In vertebrates, digestion is associated with a rise in aerobic metabolism, which is met by a rise in heart rate to secure adequate oxygen delivery, as well as intestinal nutrient absorption and transport between organs. To study the autonomic regulation of this postprandial tachycardia in an amphibian, we placed arterial catheters in individuals of the bullfrog, Lithobates catesbeianus (Shaw, 1802), to measure their blood pressure and heart rate. Heart rate in experimental subjects increased from a fasting value of 42.5 ± 2.5 to 52.5 ± 1.6 and 57.5 ± 2.1 min⁻¹ at 24 and 33 hours, respectively, after meals that corresponded to 5% of their body weight. Using an infusion of atropine and propranolol, we demonstrated that part of the postprandial tachycardia can be ascribed to a progressive decrease in parasympathetic tone (26 ± 5% in fasting frogs to 6 ± 5% by 33 hours into digestion), whereas the sympathetic tone remained unaltered at approximately 20%. In addition to the withdrawal of vagal tone, digestion was also associated with an increase in the heart rate upon double-blockade (i.e., the combination of atropine and propranolol) from a fasting value of 44.5 ± 0.9 to 50.8 ± 1.9 min⁻¹ at 33 hours. This indicates that the postprandial tachycardia in frogs, as previously demonstrated for snakes, is partially governed by excitation of non adrenergic, non cholinergic (NANC) factors.

Invertebrates, digestion is associated with a rise in aerobic metabolism, a phenomenon commonly referred to as the specific dynamic action (SDA) (Andrade et al. 2005, Secor & Faulkner 2002, Secor et al. 2007). The SDA response of amphibians, as of other vertebrate groups, depends on the size and composition of the ingested meal, and may involve substantial increases in oxygen uptake that can last for several days (Wang et al. 1995, Powell et al. 1999, Busk et al. 2000, Secor & Faulkner 2002, Andersen & Wang 2003, Secor et al. 2007). It is also well-established that, in several species of frogs and toads, the SDA response is attended by a rise in heart rate (fₚₚ) which presumably supports the increased perfusion of the gastrointestinal organs to provide oxygen for digestive functions, to partake in the absorption of nutrients (Dumont 1990, Wang et al. 1995, Andersen & Wang 2003). Similar responses are common in other vertebrates (Wang et al. 2001a).

The tachycardia that occurs during the SDA response is mediated, at least in part, by autonomic nervous regulation, to which a withdrawal of parasympathetic (“vagal”) tone contributes significantly. The increased sympathetic tone is less important for the postprandial tachycardia, which is consistent with the long duration of the metabolic increment. In addition, a number of recent studies, particularly on snakes that exhibit very pronounced postprandial tachycardia, have shown that much of the tachycardia is mediated by increased non-adrenergic-non-cholinergic (NANC) factor on the heart (Wang et al. 2001b, Skovgaard et al. 2009, Enok et al. 2012). This NANC stimulation can be ascertained from a rise in fₚₚ after double autonomic blockade (i.e., the combination of β-adrenoceptor blockade and blockade by a muscarinic antagonist), and is caused either by the neurotransmitters released from the autonomic nerves projecting into the cardiac pacemaker, or from a circulating hormone. The latter possibility is supported by the observation that pharmacological blockade of the autonomic ganglia does not attenuate the fₚₚ response in snakes that are digesting, and plasma infusion from snakes that are digesting causes fₚₚ to increase in fasting snakes (Enok et al. 2012). The amphibian heart, like the heart of other vertebrates, is dually innervated by adrenergic and cholinergic nerves that run in a common vagosympathetic trunk (Wang et al. 1999), but the autonomic regulation of the heart during SDA remains to be clarified.

Here we investigated the regulation of the postprandial rise in the heart rate of an amphibian species, the bullfrog Lithobates catesbeianus (Shaw, 1802), by placing arterial catheters in individuals, to measure their systemic blood pressure.
and to provide a way to administrate antagonist injections. Autonomic tones on the heart were obtained by pharmacological blockade of the autonomic tonus with the β-adrenergic antagonist propranolol and the muscarinic antagonist atropine. The complete blockade of autonomic tonus on the heart during SDA was used to verify the contribution of NANC factors to postprandial tachycardia.

