Ventilatory compensation of the alkaline tide during digestion in the snake  

*Boa constrictor*

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Accepted 26 January 2004

Summary

The increased metabolic rate during digestion is associated with changes in arterial acid–base parameters that are caused by gastric acid secretion (the ‘alkaline tide’). Net transfer of HCl to the stomach lumen causes an increase in plasma HCO₃⁻ levels, but arterial pH does not change because of a ventilatory compensation that counters the metabolic alkalosis. It seems, therefore, that ventilation is controlled to preserve pH and not PCO₂ during the postprandial period. To investigate this possibility, we determined arterial acid–base parameters and the metabolic response to digestion in the snake *Boa constrictor*, where gastric acid secretion was inhibited pharmacologically by oral administration of omeprazole.

The increase in oxygen consumption of omeprazole-treated snakes after ingestion of 30% of their own body mass was quantitatively similar to the response in untreated snakes, although the peak of the metabolic response occurred later (36 h versus 24 h). Untreated control animals exhibited a large increase in arterial plasma HCO₃⁻ concentration of approximately 12 mmol l⁻¹, but arterial pH only increased by 0.12 pH units because of a simultaneous increase in arterial PCO₂ by about 10 mmHg. Omeprazole virtually abolished the changes in arterial pH and plasma HCO₃⁻ concentration during digestion and there was no increase in arterial PCO₂. The increased arterial PCO₂ during digestion is not caused, therefore, by the increased metabolism during digestion or a lower ventilatory responsiveness to ventilatory stimuli during a presumably relaxed state in digestion. Furthermore, the constant arterial PCO₂, in the absence of an alkaline tide, of omeprazole-treated snakes strongly suggests that pH rather than PCO₂ normally affects chemoreceptor activity and ventilatory drive.

Key words: reptile, snake, *Boa constrictor*, feeding, postprandial period, acid–base balance, gastric acid secretion, alkaline tide, omeprazole, ventilation, ventilatory control.

Introduction

Many ectothermic vertebrates feed at irregular intervals, but are able to ingest meals that are very large relative to their own body mass (e.g. Greene, 1997; Shine et al., 1998). Digestion of these large meals is associated with considerable increments in oxygen uptake that last for several days (Benedict, 1932; Secor and Diamond, 1998; Wang et al., 2001). Digestion is also associated with changes in acid–base parameters, as gastric parietal cells are stimulated to secrete acid secretion into the stomach lumen (e.g. Hersey and Sachs, 1995; Niv and Fraser, 2002). The net transfer of HCl to the stomach leads to a reduction in plasma [Cl⁻] and a concomitant increase in strong ion difference (SID; the mmolar differences between strong cations and anions; Stewart, 1983), causing plasma [HCO₃⁻] concentration to increase (e.g. Wang et al., 2001; Niv and Fraser, 2002). This alkalination of blood and tissue is termed ‘the alkaline tide’ and is, at least for mammals, also accompanied by excretion of alkaline urine (Rune, 1965, 1966; Niv and Fraser, 2002). The alkaline tide is large in reptiles and amphibians (e.g. Coulson et al., 1950; Wang et al., 2001), but all species studied with indwelling catheters (to reduce stress associated with blood sampling) have shown that pHa only increases modestly because PaCO₂ rises during digestion (Wang et al., 1995, 2001; Overgaard et al., 1999; Busk et al., 2000a,b; Andersen and Wang, 2003). The postprandial period is characterised, therefore, by a metabolic alkalosis that is almost fully compensated by an increased PaCO₂, that appears to be caused by hypoventilation, as ventilation does not increase proportionally to CO₂ production (Glass et al., 1979; Hicks et al., 2000; Secor et al., 2000; Wang et al., 2001). Smaller but qualitatively similar respiratory compensations have also been observed in mammals (Higgins, 1914; Erdt, 1915; Van Slyke et al., 1917; Ou and Tenney, 1974).

