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Effects of inhibition gastric acid secretion on arterial acid–base status during digestion in the toad *Bufo marinus*

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Abstract

Digestion affects acid–base status, because the net transfer of HCl from the blood to the stomach lumen leads to an increase in HCO_3^- levels in both extra- and intracellular compartments. The increase in plasma [HCO_3^-], the alkaline tide, is particularly pronounced in amphibians and reptiles, but is not associated with an increased arterial pH, because of a concomitant rise in arterial P_{CO_2} caused by a relative hypoventilation. In this study, we investigate whether the postprandial increase in P_{aCO_2} of the toad *Bufo marinus* represents a compensatory response to the increased plasma [HCO_3^-] or a state-dependent change in the control of pulmonary ventilation. To this end, we successfully prevented the alkaline tide, by inhibiting gastric acid secretion with omeprazole, and compared the response to that of untreated toads determined in our laboratory during the same period. In addition, we used vascular infusions of bicarbonate to mimic the alkaline tide in fasting animals. Omeprazole did not affect blood gases, acid–base and haematological parameters in fasting toads, but abolished the postprandial increase in plasma [HCO_3^-] and the rise in arterial P_{CO_2} that normally peaks 48 h into the digestive period. Vascular infusion of HCO_3^-, that mimicked the postprandial rise in plasma [HCO_3^-], led to a progressive respiratory compensation of arterial pH through increased arterial P_{CO_2}. Thus, irrespective of whether the metabolic alkalosis is caused by gastric acid secretion in response to a meal or experimental infusion of bicarbonate, arterial pH is being maintained by an increased arterial P_{CO_2}. It seems, therefore, that the elevated P_{CO_2}, occuring during the postprandial period, constitutes of a regulated response to maintain pH rather than a state-dependent change in ventilatory control.

Keywords: Toad; *B. marinus*; Digestion; Acid–base status; Alkaline tide; Gastric acid secretion; Omeprazole; Ventilatory control

1. Introduction

Many ectothermic vertebrates eat large meals at infrequent intervals and the ensuing digestion is associated with physiological and metabolic changes that can persist for many days (Wang et al., 2001). Digestion causes a rise in metabolic rate, the 'specific dynamic action of food' (SDA), and affects acid–base status (Wang et al., 2001; Busk et al., 2000a,b). Thus, ingestion and the presence of food in the stomach stimulate a net HCl secretion from the blood to the stomach lumen that leads to an increase in HCO_3^- levels in extra- and intracellular compartments (see Wang et al., 2001; Niv and Fraser, 2002) The increase in plasma [HCO_3^-], the so-called 'alkaline tide', is

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pronounced in amphibians and reptiles, but the rise in arterial pH (pHa) is greatly dampened by a concomitant elevation in arterial P_{CO_2} (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 a respiratory acidosis apparently caused by a relative hypoventilation (Glass et al., 1979; Hicks et al., 2000; Secor et al., 2000; Wang et al., 2001). Smaller, but qualitatively similar respiratory compensations of the alkaline tide have been observed in mammals (Higgins, 1914; Erdt, 1915; Van Slyke et al., 1917; Ou and Tenney, 1974). These studies indicate that the regulation of ventilation during digestion is geared at maintaining pH rather than P_{CO_2}. However, because digestion is associated with large metabolic increments, it is possible that the rise in arterial P_{CO_2} (P_{aCO_2}) simply reflects an ineffective ventilatory compensation to the increased metabolic rate, leading to an un-regulated maintenance of pH. Alternatively, it is possible that the increased P_{aCO_2} during digestion is caused by induction of a more relaxed state with low responsiveness to ventilatory stimuli during the postprandial period, (e.g. Higgins, 1914).

To study whether toads regulate pHa or P_{aCO_2} during digestion, we measured acid–base parameters of animals, where gastric acid secretion was inhibited by the specific proton-pump inhibitor omeprazole. Omeprazole has been previously shown to uncouple H^+ and Cl^- secretion in the gastric mucosa in the frog *Rana catesbeiana* (Starlinger et al., 1986). The present study was performed on the marine toad (*Bufo marinus*), which has been extensively studied with regards to its acid–base regulation and from which we have data of the responses to digestion (Wang et al., 1995; Andersen and Wang, 2003).

