reviewed and approved by the Animal Experimentation Board under the Danish Ministry of Justice.

Surgery and Instrumentation

Anesthetized snakes. Five pythons were anesthetized by an intramuscular injection of a pentobarbital sodium (25 mg/kg; Mebumal, Sygehusapotekerne, Denmark). All reflexes disappeared within 30 min, and the animals were then tracheotomized for artificial ventilation at 10 breaths/min and a tidal volume of 50 ml/kg using a Harvard Apparatus mechanical ventilator (Cambridge, MA). A 5-cm latroventral incision was made cranial to the heart, and a polyethylene PE50 catheter filled with heparinized saline (50 IU/ml) was advanced into the vertebral artery for measurements of systemic blood pressure (Psys). 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 PE50 catheter for measurements of pulmonary blood pressure (Ppul). For measurements of blood flows, transit-time ultrasonic blood flow probes (model 1.5R; Transonic System, 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 anesthetized by ventilation with 2–3% isoflurane (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 (model UC 2; Statham, Oxnard, CA), 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 (in mM): 95 NaCl, 25 NaHCO₃, 1.0 NaH₂PO₄, 2.5 KCl, 1.0 MgSO₄, 1.5 CaCl₂, and 5 glucose; gassed with 2% CO₂-98% O₂ (pH ~ 7.5), which was delivered by a gas mixing pump (Wösthoff, Bochum, Germany). The mounted preparations were left for 30 min to stabilize and were 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 stabilize contractions. Signals from the force transducer were recorded with an MP100 data acquisition system (Biopac Systems, Goleta, CA) at 100 Hz.

Recovered snakes. Anesthesia was induced through inhalation of ~5% isoflurane (Isofluran; Baxter). The snakes were then intubated and maintained at 1–2% isoflurane during surgery using a Harvard Apparatus mechanical ventilator (10 breaths/min and a tidal volume of 50 ml/kg). A 4-cm ventrolateral incision was made posterior to the kidney, and a PE50 catheter, filled with heparinized saline, was advanced into the aorta for measurements of systemic blood pressure. The catheter was externalized, 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 disposable pressure transducers (model PX600; Baxter Edwards, Irvine, CA), and the signals were...
amplified using an in-house-built preamplifier. The pressure transducers were positioned at the 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 (model T206). Signals from the pressure transducers and the blood flow meter were recorded with a Biopac MP100 data acquisition system (Biopac Systems) at 100 Hz.

**Experimental Protocol**

**Anesthetized 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 1 ml/kg injection of 0.9% (wt/vol) 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. Hemodynamic variables were allowed to return to baselines between each injection. All drugs were given in 1.0 ml/kg 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). The efficacy of the autonomic blockade was verified through injections of acetylcholine (5 μg/kg) and adrenaline (2 μg/kg) before and after the antagonists. Also, a bolus of histamine (100 nmol/kg) was given before and after double blockade and the histamine H2-receptor antagonist ranitidine, 40 mg/kg. In initial experiments, a H1-receptor antagonist (diphenhydramine or mepyramine, 40 mg/kg) was given. The antagonists were allowed 20 min to take effect before subsequent injections of histamine. Experiments were carried out at 30°C. After the protocol ended, 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 stabilize. Preparations were then subjected to the following protocol: H1-agonist {2-[(3-trifluoromethyl)phenyl]histamine dimaleate, 10\(^{-5}\) M}, histamine (10\(^{-6}\) M) and H2-agonist (amthamine dihydrobromide, 10\(^{-5}\) M). The chamber was washed twice, the cardiac strips were incubated with H2-antagonist ranitidine.