While it seems plausible that the respiratory compensation of pH during the postprandial period serves a homeostatic function by preventing disturbances of acid–base balance to protect enzyme function and metabolic processes, the underlying regulation of acid–base balance is not well understood. In fact, because digestion is associated with large
metabolic increments it is possible that the rise in \( P_{aCO_2} \) merely reflects an ineffective ventilatory compensation to the increased metabolic rate that fortuitously acts to regulate pHa. As an alternative, it has been suggested that increased \( P_{aCO_2} \) during digestion in humans is caused by the induction of a more relaxed state with low responsiveness to ventilatory stimuli (e.g. Higgins, 1914), as has been observed during sleep.

To study whether pHa or \( P_{CO_2} \) constitute the regulated variable during digestion, we used pharmacological inhibition of gastric acid secretion. We studied the snake *Boa constrictor* because it is able to ingest large meals and exhibits large postprandial increases in metabolism (Secor and Diamond, 2000; Toledo et al., 2003). Gastric acid secretion was inhibited by oral administration of the specific proton-pump inhibitor omeprazole. Omeprazole is a weak base with a pKₐ of about 2000; Toledo et al., 2003). Gastric acid secretion was inhibited by oral administration of the specific proton-pump inhibitor omeprazole. Omeprazole was dissolved in methylcellulose (1.5%) and administrated orally through a soft rubber tube inserted into the stomach through the mouth. We applied a dose of 60 μmol kg⁻¹ (22 mg kg⁻¹, given as 2 ml kg⁻¹ snake) every 48 h for 8 days (i.e. four administrations). A final dose was administered a few hours before feeding.

**Materials and methods**

**Animals**

Experiments were performed on 22 specimens of *Boa constrictor* L. that had been bred and reared in captivity for approximately 2 years at the Jacarezinho at UNESP, Rio Claro (SP, Brazil). During this period, they were fed a mixed diet of rodents and chickens, and experienced temperatures of 22–32°C under a natural light cycle. All animals appeared healthy and had been fasted for 2–3 weeks before the experiments were conducted. The study consisted of two series of experiments performed on separate groups of snakes. For the first experiment (conducted in October, 2001), we measured arterial blood composition prior to and during digestion of six untreated snakes and six snakes treated with omeprazole (1.62±0.21 and 1.86±0.46 kg, respectively). For the second experiment (performed in August 2002), we determined the rate of oxygen uptake prior to and during digestion of five untreated snakes and five snakes treated with omeprazole (0.17±0.02 and 0.56±0.10 kg, respectively).

**Administration of omeprazole**

Eleven snakes were treated with omeprazole to inhibit gastric acid secretion. Omeprazole was dissolved in
(Baytril®; 2–3 mg kg⁻¹) to prevent infection and was allowed to recover for a minimum of 18 h at 30°C before blood samples were taken.

Experimental protocol for blood gas determinations during digestion

Snakes were maintained individually within plastic boxes (60 cm x 30 cm x 15 cm) at 30±1°C (the preferred body temperature of Boa constrictor; McGinnis and Moore, 1969) within a temperature-controlled chamber. To minimise disturbance of the snakes, the catheters were passed through an opening in the top of the box and out of the climatic chamber at least 60 min prior to blood sampling. When blood samples had been collected from fasting snakes, each snake was fed a meal of freshly killed rats, which they struck and constricted before swallowing. The snakes normally ate two rats, and the meals constituted 28±7% (range 18–45%) of body mass. Some of the omeprazole-treated snakes did not eat voluntarily and had to be force-fed.

