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Arterial acid–base status during digestion and following vascular infusion of $NaHCO_3$ and HCl in the South American rattlesnake, *Crotalus durissus*

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Abstract

Digestion is associated with gastric secretion that leads to an alkalinisation of the blood, termed the "alkaline tide". Numerous studies on different reptiles and amphibians show that while plasma bicarbonate concentration ($[HCO_3^-]_{pl}$) increases substantially during digestion, arterial pH (pHa) remains virtually unchanged, due to a concurrent rise in arterial PCO_2 ($PaCO_2$) caused by a relative hypoventilation. This has led to the suggestion that postprandial amphibians and reptiles regulate pHa rather than $PaCO_2$.

Here we characterize blood gases in the South American rattlesnake (*Crotalus durissus*) during digestion and following systemic infusions of $NaHCO_3$ and HCl in fasting animals to induce a metabolic alkalosis or acidosis in fasting animals. The magnitude of these acid–base disturbances were similar in magnitude to that mediated by digestion and exercise. Plasma $[HCO_3^-]$ increased from 18.4±1.5 to 23.7±1.0 mmol L^{-1} during digestion and was accompanied by a respiratory compensation where $PaCO_2$ increased from 13.0±0.7 to 19.1±1.4 mm Hg at 24 h. As a result, pHa decreased slightly, but were significantly below fasting levels 36 h into digestion. Infusion of $NaHCO_3$ (7 mmol kg^{-1}) resulted in a 10 mmol L^{-1} increase in plasma $[HCO_3^-]$ within 1 h and was accompanied by a rapid elevation of pHa (from 7.58±0.01 to 7.78±0.02). $PaCO_2$, however, did not change following HCO_3^- infusion, which indicates a lack of respiratory compensation. Following infusion of HCl (4 mmol kg^{-1}), plasma pHa decreased by 0.07 units and $[HCO_3^-]_{pl}$ was reduced by 4.6 mmol L^{-1} within the first 3 h. $PaCO_2$, however, was not affected and there was no evidence for respiratory compensation.

Our data show that digesting rattlesnakes exhibit respiratory compensations to the alkaline tide, whereas artificially induced metabolic acid–base disturbances of same magnitude remain uncompensated. It seems difficult to envision that the central and peripheral chemoreceptors would experience different stimuli during these conditions. One explanation for the different ventilatory responses could be that digestion induces a more relaxed state with low responsiveness to ventilatory stimuli.

Keywords: Arterial acid–base status; Vascular infusion; *Crotalus durissus*

1. Introduction

Digestion causes metabolism to rise, the so-called specific dynamic action (SDA) of food, and induces gastric secretion that leads to an alkalinisation of the blood, the "alkaline tide" (e.g. McCorvie, 1925; Rune, 1965; Wang et al., 2001; Niv and Fraser, 2002; Wood et al., 2005). The SDA response and the alkaline tide are more pronounced in animals, such as reptiles, that ingest large meals relative to their own body mass (recently reviewed by Andrade et al., 2005; Wang et al., 2005).

Numerous studies on different reptiles and amphibians show that plasma bicarbonate concentration ($[HCO_3^-]_{pl}$) may increase by up to 10 mmol L^{-1} during the alkaline tide concurrent with a rise in arterial PCO_2 ($PaCO_2$), so that arterial pH (pHa) remains virtually unchanged from that of fasting animals (Overgaard et al., 1999; Busk et al., 2000a,b; Wang et al., 2001, 2005; Andersen et al., 2003; Andersen and Wang, 2004; Andrade et al., 2004b). The rise in $PaCO_2$ is accomplished through a relative hypoventilation where ventilation does not increase proportionally to the rise in metabolism (Glass et al., 1979; Wang et al., 1995; Hicks et

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al., 2000; Secor et al., 2000). Smaller, but qualitatively similar respiratory compensations also occur in mammals (Higgens, 1914; Erdt, 1915; Van Slyke et al., 1917; Ou and Tenney, 1974; Niv and Fraser, 2002; cf. Johnson et al., 1995).

The respiratory compensation of pH during digestion is likely to represent a homeostatic response that prevents pH changes from affecting enzyme function and metabolic processes. However, the underlying regulation is not well understood and the postprandial rise in $PaCO_2$ may reflect an ineffective ventilatory response to the increased metabolism during digestion or digestion per se could induce a more relaxed state with low responsiveness to ventilatory stimuli (e.g. Higgens, 1914). However, all animals studied exhibit a respiratory compensation to the increased $[HCO_3^-]_{pl}$ during digestion, and $PaCO_2$ does not increase when gastric acid secretion is pharmacologically inhibited, so that the alkaline tide is abolished (Andersen et al., 2003; Andrade et al., 2004b). These observations have lead to the suggestion that postprandial amphibians and reptiles in contrast to mammals regulate pHa rather than $PaCO_2$ (Wang et al., 2005).