![Fig. 2. Effects of bolus intra-arterial injections of histamine in anesthetized pythons on the maximum changes in hemodynamic parameters: Psys (A); Ppul (D); Qsys (B); Qpul (E); and Gsys and Gpul, systemic and pulmonary vascular conductance (C and F). Data are means ± SE. (n = 5). *Significant difference from preinjection values (P < 0.05) evaluated by a 1-way ANOVA for repeated measurements followed by a Dunnett’s post hoc test.](http://ajpregu.physiology.org/Download)
injections of acetylcholine (10^{-3} \text{M}) for 30 min, and the protocol was repeated. Finally, the sinus venosus-atrial preparations were incubated with the H_{1}-antagonist diphenhydramine (10^{-3} \text{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 1 to 2 h 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) of body weight, and snakes digesting 25% (25.7% ± 0.3%; n = 8) of body weight. The digesting snakes were force-fed with freshly killed adult mice or preweaned rats. All three groups were left for 24 h before measurements and blood sampling; however, four snakes digesting 25% were not measured until 48 h 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 24 or 48 h after feeding, drugs were administered according to the following protocol in all 22 snakes: \(\beta\)-adrenergic antagonist propranolol and cholinergic antagonist atropine (3 mg/kg each; the order of injection of propranolol and atropine was alternated), histamine (10 nmol/kg), histamine H_{2}-receptor antagonist ranitidine (40 mg/kg), and histamine (10 nmol/kg). The efficacy of the autonomic blockade was verified in both fasting and digesting snakes through injections of acetylcholine (5 \mu g/kg) and adrenaline (2 \mu g/kg) 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 24 h after instrumentation and immediately prior to the injection of drugs. All blood samples (200 \mu l) were taken in microvets coated with EDTA (Microwette 300; Sarstedt, Numbrecht, Germany), spun down at 5,000 rpm for 2 min (Sigma-3MK), 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, Montigny le Bretonneux, France).

**Data Analysis and Statistics**

Calculations of blood flows, stroke volume and vascular conductance in anesthetized 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 anesthetized pythons, total systemic blood flow (Q_{sys}) can be estimated as 2.5 times left aortic blood flow (Q_{LAA}) (37). Total cardiac output (Q_{out}) was calculated as Q_{sys} + Q_{pul}. Heart rate (f_{HR}) was calculated from the instantaneous blood flow trace from the left aortic arch and total stroke volume (V_{Stot}; pulmonary + systemic) was calculated as Q_{out}/f_{HR}. 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_{HR}) 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 (f_{HR}) 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 H_{2}-receptor blockade).

![Fig. 3. Effects of bolus intra-arterial injections of histamine in anesthetized pythons on the maximum changes in hemodynamic parameters: Q_{out}, total cardiac output (A); f_{HR}, heart rate (B); and V_{Stot}, total stroke volume (C). Data are means ± SE (n = 5). *Significant difference from preinjection values (P < 0.05) evaluated by a 1-way ANOVA for repeated measurements followed by a Dunnett’s post hoc test.](http://ajpregu.physiology.org/)
All data recordings were analyzed 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 means ± SE.

RESULTS

Anesthetized Snakes

The effects of a 100 nmol/kg bolus intra-arterial injection of histamine in a single animal are depicted in Fig. 1. Histamine caused a transient reduction in $P_{sys}$ attended by a rise in $Q_{LAo}$. Also, there was a prolonged increase in $f_{H}$ reaching maximum values after the peak hypotensive response. The increase in $f_{H}$ was accompanied by an increase in $P_{pa}$ and $Q_{pa}$. The effects of increasing doses of histamine on maximum changes in hemodynamic parameters are presented in Figs. 2 and 3. Bolus injections of histamine produced a dose-dependent and immediate systemic vasodilatation at doses above 3 nmol/kg, which was associated with a decrease in $P_{sys}$ and a rise in $Q_{sys}$ causing $G_{sys}$ to increase (Fig. 2, A–C). There were no effects on $G_{pul}$, but $Q_{pul}$ and $P_{pul}$ increased concurrently with a rise in $Q_{tot}$ (Figs. 2, D–F and 3A). There was a large increase in $f_{H}$ (3B); however, the heart rate response was delayed relative to the systemic dilation reaching a maximum change of 15.7 ± 1.4 min$^{-1}$ at 1,000 nmol/kg (data not shown) after ~3 min.