**MATERIAL AND METHODS**

Thirty-four bullfrogs of both sexes and average mass of 206 ± 8 g were purchased from Ranário São Vicente de Paula (Conchal in the state of São Paulo, Brazil) and kept in an outdoor enclosure at the Jacarezário (UNESP Rio Claro, São Paulo, Brazil) under natural photoperiod and temperature regime (January to March – summer). Prior to being fasted for ten days, the frogs were weighted and force-fed bovine liver corresponding to 10% of their body mass to ensure that all animals were at a similar digestive state before we started the measurements.

The bullfrogs were anesthetized by emersion in benzoicaine (1.5 g/L), until their corneal reflex disappeared. They were then wrapped in humid tissue and placed on a surgical table. A 1.5 cm incision between the triceps femoris and gracilis minor gained access to the femoral artery, where a polyethylene catheter (PE50), filled with heparinized physiological saline, could be inserted by occlusive cannulation. The skin was closed with silk suture (Kruuse) and the catheter was secured to the back of the frog. The procedure lasted less than 30 min and the animals were placed under running water until they regained spontaneous ventilation (ANDERSEN & WANG 2002). They were then placed in a transparent box (34x23x10 cm) with clean water under natural photoperiod at 28°C. On the following day, the arterial catheter was connected to a disposable pressure transducer (Model PX600; Baxter Edwards, Ervine, CA), calibrated daily against a static water column, and recorded with a Biopac MP100 data acquisition system (Biopac Systems, Inc., Goleta, CA, USA) at 200 Hz. Heart rate (f_H) was derived with a Biopac MP100 data acquisition system (Biopac Systems, Inc., Goleta, CA, USA) at 200 Hz. Heart rate (f_H) was derived from the pulsating pressure signal, and mean arterial blood pressure (MAP) was calculated using AcqKnowledge 3.9.1 software (Biopac Systems, Inc., Goleta, CA, USA). To avoid disturbing the frogs during recordings, and to ensure that their heart rate and blood pressure were back to lower stable values, all measurements were taken 30 min after the catheter had been connected to the transducer.

In eight frogs, MAP and f_H were recorded during fasting and then at 15, 24, 33, 48, 57 and 72 hours after they were fed bovine liver corresponding to 5% of their body mass. In another series of experiments, autonomic regulation of f_H was revealed in fasting and digesting bullfrogs at 24 and 33 hours past feeding (fasting n = 9, digesting 24 hours n = 9 and digesting 33 hours n = 8) by measuring f_H upon administration of atropine (7.0 mg/kg) followed by a subsequent injection of propranolol (8.8 mg/kg) when f_H had stabilized. At 24 and 33 hours, SDA had provoked a significant increase in f_H. Atropine and propranolol were injected so that a double blockade coincided with 24 and 33 hours of digestion at the respectively groups. These autonomic blockades were verified by the lack of heart rate responses to β-adrenoreceptors and muscarinic antagonists.

Cholinergic and adrenergic tones were calculated on basis of the R-R interval (1/f_H) using the following formulas (AUTIMIRAS et al. 1997):

\[
\text{Cholinergic tone}\% = \frac{RR_{atropine} - RR_{control}}{RR_{double\ block}} \times 100\%
\]

\[
\text{Adrenergic tone}\% = \frac{RR_{atropine} - RR_{double\ block}}{RR_{control}} \times 100\%
\]

where RR_{control} is the RR interval of fasting frogs, RR_{atropine} is the RR interval after infusion of atropine, while RR_{double\ block} is the RR interval after subsequent injection of propranolol.

Significant differences between groups were detected by one-way analysis of variance (ANOVA) and one way repeated measures ANOVA for normal distributed data and by Mann-Whitney and Holm-Sidak tests for non-parametric data. Differences were considered significant when p < 0.05. Data is presented as mean ± SEM.

**RESULTS**

A heart rate peak was achieved at 33 hours into digestion and the prolonged and significant rise in f_H during digestion is shown in Fig. 1 (p < 0.001 for all groups; one-way ANOVA). By contrast, digestion did not influence MAP (Fig. 2). During measurements, frogs remained resting and undisturbed, to guarantee that the change in f_H can be attributed to SDA.