Here we characterize blood gases during digestion in the South American rattlesnake (*Crotalus durissus*). This species occur widely across arid environments in tropical, subtropical and temperate South America and exhibit pronounced metabolic responses to digestion (Andrade et al., 1997). In addition, blood gases have previously been characterised in this species (Wang et al., 1998) and *C. durissus* exhibit marked ventilatory responses to hypercapnia (Andrade et al., 2004a). To gain further insight into ventilatory compensation of arterial pH, we investigated whether respiratory compensations to metabolic acid–base disturbances occur in fasting animals. This was achieved by inducing a metabolic alkalosis and acidosis in fasting animals by systemic injections of $NaHCO_3$ and HCl, respectively. The magnitude of these acid–base disturbances were similar to that mediated by digestion ($NaHCO_3$) and exercise (HCl).

2. Materials and methods

2.1. Animals

Thirty-one South American rattlesnakes (*C. durissus*) that had been collected at several localities within the state of São Paulo were obtained from the Butantan Institute (São Paulo, Brazil), and transported to the Jacarezário, UNESP, Rio Claro, SP, Brazil. Here the snakes were kept in separate containers ($20 \times 30 \times 25$ cm) and maintained at a 12 h/12 h L/D cycle at a temperature of 30 °C (±3 °C). The animals had free access to water and were fed rodents on a weekly basis. At the time of experimentation, the snakes weighed between 280 and 685 g (390±100 g) and all appeared to be in good health. Food was withheld for 2–3 weeks before commencing the experiments.

2.2. Surgical procedure

All snakes were instrumented with an arterial catheter for blood sampling and infusions. To place the catheter, the snakes were anaesthetised by inhalation of CO_2 (Wang et al., 1993) until they ceased to exhibit reflexes when pinched. A ventrolateral incision was made about 3 cm cranial to the heart, so the vertebral artery could be occlusively cannulated with PE60 catheter containing heparinized saline. The tip of the catheter was pushed towards the right aortic arch and the catheter was exteriorised through the back of the snake and secured with two or three sutures. Then, the incision was closed and the snake allowed to recover for at least 24 h. The surgery normally lasted 15–20 min, and most animals spontaneously resumed ventilation immediately after termination of surgery.

2.3. Experimental protocols

Arterial blood gases, haematological parameters and plasma concentrations of chloride ($[Cl^-]_{pl}$), potassium ($[K^+]_{pl}$) and sodium ($[Na^+]_{pl}$) concentrations were measured in four experimental groups: digesting snakes ($N=5$); HCO_3^- infused ($N=9$); H^+ infused ($N=6$); and a control group that was injected with saline ($N=3$).

Blood samples were taken prior to feeding and at 12, 24, 36 and 48 h after the snake had voluntarily ingested a rat equal to 15±2% of body mass. HCO_3Na infused animals were sampled before and at 1, 3, 6, 12, 24, and 48 h after being infused with a dose of 7 mmol $NaHCO_3$ kg^{-1}. Acid infused snakes received a dose of 4 mmol HCl kg^{-1} over three infusions of 1.33 mmol kg^{-1} in each. Each infusion was performed over 10 min with 20 min between infusions. To minimize the acute disturbance during HCO_3^- and H^+ infusions, 3 mL of blood was withdrawn into the syringe, and mixed with the solution prior to injection. To assess the possible effects of blood sampling, blood was withdrawn three times at 24 h intervals in fasting (control) snakes. All animals were kept in individual plastic boxes inside a climatic chamber at 30 °C (±1 °C) during experimentation.

2.4. Measurements of blood gases and plasma ions

Arterial blood samples were drawn anaerobically and analysed immediately after collection, except for plasma ion samples which were frozen for subsequent measurement. Arterial PO_2 and pH were measured using Radiometer (Copenhagen, Denmark) electrodes mounted in a BMS Mk3 unit. Electrodes were kept at 30 °C by a custom-made adaptation of the BMS Mk3 unit and electrodes were calibrated before each sample analysis. Outputs from the electrodes were displayed on a Radiometer PHM 73. Haematocrit was determined as the fractional red cell volume after centrifugation (12,000 rpm for three min) and monomeric haemoglobin concentration, [Hb], was measured after conversion to cyanmethaemoglobin, applying a millimolar extinction coefficient of 10.99 and measured at 540 nm (Zijlstra et al., 1983). Arterial $[O_2]$ was measured as described by Tucker (1967), with the correction pointed out by Bridges et al. (1979). The Tucker chamber was thermostated at 40 °C. Haemoglobin bound oxygen (HbO_2) was calculated as $[O_2]-(\alpha_{O_2} * Pa_{O_2})$, where α_{O_2} is the blood oxygen solubility (Christoforides and Hedley-Whyte, 1969), and haemoglobin oxygen saturation was subsequently calculated as: $HbO_2sat = HbO_2/[Hb]$, under the assumption that all Hb was functional.