2. Materials and methods

2.1. Animals and surgery

Toads, *Bufo marinus* (Linnaeus, 1758) of undetermined sex and body masses between 230–522 g (355 ± 24 g, mean ± 1 S.E.M.) were obtained from Lemberger (Oshkosh, WI, USA) and kept at the University of Aarhus for several months. The toads were kept at 23–28 °C in large containers with access to running water and dry areas and fed mealworms daily. Toads were anaesthetised by immersion into a 1.0 g l^{-1} benzocaine solution (ethyl *p*-amino benzoate Sigma® E 1501), and surgery started when the corneal reflex disappeared. The right femoral artery was occlusively cannulated through an incision in the leg, and the catheter was secured to the back of the animal by three or four sutures. The surgery normally lasted less than 30 min and all toads regained normal righting reflexes within 30 min after being placed under running tap water. All toads were treated with enrofloxacin (Baytril; 2 mg kg^{-1}, i.p.) to prevent infections. When the toads had regained normal reflexes, each individual animal was transferred to an experimental chamber ($40 \times 30 \times 20$ cm) containing wet paper towels and a dry area. These containers were maintained within a climatic chamber at a constant temperature of 25 °C, the preferred body temperature of toads (Wood and Malvin, 1991). Toads were visually and audibly shielded from disturbances.

2.2. Experimental protocols

2.2.1. Effects of omeprazole on blood gas composition during digestion

To inhibit gastric acid secretion, omeprazole was given orally to six fasting toads prior to the experiments. Omeprazole was dissolved in methylcellulose (1.5%) and administrated through a soft rubber tubing inserted into the stomach through the mouth. A dose of 0.06 mg kg^{-1} (2 ml of 28 mg kg^{-1} pr kg toad) omeprazole was applied daily over 4 days before cannulation, and a final dose was administered a few hours before feeding.

A blood sample from fasting animals was withdrawn 24 h after surgery, as we have previously shown that arterial blood gases and acid–base parameters of *B. marinus* have stabilised at this time (Andersen and Wang, 2002), and analysed immediately (see below). Then, the animals were force-fed rat pups amounting to $7.0 \pm 0.3\%$ of body mass. Subsequent blood samples were taken 24 and 48 h after feeding.

A group of un-treated toads, where blood samples were taken at the same time into the digestive period, were included for comparison. These data have been published previously (Andersen and Wang, 2003), but the experiments were performed during the same period as those described in the present study and using the same batch of toads kept under the same conditions.

2.2.2. Effects of bicarbonate injection on arterial acid–base status

A control blood sample was withdrawn 24 h after surgery. Then, bicarbonate was injected as a 1.5 to 3.5 ml bolus, depending on the mass of the animal, of 1 mol l^{-1} $NaHCO_3$ giving a final concentration of 6.9 ± 0.04 mmol kg^{-1} toad. Blood samples were withdrawn 1, 2, 6, 12 and 24 h after injection.

3. Blood gas analysis

Arterial blood was analysed for oxygen tension (P_{aO_2}), pH, haematocrit, blood haemoglobin concentration ($[Hb_4]$), oxygen content ($[O_2]$) and total carbon dioxide content of plasma ($[CO_2]$). P_{aO_2} and pHa were measured with Radiometer (Copenhagen, Denmark) electrodes maintained in a BMS 3 electrode set-up at 25 °C while displaying the output on a Radiometer PHM 73. Haematocrit was determined in duplicate as the fractional red cell volume after centrifugation (12 000 rpm for 3 min) and $[Hb_4]$ was measured in triplicate after conversion to cyanmethaemoglobin, applying a millimolar extinction coefficient of 10.99 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), and plasma $[CO_2]$ was measured according to Cameron (1971). Both Tucker and Cameron chambers were maintained at 40 °C. Haemoglobin bound oxygen (HbO_2) was calculated as $[O_2]a - (\alpha_{O2} \cdot PaO_2)$, where α_{O2} is the blood oxygen solubility (determined by Christoforides and Hedley-Whyte, 1969), and haemoglobin saturation (HbO_2sat) was calculated as: $HbO_2/[Hb]$, under the assumption that all Hb was functional. Arterial carbon dioxide tension (P_{aCO_2}) was calculated from pH and plasma $[CO_2]$ using the Henderson–Hasselbalch equation and the plasma solubility of CO_2 (α_{co2}) was provided by Boutilier et al. (1979). Assuming that the carbonate concentration is negligible, plasma $[HCO_3^-]$ was calculated as: $[HCO_3^-] = [CO_2] - (\alpha_{CO2} \cdot PCO_2)$.