The effects of the autonomic antagonists on f_H are shown in Fig. 3 and Table 1 for both fasting and digesting frogs. Again, there was a significant rise in f_H during digestion, with no change in MAP. Consistent with the removal of the inhibitory parasympathetic tone, atropine caused significant tachycardia, whereas double blockade after injection of propranolol caused a subsequent decline in f_H in accordance with the inhibition of the excitatory role of the sympathetic innervation. The calculated tones based on these changes in f_H are shown in Figs. 4-5 and Table 2, with a significant decline in the cholinergic tone during digestion (p = 0.027 and 0.007 at 24 and 33 hours, respectively; Mann-Whitney test), whereas the adrenergic tone was unaffected by digestion (Fig. 5).

Of particular relevance to the goals of the present study, the f_H of frogs following double autonomic blockade was significantly elevated during digestion (p = 0.001 and 0.007 at 24 and 33 hours, respectively; Holm-Sidak test), indicating the presence of a NANC factor exerting a positive chronotropic effect on f_H (Fig. 3). Thus, the double-blocked f_H of fasting frogs was 44.5 ± 0.9 min⁻¹, and increased to 48.8 ± 0.5 and 50.8 ± 1.9 min⁻¹ at 24 and 33 hours, respectively.
The bullfrogs in the present study exhibited a pronounced and sustained rise in \( f_H \) during digestion. We did not measure the rise in oxygen consumption and thus the duration of SDA, but meals of similar magnitudes have been reported to double aerobic metabolism in anurans with maximal values occurring several days into the postprandial period (WANG et al. 1995, BUSK et al. 2000, SECOR & FAULKNER 2002, SECOR et al. 2007). Given that the rise in \( f_H \) did not match the expected rise in metabolism, it is likely that stroke volume increased and that extraction of oxygen from the venous blood were elevated during digestion. As also reported for pythons, MAP did not change during the SDA response, which indicates a lower peripheral vascular resistance during digestion, as perfusion of the gastrointestinal organs is elevated by vasodilatation (SKOVGAARD et al. 2009). Previous studies on toads revealed variable increases in \( f_H \) during digestion (DUMSDAY 1990, WANG et al. 1995, ANDERSEN & WANG 2003).

Our study shows that the postprandial tachycardia in bullfrogs is mediated by a release in vagal tone and a rise in

### Table 1. Heart rate (bpm) and systemic blood pressure (cm H\( _2 \)O) for control (fasting) and digesting animals, before and after manipulation with atropine and propranolol.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Atropine</th>
<th>Propranolol</th>
<th>Control</th>
<th>Atropine</th>
<th>Propranolol</th>
<th>Control</th>
<th>Atropine</th>
<th>Propranolol</th>
<th>Control</th>
<th>Atropine</th>
<th>Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>42.5 ± 2.5</td>
<td>54.2 ± 2.1</td>
<td>44.5 ± 0.9</td>
<td>52.5 ± 1.6*</td>
<td>58.7 ± 1.6</td>
<td>48.8 ± 0.5***</td>
<td>57.5 ± 2.1*</td>
<td>60.8 ± 2.2**</td>
<td>50.8 ± 1.9***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( P_{sys} )</td>
<td>30.8 ± 2.6</td>
<td>28.6 ± 3.7</td>
<td>29.3 ± 2.6</td>
<td>31.7 ± 2.2</td>
<td>31.6 ± 1.9</td>
<td>31.6 ± 1.3</td>
<td>40.5 ± 3.3</td>
<td>32.9 ± 3.1</td>
<td>33.5 ± 2.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significantly differences from fasting values.

### Table 2. Cholinergic and adrenergic tones on the heart during fasting and digestion. Asterisk shows significantly differences from fasting values.