Measurements of arterial acid–base parameters, PO₂ and haematocrit

All blood samples (0.8–1.0 ml) were sampled anaerobically and analysed within 2 min after being collected. Blood pH was measured with a capillary pH electrode connected to a PHM 73 (Radiometer, Copenhagen, Denmark) maintained at 30°C in a BMS Mk 3 electrode unit (Radiometer). The pH electrode was calibrated several times a day with Radiometer precision buffers (S1500 and S1510). Total CO₂ content of freshly separated plasma (ct[CO₂]ₚl) was measured according to Cameron (1971). Arterial Po₂ (PaCO₂) was calculated using the rearranged Henderson–Hasselbalch equation:

\[ \text{PaCO}_2 = \text{ct}[\text{CO}_2]_p / \left[ \alpha_{\text{CO}_2} \times (1 + 10^{(\text{pH} - \text{pK})}) \right], \]  

(1)

using a CO₂ solubility in the plasma (αCO₂) of 0.0366 mmol l⁻¹ mmHg⁻¹ (Heisler, 1986) and the apparent pK of Python plasma at 30°C (pK = 0.0763 pH+6.7283; Overgaard and Wang, 2002). Plasma bicarbonate concentration ([HCO₃⁻]) was then calculated as:

\[ [\text{HCO}_3^-] = \text{ct}[\text{CO}_2]_p - (\text{PaCO}_2 \times \alpha_{\text{CO}_2}). \]  

(2)

PaCO₂ was measured at 30°C with a Radiometer E5046-0 O₂ electrode maintained and calibrated at 30°C in the BMS Mk3 electrode assembly. The zero reading of the PO₂ electrode was verified daily by flushing the chamber with pure nitrogen and it was calibrated using humidified room air before each measurement. Haematocrit was determined following 3 min centrifugation at 12 000 revs min⁻¹ in capillary tubes.

Data analysis and statistics

Effects of digestion on blood gas composition and oxygen consumption, within each set of experimental treatments, were analysed with a one-way analysis of variance (ANOVA) for repeated measures, followed by a post hoc Student–Newman–Keuls test to identify means that were significantly different. Differences in fasting values for untreated control snakes and snakes treated with omeprazole were compared with a Student’s t-test. A Student’s t-test was also used when comparing maximal metabolic changes during digestion in untreated and omeprazole-treated snakes. Differences were considered to be statistically significant when P<0.05 and all results are presented as means ± 1 S.E.M.

Acid–base regulation during digestion in Boa

Results

The metabolic response to digestion for untreated and omeprazole-treated Boa is shown in Fig. 1. RMR of untreated animals was 1.63±0.32 ml O₂ kg⁻¹ min⁻¹, which was significantly higher than RMR of omeprazole-treated snakes (0.81±0.14 ml O₂ kg⁻¹ min⁻¹). In both groups of snakes, digestion was associated with a three- to fourfold increase in metabolic rate compared to fasting levels. Peak metabolic rate occurred about 20 h later in the omeprazole-treated animals.

Plasma HCO₃⁻ concentration of untreated control animals increased from a fasting level of 13.9±1.0 mmol l⁻¹ to 25.9±0.9 mmol l⁻¹ within 12 h after ingestion and remained elevated for the remainder of the experiment (see blue line in Fig. 2). Arterial pH of fasting untreated control animals was 7.519±0.016, and increased significantly to a maximal value of 7.641±0.014 at 24 h after ingestion. Digestion was also associated with a significant increase in PaCO₂ from a fasting level of 16.3±0.9 to 26.4±1.1 mmHg at 12 h post feeding. Using a Davenport diagram (Fig. 3), we estimate that pH would have increased to approximately 7.75 if PaCO₂ had remained at the fasting level during digestion (see red line in Fig. 3). Conversely, to maintain pH at the fasting level, PaCO₂ would have had to increase to 34 mmHg (see blue line in Fig. 3).