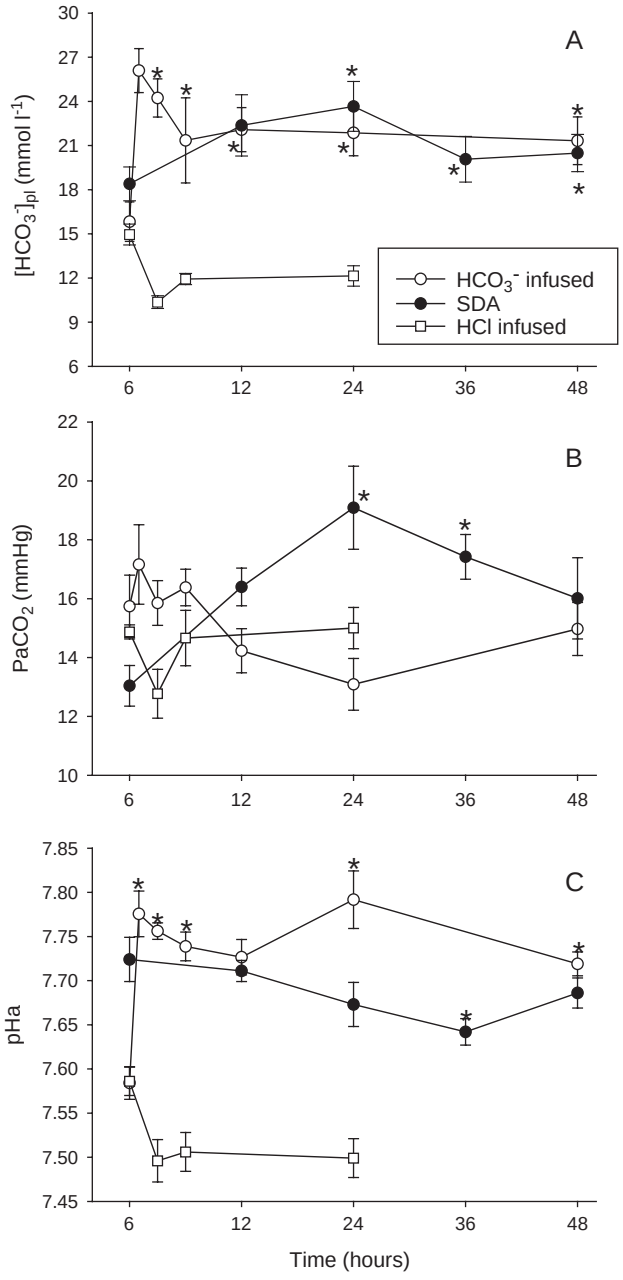

Fig. 1. Arterial acid–base variables in South American rattlesnakes (*Crotalus durissus*) in four experimental groups: digesting snakes ($N=5$); HCO_3^- infused ($N=9$); HCl infused ($N=6$). Data at 0 h show fasting levels, values before HCO_3Na (7 mmol kg^{-1}) or HCl (4 mmol kg^{-1} of HCl dispersed over three infusions of 1.33 mmol kg^{-1}) infusion. (A) Arterial pH, (B) plasma carbon dioxide tension (PCO_2), (C) plasma bicarbonate concentration ($[HCO_3^-]_{pl}$). Data are presented as means ± S.E.M. and values that are significantly different from 0 h levels are marked with an asterisk.

Plasma carbon dioxide concentration ($[CO_2]$) was measured according to Cameron (1971) at 40 °C. $PaCO_2$ was calculated from pH and $[CO_2]$ of the plasma using the Henderson–Hasselbalch equation, with the plasma solubility of CO_2 (α_{CO2}) of 0.0366 mmol L^{-1} (Heisler, 1984). The apparent pK′at 30 °C was taken from Overgaard and Wang (2002). Plasma chloride was measured by colorimetric titration (Radiometer CMT 10), while sodium and potassium was measured by flame photometry (FLM 3 Flame Photometer, Radiometer). Osmolality was determined by freezing point depression (Knauer semimicro osmometer; Berlin, Germany).