3.1. Data analysis, statistical analysis and presentation

Significant effects of digestion were found by the use of a one-way ANOVA for repeated measures. A two-way ANOVA was employed to identify significant effects of omeprazole on the SDA response compared to untreated animals. The SDA response of untreated *B. marinus* was used from a previous study using the same batch of animals and performed at the same time (Andersen and Wang, 2003). To evaluate the effects of the bicarbonate injection, a one-way ANOVA for repeated measures was employed. All differences among means were assessed by a SNK post-hoc test. The level of significance was chosen at the $P < 0.05$ level. All data in text and figures are presented as mean ± 1 S.E.M.

4. Results

4.1. Arterial acid–base status during digestion and effects of omeprazole

In the untreated control toads, digestion was associated with a 12 mmol l^{-1} increase in plasma $[HCO_3^-]$ that persisted for at least 48 h after ingestion (Fig. 1). The increase in plasma $[HCO_3^-]$ was tailored by a simultaneous increase in P_{aCO_2}, so that arterial pH did not change during the first 48 h after ingestion (Figs. 1 and 2). Arterial acid–base status of fasting animals was not significantly affected by omeprazole treatment (Figs. 1 and 2), but the postprandial response was affected. Thus, in the omeprazole-treated toads, arterial pH, P_{CO_2} and plasma $[HCO_3^-]$ did not change significantly during digestion when compared to fasting levels (Fig. 1). Plasma $[HCO_3^-]$ remained relatively constant and significantly lower than in the untreated toads throughout the digestive period (Fig. 1). The difference between omeprazole-treated and untreated animals becomes even more apparent when depicting arterial acid–base parameters in a Davenport diagram (Fig. 2). The omeprazole-treated animals show a minor respiratory disturbance, whereas the untreated animals show a metabolic alkalosis, which is compensated by a respiratory acidosis, thereby keeping pHa unaltered.

Digestion was not associated with changes in blood oxygen levels and haematological parameters (Table 1).

4.2. Acid–base status after vascular injections of bicarbonate

Vascular injection of bicarbonate caused a significant elevation of plasma $[HCO_3^-]$ from 26.5 ± 1.37 to 36.0 ± 1.77 mmol l^{-1} 1 h after