The effects of a bolus injection of histamine (100 nmol/kg) in untreated animals, after autonomic double block and after $H_{2}$-receptor block are shown in Figs. 4 and 5. Double autonomic block did not abolish the hemodynamic effects of histamine. An increase in total stroke volume 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 $f_{H}$ and $V_{S\,tot}$. Furthermore, there was a small pressor response.
effect of histamine after H2-receptor blockade (Fig. 5A). Administration of either of the H1-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. 6, A–B). The H1-agonist had no effects on frequency or twitch force before the H2-antagonist (Fig. 6C); however, after H2-blockade there was a decrease in frequency ($P = 0.014$). The H2-agonist caused an increase in both frequency and twitch force similar to the effects of histamine (Fig. 6, C–D). Incubation with the H2-antagonist abolished the effect of histamine. However, the effect of the H2-agonist on frequency persisted after H2-block, and there was a doubling in twitch-force. Incubation with the H1-antagonist stopped the spontaneous frequency of the preparation.

**Recovered Snakes**

Mean blood pressures and heart rates during the experimental protocol are depicted in Fig. 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. 7, A–C). Fasting heart rates were similar in the three experimental groups, and digestion elicited a rise in heart rate depending on meal size from $25.2 \pm 2.4$ min in fasting animals to $55.5 \pm 4.3$ min after 24 h in snakes digesting 25%. The postprandial rise in heart rate was partially due to a doubling of the intrinsic heart rate from $27.5 \pm 2.2$ min to $55.0 \pm 3.6$ min (Fig. 7, D–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 H2-receptor antagonist ranitidine after double autonomic blockade did not affect heart rate of fasting animals, but decreased heart rate in animals digesting 25% (24 h) from $57.0 \pm 3.0$ min to $39.8 \pm 2.3$ min (Fig. 8). This value was, however, still elevated above the fasting value of $27.7 \pm 1.9$ min. Bolus injections of histamine (10 nmol/kg) exerted a positive chronotropic effect after double autonomic blockade (Fig. 9). This effect was attenuated in digesting animals and was completely abolished after H2-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%, 24 h) 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 toward a decrease in adrenergic tone and a large increase in histaminergic tone from $0.1 \pm 4.3\%$ to $30.2 \pm 1.3\%$. Fig. 11 shows double-blocked heart rate and histaminergic tones in fasting and digesting (25%) snakes 24 and 48 h after feeding. After 48 h 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 24 and 48 h 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, suggest-

Fig. 5. Cardiac effects of histamine are mediated directly through histamine H2-receptors. Effects of a bolus intra-arterial injection of histamine (100 nmol/kg) in anesthetized pythons, in untreated animals, after double block (atropine and propranolol, 3 mg/kg), and after triple block including the H2-receptor antagonist ranitidine (40 mg/kg): $Q_{\text{tot}}$, total cardiac output (A); $f_{\text{H}}$, heart rate (B); and $V_{\text{S}_{\text{tot}}}$, total stroke volume (C). Preinjection values are black bars and maximum responses are white bars. Data are means $\pm$ SE. ($n = 5$). *Significant difference from preinjection values ($P < 0.05$) evaluated by a paired $t$-test.
ing 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 anesthetized 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 min and 27.5 ± 2.2 min, 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 H₂-agonist on frequency in the sinus/right atrial preparation, and twitch force was doubled. These differences may be explained by the 10-fold higher concentration of the H₂-receptor agonist in the bath, which may compromise the competitive binding of the H₂-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 H₂-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 H₁ and H₂ are located in the cardiac tissue, whereas H₃ is a prejunctional 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 H₂-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 H₁- and/or H₂-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 H₁- or H₂-receptors (9, 21, 27). The chronotropic effect is often weak or even absent, although histamine causes a marked increase in frequency of the spon-