2.5. Statistical analysis and data presentation

A one-way ANOVA for repeated measurements was employed to test for significant differences in the measured

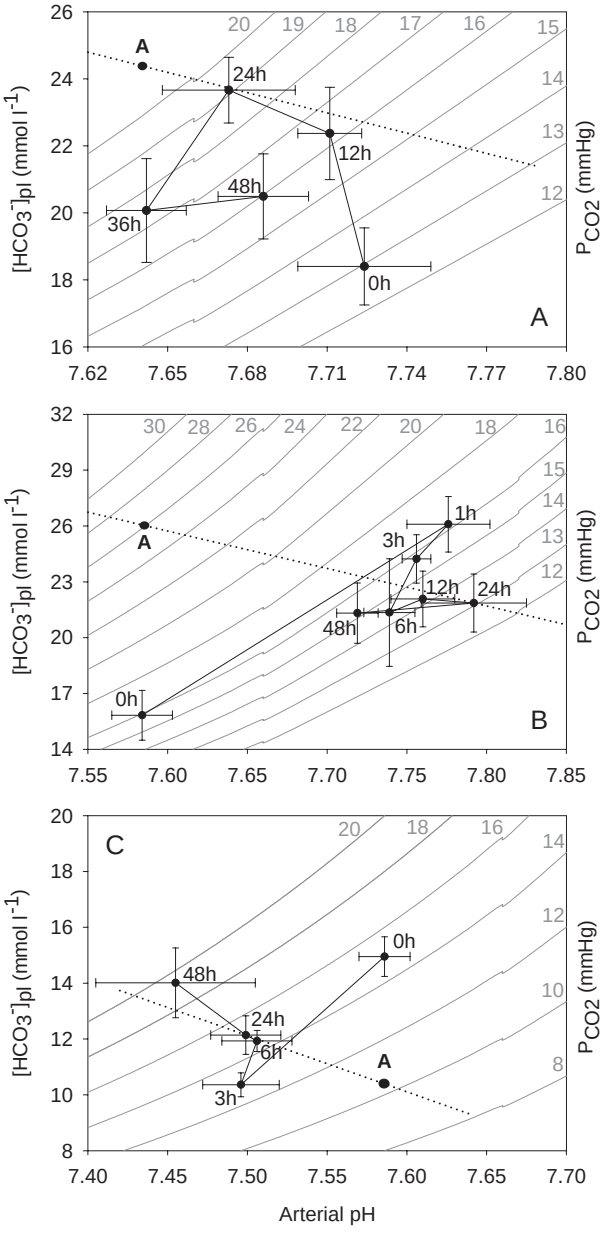

Fig. 2. Davenport diagram, showing plasma bicarbonate concentration ($[HCO_3^-]$) and arterial pH in South American rattlesnakes (*Crotalus durissus*). (A) Before and during digestion. (B) pH before and after infusion of bicarbonate (7 mmol kg^{-1}) ($N=9$). (C) Before and after infusion of HCl (4 mmol kg^{-1} of HCl dispersed over three infusions of 1.33 mmol kg^{-1}) ($N=7$). The Davenport diagram includes carbon dioxide isoclines for the partial CO_2 tension/pressure in arterial blood ($PaCO_2$, curved lines) and an in vitro buffer-line (dotted lines, $\beta_{noncarb}=-20.16$) determined by Overgaard and Wang (2002). Point A in A, B, and C illustrates the $PaCO_2$ it would require to fully compensate pH during alkaline tide, after HCO_3Na and HCl infusion. Data are presented as means ± S.E.M.

Table 1
Arterial oxygen levels and haematological variables in rattlesnakes prior to and during digestion of a meal equal to 15% of their body mass ($N=5$)

	Time (h)				
	0	12	24	36	48
PaO_2 (mm Hg)	77.6±3.3	57.1±1.9*	58.9±2.0*	57.2±1.8*	55.0±2.8*
HbO_2sat	0.95±0.02	1.03±0.05	0.97±0.05	0.98±0.05	0.88±0.01
Haematocrit	0.18±0.01	0.18±0.00	0.16±0.01	0.16±0.01	0.15±0.01*
$[Hb_4]$ (mmol L^{-1})	0.94±0.04	0.77±0.02*	0.65±0.04*	0.72±0.03*	0.71±0.04*
Hb (mmol L^{-1})	3.76±0.17	3.09±0.09*	2.60±0.17*	2.88±0.11*	2.86±0.16*
MCHC (mmol L^{-1})	5.16±0.11	4.37±0.17*	3.99±0.18*	4.41±0.15*	4.73±0.14

Values that are significantly different from 0 h levels are marked with an asterisk.

parameters before and at different moments after feeding. A Bonferroni post-hoc test was used to identify mean values that differed from the control condition. Differences were considered statistically significant at the level of $P \leq 0.05$, and all data are presented as mean±S.E.M.