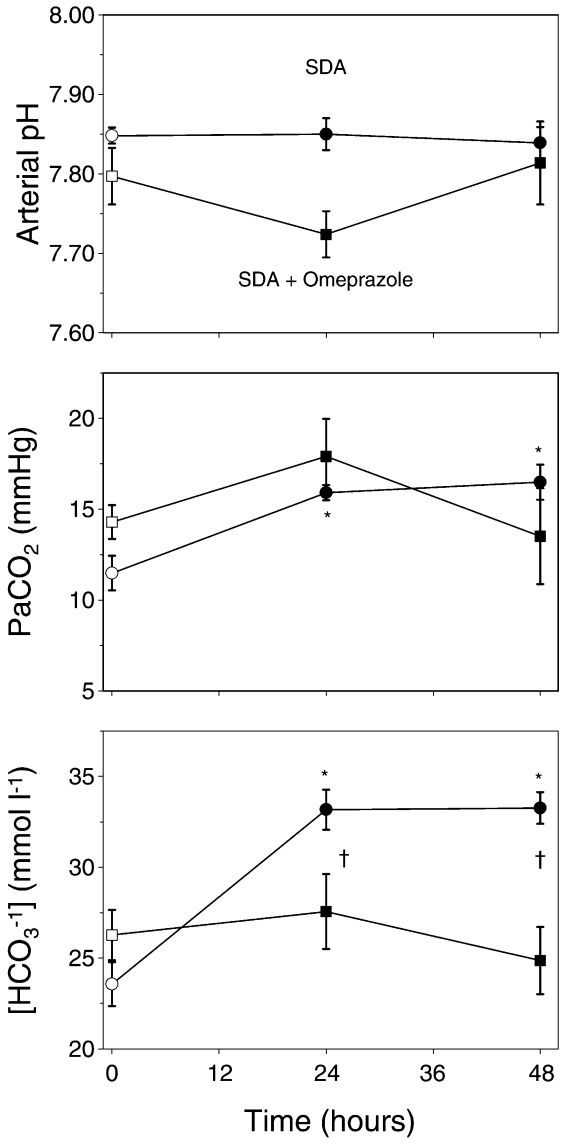

Fig. 1. Arterial acid–base parameters in the toad *Bufo marinus* before and during digestion. The circles show the response of untreated control animals, while the response of omeprazole-treated toads is shown by the squares. Fasting values are presented as open symbols. (a) arterial pH; (b) plasma bicarbonate concentration, $[HCO_3^-]$; (c) plasma carbon dioxide tension, P_{aCO_2}. Means that are different from the fasting value are marked with an asterisk, while significant effects of omeprazole treatment are marked with a dagger. Data are presented as mean ± 1 S.E.M. ($N=6$ in each group).

infusion (Fig. 3). There was no significant reduction during the next 24 h. This increase in plasma $[HCO_3^-]$ concentration was of similar magnitude to changes elicited by digestion in untreated toads (Fig. 1). Initially, the increased plasma $[HCO_3^-]$ was associated with a substantial increase in pHa from 7.74 ± 0.03 to 7.91 ± 0.02, at unchanged P_{aCO_2}. However, as seen in the Davenport diagram (Fig. 4), the metabolic alkalosis was progressively compensated by an increased P_{aCO_2}, (i.e. a respiratory acidosis). Plasma $[HCO_3^-]$ was still significantly elevated 24 h after the infusion, while pHa was fully compensated. The bicarbonate injection had no effect on blood oxygen levels and haematological parameters (Table 2).

5. Discussion

Our aim of this study was to investigate whether the increased P_{aCO_2} during digestion in *Bufo* represents a compensatory response to increased plasma $[HCO_3^-]$, (i.e. the alkaline tide) or whether the increased P_{aCO_2} represents a state-dependent change in the control of pulmonary ventilation. To this end, we successfully prevented the alkaline tide by inhibiting gastric acid secretion with omeprazole and, in addition, vascular infusions of bicarbonate mimicked the alkaline tide in fasting animals.

5.1. Effects of digestion on arterial blood gases in B. marinus

In untreated toads, digestion was associated with a marked increase in plasma $[HCO_3^-]$, but, because of the simultaneous increase in P_{aCO_2}, pHa did not change during digestion. A similar respiratory compensation of the postprandial metabolic alkalosis, (i.e. the alkaline tide) has been observed in all amphibians and reptiles where blood samples have been obtained from undisturbed animals using indwelling catheters (Overgaard et al., 1999; Busk et al., 2000a,b; Wang et al., 2001; Andersen and Wang, 2003; Andrade et al., in review).

The postprandial rise in plasma $[HCO_3^-]$ of *B. marinus* and other amphibians and reptiles is numerically larger than in mammals. This is partially due to the smaller meal size ingested by mammals and a consequence of a more regular feeding pattern where gastric acid secretion is continuously countered by pancreatic base secretion to the small intestine. Furthermore, the mammalian kidney responds effectively to metabolic acid–base disturbances and the alkaline tide is rapidly reduced by increased base output in the urine (e.g. Niv and Fraser, 2002). This is not the case in amphibians where transport of acid–base