![Fig. 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 H₂-receptor blockade on spontaneously beating sinus venosus-atrial preparations. Black bars show the effects of histamine agonists before, and white bars after, blockade of H₂-receptors. Grey bars show effect of the H₂-receptor blockade. Data are means ± SE. *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 2-way ANOVA followed by a Tukey post hoc test (\( n = 5 \)) (C–D).](http://apregul.physiology.org/doi/10.220.32.246/372067)
We studied the effects of the H1-antagonists diphenhydramine and mepyramine in preliminary in vivo and in vitro studies, but both H1-antagonists caused immediate cardiac arrest in the pythons. In mammals, H1-antagonists are notorious for their cardiotoxic effects (22). Nevertheless, the H1-agonist decreased the frequency in the isolated sinus/right atrial preparation, which was intensified after the H2-receptor blockade, indicating that H1-receptors are, in fact, present in the heart, mediating a negative chronotropic effect.

Vascular Effects of Histamine in Anesthetized Animals

Hemodynamic variables of the anesthetized pythons studied here were similar to previous reports (37, 48), and the higher heart rate, compared with recovered and awake pythons (34), is caused by depression of autonomic and barostatic functions during anesthesia. This makes anesthetized animals suitable for studies on local regulatory mechanisms, 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 H2-receptor blockade. The response is likely, therefore, to be caused by stimulation of H3-receptors in the vasculature, and while there was no direct evidence for vascular H1-receptors in pythons, the depressor effect of histamine was reversed to a slight pressor response after H2-receptor blockade. In vitro studies on other reptiles have revealed a dominant inhibitory effect of histamine causing vasodilation through H2-receptors and a stimulatory effect causing vasoconstriction mediated through H1-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 nitric oxide in the pulmonary circulation of most reptiles studied so far (e.g., 38).

Role of Histamine During Digestion in Pythons

Consistent with previous studies on snakes (34, 47), digestion caused a large and prolonged rise in the 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 chronotropic action during digestion. This NANC stimulation of the heart could arise from increased circulating levels of a hormone that either acts directly on the heart or presynaptically 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 24 h 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 ∼7.5 to ∼2.0 during digestion (36). Histamine is released from specialized enterochromaffin-like 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 sympathethic tone (8, 47), 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 (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 specialized cells, released into the bloodstream, and carried to the enterochromaffin-like cells in the gastric mucosa, where it stimulates the release of histamine through cholecystokinin 2 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
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 are mediated through a direct effect on cardiac H$_2$-receptors, but the stomach is an unlikely source of the histamine. It seems, therefore, that other regulatory peptides or hormones cause 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 H$_2$-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 vs. 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 specific dynamic action response.

In line with the August Krogh principle, which dictates 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 cardiac function in many other species that are more easily accessible.

**Table 1. Histamine plasma concentrations (nM) in fasting and digesting pythons (Python regius) at 30°C**

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of pythons</strong></td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>13.3±2.0</td>
<td>7.5±3.0</td>
<td>15.1±6.9</td>
</tr>
<tr>
<td><strong>Fasting</strong></td>
<td>10.1±1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Postprandial, 24 h</strong></td>
<td></td>
<td>5.7±1.2</td>
<td></td>
</tr>
<tr>
<td><strong>Postprandial, 48 h</strong></td>
<td></td>
<td></td>
<td>12.4±0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. Differences from control values were evaluated with a paired t-test (P < 0.05).
of digestion that apply to all vertebrates. Moreover, the python heart may have potential as a model to further explore the signaling and mechanical mechanisms that underlie human heart diseases. Postprandial angina pectoris in humans has been known for more than two centuries, and 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.

ACKNOWLEDGMENTS
The authors thank Doris Ossen for technical assistance.

GRANTS
This study was supported by the Danish Research Council.

REFERENCES