3. Results

3.1. Effects of digestion

Plasma bicarbonate concentration increased from a fasting level of 18.4±1.5 mmol L^{-1} within 12 h after ingestion, and reached the maximal level of 23.7±1.0 mmol L^{-1} by 24 h, and then declined towards fasting level (Fig. 1A). This rise in $[HCO_3^-]_{pl}$ was accompanied by a rise in $PaCO_2$, which increased from a fasting value of 13.0±0.7 to 19.1±1.4 mm Hg at 24 h (Fig. 1B). As a result, pHa decreased slightly and was significantly below fasting levels 36 h into digestion (Fig. 1C). When depicted in a Davenport diagram (Fig. 2A), it is evident that the metabolic alkalosis was slightly overcompensated by the respiratory acidosis, i.e. pHa would have increased to 7.79 if there had been no respiratory compensation, and plasma $[HCO_3^-]$ would have been 21.8 mmol L^{-1} (Fig. 2A). Thus, the maximal change in ion difference, reflected maximal change in plasma $[HCO_3^-]$ at unchanged $PaCO_2$, can be estimated to be approximately 4 mmol L^{-1}.

Arterial PO_2 decreased significantly within 12 h after ingestion and remained low throughout the experiment (Table 1). Both haematocrit and $[HbO_2]_a$ decreased significantly within 24 h of digestion (Table 1). Plasma $[Na^+]$ increased significantly after 24 h of digestion and persisted increased for the next 24 h. On the other hand, feeding did not cause any significant changes in plasma osmolality, $[K^+]_{pl}$ or $[Cl^-]_{pl}$ (Fig. 3).

3.2. Effects of HCO_3^- infusion

The vascular infusion of $NaHCO_3$ in fasting rattlesnakes caused plasma $[HCO_3^-]$ to increase by approximately 10 mmol L^{-1} within 1 h and was accompanied by a rapid elevation of pHa from 7.58±0.01 to 7.78±0.02 (Fig. 1A). $PaCO_2$, however, did not change following HCO_3^- infusion, which indicates a

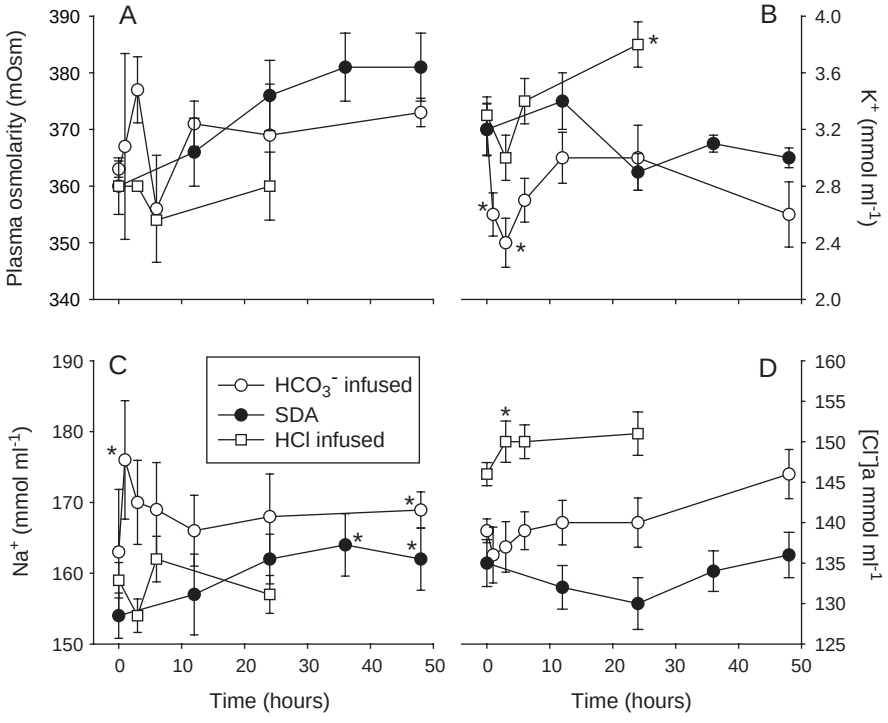

Fig. 3. Plasma ions and osmolality in South American rattlesnakes (*Crotalus durissus*) in four experimental groups: digesting snakes ($N=5$); HCO_3Na infused ($N=9$); HCl infused ($N=6$); and a control group that was injected with saline ($N=3$). Data at 0 h show fasting levels, values before HCO_3Na (7mmol kg^{-1}) or HCl (4 mmol kg^{-1} of HCl dispersed over three infusions of 1.33 mmol kg^{-1}) infusion. (A) Plasma osmolality (mosM); (B) plasma K^+ concentration; (C) plasma Na^+ concentration; (D) plasma Cl^- concentration. The circles at 0 h show levels before feeding, infusion of HCO_3^- and infusion of HCl. Data are presented as means±S.E.M. and values that are significantly different from fasting levels are marked with an asterisk.