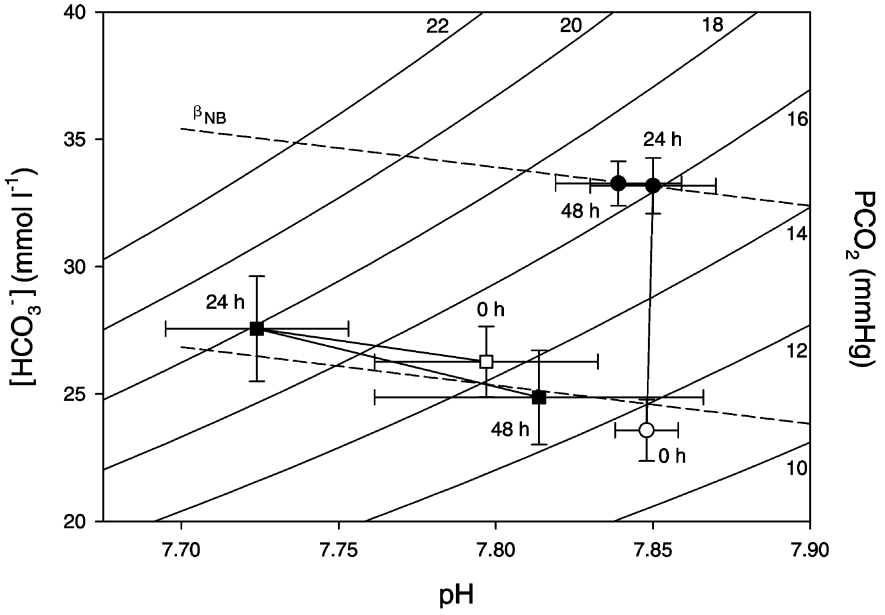

Fig. 2. Davenport diagram showing plasma [HCO_3^-] and arterial pH during fasting and digestion in the toad *Bufo marinus*. Animals treated with omeprazole are shown with the squares and untreated control animals are shown by circles. The Davenport diagram includes two in vitro non-bicarbonate buffer lines (dotted lines, β_{NB}) determined by Andersen et al. (2001), and isobars for the partial pressure of CO_2 in arterial blood (P_{aCO_2}, curved lines). Data are presented as mean ±1 S.E.M. ($N=6$ in each group).

relevant ions over the bladder and kidney is less effective than the mammalian kidney (see Tufts and Toews, 1986; Toews and Stiffler, 1989). In the present experiments this is revealed by the slow decline in plasma [HCO_3^-] after bicarbonate infusion: less than half of the extra bicarbonate, present in the plasma 1 h after infusion, had been removed 24 h into the experiment (Fig. 4). In *R. catesbeiana*, transepithelial acid–base exchange is nevertheless increased during digestion (Busk et al., 2000a).

5.2. Acid–base regulation after inhibition of gastric acid secretion with omeprazole

Omeprazole inhibits the H^+/K^+-exchange mechanism, which is the final step in the secretory process of the ATP-driven proton pump, and inhibits both basal and meal-stimulated secretion of gastric acid from the parietal cells (Sachs et al., 1995). Arterial blood gases and haematological parameters of fasting omeprazole-treated toads were not significantly different from untreated

Table 1
Effects of digestion on arterial blood gases and haematological parameters in omeprazole-treated and untreated toads (*Bufo marinus*)

Omeprazole	Fasting		24 h post feeding		48 h post feeding	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
$[O_2]$ (mM)	3.22±0.42	3.14±0.20	3.14±0.37	3.14±0.21	3.01±0.61	3.42±0.37
P_{aO_2}(mM)	76.4±5.9	80.6±3.4	84.8±4.3	81.3±3.2	70.7±7.9	84.9±2.9
HbO_2sat	0.89±0.04	0.94±0.02	0.90±0.06	0.92±0.05	0.88±0.11	1.00±0.05
Hct	0.20±0.02	0.18±0.01	0.22±0.03	0.19±0.01	0.19±0.02	0.20±0.01
$[Hb_4]$ (mM)	0.87±0.08	0.80±0.04	0.84±0.10	0.82±0.06	0.79±0.10	0.83±0.09
MCHC (mM rbc)	4.3±0.06	4.5±0.11	4.2±0.20	4.4±0.21	4.2±0.12	4.1±0.18

Oxygen concentration ($[O_2]$), oxygen tension (P_{aO_2}), haemoglobin oxygen saturation (HbO_2sat), haematocrit (Hct), haemoglobin concentration ($[Hb_4]$), mean cellular haemoglobin concentration, plasma pH, plasma carbon dioxide (P_{aCO_2}) and bicarbonate concentration ([HCO_3^-]).

Values are mean ±1 S.E.M ($N=6$ in each group)