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>24 hours</th>
<th>33 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholinergic tone (%)</td>
<td>25.9 ± 4.6</td>
<td>12.6 ± 1.2*</td>
<td>6.3 ± 5.1*</td>
</tr>
<tr>
<td>Adrenergic tone (%)</td>
<td>22.2 ± 5.1</td>
<td>20.3 ± 2.6</td>
<td>20.0 ± 3.1</td>
</tr>
</tbody>
</table>

DISCUSSION

The bullfrogs in the present study exhibited a pronounced and sustained rise in \( f_H \) during digestion. We did not measure the rise in oxygen consumption and thus the duration of SDA, but meals of similar magnitudes have been reported to double aerobic metabolism in anurans with maximal values occurring several days into the postprandial period (WANG et al. 1995, BUSK et al. 2000, SECOR & FAULKNER 2002, SECOR et al. 2007). Given that the rise in \( f_H \) did not match the expected rise in metabolism, it is likely that stroke volume increased and that extraction of oxygen from the venous blood were elevated during digestion. As also reported for pythons, MAP did not change during the SDA response, which indicates a lower peripheral vascular resistance during digestion, as perfusion of the gastrointestinal organs is elevated by vasodilatation (SKOVGAARD et al. 2009). Previous studies on toads revealed variable increases in \( f_H \) during digestion (DUMSDAY 1990, WANG et al. 1995, ANDERSEN & WANG 2003).

Our study shows that the postprandial tachycardia in bullfrogs is mediated by a release in vagal tone and a rise in
the double-blocked \( f_{\text{H}} \). The adrenergic tone was relatively low in fasting frogs and did not contribute to the postprandial tachycardia. In a previous study using the snake *Boa constrictor* Linnaeus, 1758, a small reduction in the sympathetic tone during digestion was reported (WANG et al. 2001b), but the reduction in the cholinergic tone seems to be the primary mediator in the vertebrates studied so far (WANG et al. 2001b, SKOVGAARD et al. 2009, IVERSEN et al. 2010). In this study, \( f_{\text{H}} \) increased approximately 14 beats min\(^{-1}\) between fasting and digesting frogs (from 42.5 ± 5.2.1 min\(^{-1}\) at 33 hours) in the series of experiments where the autonomic tones were quantified (Figs. 4-5 and Table 2), and given that double blocked \( f_{\text{H}} \) increased by about 6 min\(^{-1}\) (from 44.5 ± 0.9 to 50.8 ± 1.9 min\(^{-1}\) at 33 hours), the NANC contribution accounts for approximately half of the rise in \( f_{\text{H}} \).

The NANC stimulation of the heart could stem from a release of excitatory neurotransmitters from the autonomic nerves innervating the cardiac pacemaker and/or represent a positive chronotropic effect exerted by circulating signal molecules, such as hormones released from the gastrointestinal organs in response to the presence of food. The latter seems to be the responsible factor in digesting snakes, since the ganglionic blocker hexamethonium does not reduce the NANC (ESOR et al. 2012). Furthermore, infusion of plasma drawn from digesting snakes exerts a clear positive chronotropic effect on \( f_{\text{H}} \) in fasting snakes, even after double autonomic blockade (ESOR et al. 2012). Humans with cardiac transplant, hence devoid of cardiac innervation, also exhibit postprandial tachycardia, pointing to a humoral origin of the postprandial NANC response (WAALER et al. 2002, 2006). If the NANC factor indeed does stem from circulating signal molecules such as regulatory peptides or hormones, released from gastrointestinal organs in concert with the stimulation of digestive processes, this response represents a type of cardiovascular regulation that occurs without the direct influence of the central nervous system. Nevertheless, although the postprandial NANC stimulation seems broadly distributed among vertebrates, there was no evidence for NANC stimulation in the European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758), where the heart rate response to digestion was exclusively mediated by a reduction in the parasympathetic tone on the heart (IVERSEN et al. 2010).

Our present experiments using frogs could not identify the origin of the NANC stimulation of the heart during digestion in these animals. Answering this question may prove extraordinarily difficult because the autonomic neurons of amphibians contain a plethora of NANC factors that could be responsible. One obvious possibility is adenosine triphosphate (ATP), which is released from sympathetic nerves innervating the frogs’ heart and exerts a positive chronotropic effect (MORRIS et al. 1981, BRAMICH et al. 1990). Thus, while we can conclude that the postprandial rise in heart rate in the bullfrog *L. catesbeianus* is mediated by the withdrawal of vagal tone and through the presence of a non-adrenergic, non-cholinergic factor, it is also evident that future studies are needed to elucidate the identity and relative contributions of the relevant hormones and neurotransmitters.

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