Fig. 2 also includes the arterial acid–base parameters of the omeprazole-treated animals. The fasting acid–base parameters
of this group of snakes differed somewhat from the control group. Thus, the omeprazole-treated animals had a significantly higher pH (7.616±0.020) as well as significantly higher $P_{a\text{CO}_2}$ and plasma [HCO$_3^-$] (19.9±1.6 mmHg and 21.0±1.8 mmol l$^{-1}$, respectively) compared with untreated snakes. Following ingestion, there were no significant changes in pH, and there only very small, and not statistically significant, increases in $P_{a\text{CO}_2}$ and plasma [HCO$_3^-$] (Fig. 2).

There were no changes in arterial $P_{O_2}$ or haematocrit during digestion in any of the two experimental groups (Table 1), but haematocrit values of the untreated control snakes were significantly higher than those of the omeprazole-treated snakes.

**Table 1. Arterial $P_{O_2}$ and haematocrit of the snake Boa constrictor before and after ingestion of a meal in untreated snakes and snakes treated with omeprazole to inhibit gastric acid secretion**

<table>
<thead>
<tr>
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<th>Untreated snakes</th>
<th>Omeprazole-treated snakes</th>
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<tr>
<td>$P_{a\text{O}_2}$ (mmHg)</td>
<td>Haematocrit (mmHg)</td>
<td>$P_{a\text{O}_2}$ (mmHg)</td>
</tr>
<tr>
<td>Fasting</td>
<td>61.1±2.7</td>
<td>26.0±1.5</td>
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<tr>
<td>Postprandial (h)</td>
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<tr>
<td>12</td>
<td>63.5±3.1</td>
<td>23.1±1.8</td>
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<tr>
<td>24</td>
<td>56.1±2.6</td>
<td>22.5±2.1</td>
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<tr>
<td>32</td>
<td>59.7±2.6</td>
<td>21.9±2.3</td>
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<tr>
<td>48</td>
<td>58.9±5.1</td>
<td>25.0±1.5</td>
</tr>
<tr>
<td>72</td>
<td>54.9±5.8</td>
<td>25.0±2.0</td>
</tr>
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$P_{a\text{O}_2}$, arterial $P_{O_2}$.
Values are means ± 1 S.E.M. (N=5–7 in each group).

The metabolic response to digestion

Boa exhibited the well-established rise in oxygen uptake after feeding (e.g. Benedict, 1932; Secor and Diamond, 1995, 2000; Andrade et al., 1997). Oxygen uptake of untreated snakes increased three-to fourfold and reached maximal levels of 4–6 ml O$_2$ kg$^{-1}$ min$^{-1}$. The factorial increase and the peak rates are somewhat lower than most values reported for *Boa constrictor* and *Python molurus* following similar meal sizes
Acid–base regulation during digestion in *Boa constricta* at 30°C (Secor and Diamond, 1995; Overgaard et al., 1999, 2002; Secor et al., 2000; Toledo et al., 2003). The lower factorial scope is, at least partially, due to the relatively high RMR in our study compared to previous measurements on *Boa* (Chappell and Ellis, 1987; Secor and Diamond, 2000; cf. Toledo et al., 2003), but maximal $V_{O_2}$ during digestion was lower than reported by Secor and Diamond (2000) (see also Toledo et al., 2003).

RMR was lower in omeprazole-treated snakes, but because the untreated snakes had a lower mass than the omeprazole-treated snakes, it is unlikely that the difference in RMR is due a direct effect of omeprazole. Omeprazole-treated animals were able to digest the ingested prey and the factorial increase in metabolism was similar to that of untreated animals. However, maximal $V_{O_2}$ occurred somewhat later in omeprazole-treated snakes, which may reflect a lower rate of food degradation within the stomach in the absence of gastric acid secretion. In mammals, the rate and efficiency of digestion is not markedly affected by omeprazole (e.g. Evenopoel, 2001), and it has been suggested that gastric acid secretion is more important for reducing infections transmitted over the gut than for digestion (e.g. Sanford, 1992). However, in snakes and other animals that ingest large and intact prey, it seems likely that gastric acid secretion serves an important role in digestion and by initiating the breakdown of the large meals within the stomach.