Table 2
Blood gases and plasma ion composition in rattlesnakes receiving a sham infusion of saline to determine the effects of blood sampling ($N=3$)

	Time (h)		
	0	24	48
pH	7.647±0.003	7.633±0.009	7.630±0.010
PCO_2 (mm Hg)	17.22±1.49	18.78±1.63	18.70±1.61
HCO_3^- (mmol L^{-1})	20.04±1.86	21.06±1.67	20.86±1.97
$[K^+]$ (mmol L^{-1})	3.1±0.04	3.0±0.03	3.3±0.25
$[Na^+]$ (mmol L^{-1})	155±3.00	160±3.00	156±0.73
$[Cl^-]$ (mmol L^{-1})	141±0.70	142±2.00	145±5.00
Osm (mosM)	380±10.00	370±10.00	365±5.00
PaO_2 (mm Hg)	67.1±7.8	67.6±2.9	73.2±4.0
HbO_2sat	0.73±0.13	0.78±0.04	0.81±0.10
Haematocrit	0.18±0.01	0.15±0.01	0.14±0.01
[Hb4] (mmol L^{-1})	0.85±0.09	0.85±0.11	0.64±0.06

lack of respiratory compensation. To fully compensate pHa after HCO_3^- infusion would require that $PaCO_2$ should have reached 29 mm Hg (point A in Fig. 2B).

Bicarbonate infusion had no effect on osmolality and $[Cl^-]_{pl}$ but caused $[K^+]_{pl}$ to decrease significantly during the first 3 h after the infusion. The concentration of sodium in plasma was significantly increased 1 h after infusion (Fig. 3C).

3.3. Effects of H^+ infusion

Infusion of HCl significantly reduced plasma pHa by 0.07 units and $[HCO_3^-]_{pl}$ by ~4.6 mmol mL^{-1} within the first 3 h after infusion. The HCl infusion had no effect on $PaCO_2$ (Figs. 1B and 2C), hence, no respiratory compensation was observed (Figs. 1 and 2).

Plasma chloride concentration increased significantly by 4.1 mmol L^{-1} within 3 h after infusion and remained elevated thereafter (Fig. 3A). Plasma $[Na^+]$ and $[K^+]$ concentrations did not change significantly immediately after injection, but 24 h after infusion, $[K^+]_{pl}$ was significantly elevated (Fig. 3B).

3.4. Effects of infusion and handling: control group

There were no changes in arterial acid–base parameters, plasma ion concentrations or haematological variables in the three snakes where blood samples were taken 24 and 48 h after cannulation (Table 2). The overall values of these three animals were similar to those studied during feeding and after metabolic acid–base disturbances.

4. Discussion

4.1. Acid–base parameters during digestion and the effects of inducing metabolic acid–base disturbances

Arterial acid–base status of digesting rattlesnakes follows a pattern that is similar to that of other snakes as well as alligators, frogs and toads (Overgaard et al., 1999; Busk et al., 2000a,b; Wang et al., 2001, 2005; Andersen et al., 2003; Andersen and Wang, 2004; Andrade et al., 2004b). In all cases, plasma $[HCO_3^-]$ increases shortly after ingestion and reaches a maximal value approximately 24 h into the postprandial period. In mammals, which generally eat relatively small meals, plasma $[HCO_3^-]$ rarely increases more than a few mmol L^{-1} (Rune, 1965; Rune and Lassen, 1968; Niv and Fraser, 2002), but plasma $[HCO_3^-]$ increases 5–15 mmol L^{-1} in amphibians and reptiles (Wang et al., 2005). *Crotalus*, therefore, seems to have a relatively small alkaline tide compared to other reptiles. The larger alkaline tide of reptiles compared to mammals probably relates to the larger meals that presumably requires large gastric acid secretion. Secondly, in mammals, food enters the intestine earlier than in reptiles (Secor and Diamond, 1995; Rune, 1965) and the feeding pattern is more continuous. Thus, there is more of a temporal overlap between gastric acid and pancreatic base secretions, which dampens the alkaline tide. Finally, in mammals, the kidneys effectively excrete HCO_3^- through the urine (e.g. Vaziri et al., 1980), whereas the reptilian kidney does not seem effective in countering acid–base disturbances (Jackson, 1986; Silver and Jackson, 1985; Glass and Heisler, 1986).

As in all other reptiles and amphibians studied to date, the metabolic alkalosis in *Crotalus* was countered by a respiratory acidosis where $PaCO_2$ increased by 6.5 mm Hg (13.0±0.7 to 19.1±1.4 mm Hg) (Fig. 2A). In *Crotalus*, there was a small respiratory over-compensation resulting in a small but significant decline of pHa, whereas most other reptiles respond to digestion with a small rise in pHa (Busk et al., 2000b; Andrade et al., 2004b; Overgaard et al., 1999; Overgaard and Wang, 2002). The measured pHa of fasting *Crotalus* in the feeding study was, however, higher while $PaCO_2$ was lower than control values in the other experimental series (Fig. 1 and Table 2). We observed that the fasting individuals of *Crotalus* were noticeably more alert than during digestion, which may have caused hyperventilation during blood sampling. In this case, $PaCO_2$ would decrease, while pHa would increase compared to undisturbed levels. As the animals were less alert during digestion and hypoventilated to compensate for the alkaline tide, it would appear that they overcompensated, in part because the fasting pHa was elevated. Thus, it is quite possible that *Crotalus* does not differ from other reptiles.