Secor (2003) recently estimated that gastric production and secretion of HCl and enzymes account for more than half of the energetic costs of digestion in *Python*. In this case, one would expect that inhibition of gastric acid secretion should significantly reduce SDA of omeprazole-treated snakes. However, since it is possible that gastric acid secretion was restored later into the digestive period, our study may not be able to reveal anything about the costs of gastric function during digestion.

**Effects of digestion on arterial acid–base parameters and oxygen levels**

The changes in arterial acid–base balance during the postprandial period are consistent with previous studies on snakes and other ectothermic air-breathing vertebrates (Wang et al., 1995, 2001; Overgaard et al., 1999; Busk et al., 2000a,b; Overgaard and Wang, 2002; Andersen and Wang, 2003). Plasma HCO$_3^-$ concentration of untreated snakes increased by approximately 13 mmol l$^{-1}$ (Fig. 2). This is larger than the 6 mmol l$^{-1}$ increase that has been observed previously in similarly-sized *Python* (Overgaard and Wang, 2002; see also Overgaard et al., 1999), but similar to that observed in *Alligator*, *Rana* and *Bufo* (Busk et al., 2000a,b; Andersen and Wang, 2003). The alkaline tide of *Boa* lasted considerably longer than in *Python*, where most of the acid–base changes occur within the first 48 h after ingestion (Overgaard et al., 1999). The magnitude of the alkaline tide represents the temporal and quantitative difference in gastric acid secretion and the subsequent base output by the pancreas and the intestine when food is passed from the stomach to the intestine. It is possible that *Boa* and *Python* differ in the speed at which these processes proceed. Indeed, it seems that *Python* does pass a larger portion of meal to the intestine within the initial 24 h of digestion compared to *Boa* (Secor and Diamond, 2000).

In spite of the increased plasma HCO$_3^-$ concentration, pH$_a$ only increased by 0.12 pH units because the elevated $P_{aco_2}$ countered the metabolic alkalosis. A similar pattern has been observed in all studies on amphibians and reptiles where blood samples were obtained through chronic cannulation on minimally disturbed animals (Wang et al., 1995, 2001; Overgaard et al., 1999; Busk et al., 2000a,b; Overgaard and Wang, 2002). A smaller, but qualitatively similar respiratory compensation, has also been observed in cats (Ou and Tenney, 1974) and humans (e.g. Higgins, 1914; Erdt, 1915; Van Slyke et al., 1917). The increased $P_{aco_2}$ seems to be caused by a
relative hypoventilation, where lung ventilation does not increase proportionally to CO\textsubscript{2} production (Glass et al., 1979; Wang et al., 1995; Hicks et al., 2000; Secor et al., 2000). We have previously speculated that the relative hypoventilation implies that amphibians and reptiles control pH\textsubscript{a} and not PaCO\textsubscript{2} during the postprandial period (e.g. Wang et al., 2001).

The relatively low PaO\textsubscript{2} of Boa is common for reptiles and can be explained by admixture of systemic venous blood to the arterial blood within the undivided ventricle (Right-to-Left (R–L) cardiac shunt; e.g. Wang and Hicks, 1996). The sizable R–L cardiac shunt of reptiles means that PaCO\textsubscript{2} and PaO\textsubscript{2} are determined by different parameters, so that PaCO\textsubscript{2} can increase without concomitant decreases of PaO\textsubscript{2}. Thus, because the capacitance of CO\textsubscript{2} in blood is much higher than that of oxygen, PaCO\textsubscript{2} is primarily determined by lung PCO\textsubscript{2}, whereas PaO\textsubscript{2} is primarily determined by the degree of admixture and venous oxygen levels (reviewed by Wang and Hicks, 1996; Wang et al., 2001).