When gastric acid secretion is inhibited by the specific proton-pump inhibitor omeprazole, there is no alkaline tide in toads and snakes and these animals do not exhibit a postprandial rise in $PaCO_2$ (Andersen et al., 2003; Andrade et al., 2004a). Because metabolism does increase after treatment with omeprazole, the rise in $PaCO_2$ that normally occurs in postprandial reptiles can not be attributed to an ineffective ventilatory response (Andrade et al., 2004a). Rather, it seems that pHa is regulated towards maintaining fasting level. As in mammals, the central chemoreceptors of reptiles are bathed in cerebrospinal fluid (CSF; e.g. Hitzig and Jackson, 1978) and the blood–brain barrier, which separates the blood and CSF, is normally considered rather impermeable for ions (Fencl et al., 1966). A postprandial regulation of pHa rather than $PaCO_2$ requires, therefore, that the blood–brain barrier is somewhat permeable to ions for the metabolic acid–base disturbances to be transmitted from the blood to the CSF. Alternatively, the peripheral chemoreceptors, which in addition

to oxygen are sensitive to pH and PCO_2, could exert a dominating control of ventilation during the postprandial period, but the central chemoreceptors are considered the dominant regulator of ventilation in reptiles (e.g. Branco and Wood, 1993).

If pHa is the regulated variable, the vascular infusion of injection of HCO_3^- that resulted in plasma [HCO_3^-] similar to those observed during digestion should have been attended by a respiratory compensation. Similarly, infusion of HCl causing an increase in blood [H^+] of same magnitude as during severe exercise (e.g. Ruben, 1976) should have elicited a decline in $PaCO_2$. This was, however, not the case in *Crotalus*. $PaCO_2$ actually tended to decrease after infusion of bicarbonate ($p=0.086$) and pHa remained almost 0.2 pH units above the control value for 48 h (Fig. 1). In the case of HCl infusion, the immediate reduction in pHa after infusion was followed by an initial decline of $PaCO_2$, but returned to control levels within 6 h, and there was no respiratory compensation hereafter. The observation that the metabolic acid–base remained uncompensated for 24–48 h is consistent with previous studies on reptiles and indicates that the kidney of *Crotalus* is unimportant in acid–base regulation.

The lack of respiratory compensation to the metabolic acid–base disturbances differs from several previous studies on reptiles and amphibians. In toads, HCO_3^- infusion led to an increase in $PaCO_2$ that re-established pHa at the control level within 24 h (Andersen et al., 2003). Similarly, daily gastric infusions of either HCl or HCO_3^- in turtles resulted in a decrease or increase of $PaCO_2$, respectively (Jackson, 1969). Lizards compensate in response to slow, but not fast, infusions of lactic acid (Mitchell and Gleeson, 1985). Sturgeons, humans and dogs also show respiratory compensation to HCl infusion (Warren et al., 2003; Wasserman et al., 1975; Kaehny and Jackson, 1979; Bainton, 1978). In the mammalian studies, the acid load was administered over days, which may have allowed for diffusion across the blood–brain barrier, so that the central chemoreceptors could have been stimulated. The relative short duration of the acidosis might, therefore, explain the lack of compensation in *Crotalus*.

While the data in our study seems to differ from previous studies that have induced metabolic acid–base disturbances, it is clear that digesting rattlesnakes exhibit respiratory compensations to the alkaline tide, whereas artificially induced metabolic acid–base disturbances remain uncompensated. As the magnitude and the time-course of plasma [HCO_3^-] were similar during digestion and following HCO_3^- infusion, it seems difficult to envisage that the central and peripheral chemoreceptors would experience different stimuli during these conditions. The explanation for the different ventilatory responses must, therefore, reside elsewhere. It has been suggested that digestion induces a more relaxed state with low responsiveness to ventilatory stimuli, which could explain $PaCO_2$ to rise (Higgens, 1914; Rune and Lassen, 1968). Thus, it is possible that digestion in *Crotalus* is associated with such a change in state. In addition, while the lack of compensation could reflect an ineffective ventilatory response to the increased metabolism during digestion. This possibility, however, is not very likely since rattlesnakes can maintain low $PaCO_2$ during exercise where metabolism is elevated several times (T. Wang and D. Andrade, unpublished).