*Effects of inhibiting gastric acid secretion on arterial acid–base status during fast and digestion*

Fasting omeprazole-treated animals had higher pH, PaCO\textsubscript{2}, and plasma HCO\textsubscript{3}– concentration than untreated control snakes. While this may be a direct effect of omeprazole on acid–base balance of fasting animals, it may also be caused by seasonal changes since the two sets of experiments were conducted at different times of the year. In rats, omeprazole treatment over several months does not affect arterial acid–base status (T. Wang, P. Norlen and R. Haakanson, personal observation). Irrespective of the differences in acid–base status of fasting snakes, omeprazole greatly reduced the changes in arterial acid–base parameters seen during digestion. The marked reduction of the alkaline tide is consistent with omeprazole being effective in blocking gastric acid secretion. If gastric acid secretion was completely blocked by omeprazole, it may have been expected that the snakes would display a postprandial decrease in plasma HCO\textsubscript{3}– concentration (i.e. decreased SID) as pancreatic base production is stimulated by the entrance of chyme to the intestine. Part of the increased pancreatic base output during digestion occurs in response to acidification of the small intestine, but in some mammals, the base output increases even when gastric acid secretion is inhibited (Vaziri et al., 1980). As discussed previously, we cannot ascertain whether gastric acid secretion was fully blocked and it is possible that the stable plasma HCO\textsubscript{3}– concentration throughout digestion reflect equimolar gastric acid output and pancreatic base secretion.

Inhibition of gastric acid secretion and the alkaline tide abolished the postprandial increase in PaCO\textsubscript{2} even though metabolic rate increased similarly to untreated control snakes (Fig. 1). Thus, the increased PaCO\textsubscript{2} during digestion is not caused by inefficient ventilatory response to elevated metabolism, or a more relaxed state during the postprandial period (Higgins, 1914). Instead, our data strongly suggest that the ventilatory compensation of the alkaline tide represents a regulated response that serves to maintain pH\textsubscript{a} and not PaCO\textsubscript{2}.

The ventilatory responses to acid–base disturbances in reptiles are complex and involve different receptors (e.g. Milsom, 1995), and all of these may be involved in the respiratory compensation during digestion. It is not well known whether reptiles regulate pH or PCO\textsubscript{2}, but immediately after an acutely imposed acidosis by exercise or infusion of lactic acid in resting lizards, it seems that ventilation is geared towards regulation of PaCO\textsubscript{2} (Mitchell and Gleeson, 1985). However, with chronic alkalosis by systemic infusion of bicarbonate, pH\textsubscript{a} seems to be regulated (Jackson, 1969). Central chemoreceptors exert an important contribution to ventilatory responses to acid–base disturbances in reptiles (Hitzig and Jackson, 1978; Branco and Wood, 1993). In mammals, the central chemoreceptor is not as sensitive to metabolic acid–base disturbances of arterial blood as they are to respiratory disturbances, because the blood–brain barrier, separating blood from the cerebrospinal fluid (CSF), is rather impermeable to ions, but permeable to CO\textsubscript{2} (e.g. Fencl, 1986). It is likely that a similar mechanism operates in reptiles, but the slow time course of the alkaline tide may allow for the metabolic alkalosis to be transmitted from the blood to the CSF. Alternatively, it is possible that pH-sensitive peripheral chemoreceptors contribute to the ventilation compensation. Finally, elevated lung and end-tidal PCO\textsubscript{2} during digestion may stimulate lung and upper airway receptors that may regulate ventilation whenever metabolic rate is increased (e.g. Furilla, 1991; Furilla et al., 1991). Clearly the role of the different receptors needs to be further understood to provide a mechanistic explanation of the regulation of arterial pH during digestion in snakes.

This study was supported by FAPESP and The Danish Research Council. We are extremely grateful to Astra for donating the omeprazole and we are indebted to Prof. Rolf Haakanson for having made several suggestions during the planning of these experiments. Two anonymous reviewers provided helpful comments on an earlier version of this manuscript.

**References**


Acid–base regulation during digestion in Boa