The rattlesnakes did not tolerate the HCl infusions very well and we were only able to obtain reliable measurements on the animals within the first 24 h. When infused with higher dosages of HCl, the animals died within 30 min. In two snakes we observed a rise in $PaCO_2$ and a decrease in pHa at 48 h, but these responses may be attributed to the, at this time, aggravated physical state with an insufficient breathing. It is not clear why HCl infusions were not tolerated; the acid load was not higher than seen in exercising *Crotalus*, where lactate concentrations are up 7 mmol L^{-1} (Ruben, 1976 Kemper et al., 2001). In dogs, however, HCl infusion causes haemorrhage in the lungs, whilst similar infusion of lactate did not (Bainton, 1978). Vascular infusions of HCl have, however, been performed in green sturgeon (0.45 mmol kg^{-1}), humans and dogs (4–7 mmol kg^{-1}) without lethal or damaging effects (e.g. Warren et al., 2003; Wasserman et al., 1975; Kaehny and Jackson, 1979). We did not examine the dead snakes, but it is possible that pulmonary haemorrhage caused by HCl could explain the rise in $PaCO_2$ at 48 h.

4.2. Plasma ions and osmolality during digestion

Plasma osmolarity tended to increase during digestion in the rattlesnakes, but the change was not statistically significant (Fig. 3A). In *Bufo* and some studies on *Python*, there was no change in osmolality during digestion (Andersen et al., 2003; Secor and Diamond, 1995). However, in other studies on *Python*, *Alligator* and *Rana* plasma osmolality increased during digestion (Overgaard et al., 1999; Coulson et al., 1950; Coulson, 1985; Busk et al., 2000a). Also, as reported in other studies, the plasma had a visually milky appearance during digestion, which probably stems from fatty acids from the food. Plasma [K^+] has a tendency to decrease when the alkaline tide is at its maximum (Fig. 3B). This has also been found in *Python* (Overgaard and Wang, 2002) and may be caused by K^+ excretion by the kidneys in exchange of H^+ to compensate the alkalosis. Plasma [K^+] also decreased significantly immediately after HCO_3^- infusion where $[HCO_3^-]_{pl}$ was maximal (Fig. 3B). Oppositely, $[K^+]_{pl}$ increased after HCl (Fig. 3B), which may reflect exchange of H^+ in the kidneys. Moreover, the snakes were very irritable and easily began to rattle after the HCl infusion, so part of the rise in $[K^+]_{pl}$ could stem from K^+ loss from muscles participating in rattling.

An equimolar change in plasma HCO_3^- and Cl^- levels is expected from the stoichiometry of gastric acid secretion by the H^+–K^+-ATPase in parietal cells located in the stomach lumen, where K^+ diffuses back in exchange for Cl^-, so that the parietal cells effectively secrete HCl (e.g. Hersey and Sachs, 1995). As a result the increase in $[HCO_3^-]_{pl}$ after feeding is often mirrored by a similar decrease in $[Cl^-]_{pl}$ (Busk et al., 2000a,b; Wang et al., 2005). Nonetheless, this was not apparent in *Crotalus*, and although we do not have an explanation for the lack of equimolar changes in plasma HCO_3^- and Cl^-, the same was observed in *Bufo* (Andersen and Wang, 2004).

4.3. Arterial oxygen levels and haematological changes during digestion and acid–base infusions

Arterial PO_2 decreased during digestion in *Crotalus* (Table 1). This response is different from other reptiles and amphibians where PaO_2 normally remains unchanged or increases slightly above fasting levels (Andrade et al., 2004a; Overgaard et al., 1999; Andersen and Wang, 2004; Wang et al., 1995; Busk et al., 2000a,b). Because the fasting and alert snakes may have hyperventilated during blood sampling, it is possible that PaO_2 of fasting snakes is overestimated compared to truly resting animals, and Wang et al. (1998) did indeed report slightly lower values for PaO_2 in the same species. Furthermore, it is possible that *Crotalus* does not exhibit the decrease in right-to-left (R–L) cardiac shunt during digestion that has been inferred for other species of reptiles (Wang et al., 2001).

In some animals, increased oxygen demands are also meet by a rise in haematocrit, which was not observed in this study. The increase is very pronounced in *Rana* where haematocrit increases with 60% following feeding (Busk et al., 2000a), but much less pronounced in the toad *Bufo marinus* and dogs (Andersen and Wang, 2004; Kurata et al., 1993). As in rattlesnakes, haematocrit does not change in boas, pythons and alligators (Andrade et al., 2004b; Overgaard and Wang, 2002; Busk et al., 2000b).

5. Conclusion

The arterial acid–base composition during digestion of rattlesnakes resembles that of other reptiles and amphibians, but magnitude of the alkaline tide is relatively small. There was no compensation after systemic infusion of acid (HCl) or base ($NaHCO_3^-$), which is in contrast with previous studies on reptiles and amphibians. One explanation could be that rattlesnakes diminish their ventilatory responsiveness during digestion, which allows for $PaCO_2$ to increase.

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