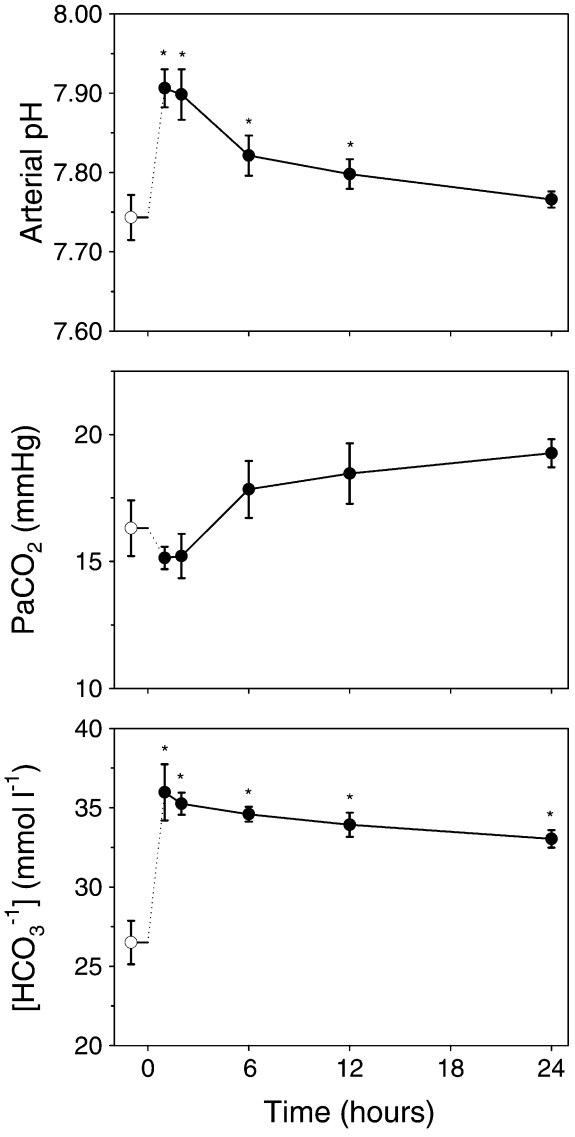

Fig. 3. Effects of a bicarbonate injection at 0h (6.9 ± 0.04 mmol kg^{-1}) on arterial acid–base parameters in the toad *Bufo marinus*. Open symbol denotes pre-injected, whereas closed symbols denotes post-injected animals. (a) arterial pH; (b) plasma bicarbonate concentration, $[HCO_3^-]$ and (c) plasma carbon dioxide tension, P_{aCO_2}. Significant differences from the pre-injected values are marked with an asterisk. Data are presented as mean ± 1 S.E.M. ($N=6$).

toads and were similar to previous studies on *B. marinus* (e.g. Andersen et al., 2001; Andersen and Wang, 2002). Plasma $[HCO_3^-]$ and P_{aCO_2}, however, appeared slightly higher in omeprazole-treated animals, which was also observed in the snake *Boa constrictor* (Andrade et al., in review), but given the lack of statistically significant effects, it remains uncertain whether acid–base status of fasting animals is affected by omeprazole. In mammals, omeprazole is considered to be very specific and without side effects (e.g. Sachs et al., 1995) and arterial acid–base parameters of fasting rats are not affected by omeprazole (Wang, Norlen and Haakanson, unpublished). Almost half of the omeprazole-treated toads vomited within 48 h after force feeding, and while their blood gas composition did not differ from those completing digestion (data not shown), we excluded these animals from the study. It is likely that inhibition of gastric acid secretion impaired the digestive ability and stimulated the emetic reflex, and secondary adverse effects of omeprazole cannot be ruled out.

Plasma $[HCO_3^-]$ did not increase after feeding in omeprazole-treated toads, which indicates an effective inhibition of the proton pump of the parietal cells in the gastric mucosa. The inhibition of the alkaline tide by omeprazole is consistent with the postprandial increase in plasma $[HCO_3^-]$ being caused by a rise in plasma strong ion difference, as protons and chloride are secreted into the stomach lumen. Omeprazole also inhibited the postprandial rise in plasma $[HCO_3^-]$ in the snake *B. constrictor* (Andrade et al., in review).

The inhibition of the postprandial rise in plasma $[HCO_3^-]$ allows us to investigate whether the respiratory acidosis reflects a ventilatory compensation to maintain pHa. 48 h into the postprandial period, omeprazole had completely abolished the increased P_{aCO_2}. This indicates that the relative hypoventilation during the postprandial period is a regulated response that act to maintain pHa by modulating P_{aCO_2}. A similar conclusion was reached in experiments on *B. constrictor*, where omeprazole fully abolished the increase in P_{aCO_2} during digestion (Andrade et al., in review). Ventilatory regulation of pHa, rather than P_{aCO_2}, is further supported by the observation that vascular bicarbonate infusion led to an increased P_{aCO_2} that re-established pHa at the control level 24 h after infusion, however, the response at 24 h after feeding was less clear, because there was a tendency, albeit not statistically significant, for an increased P_{aCO_2}.

Our study cannot reveal, which chemoreceptors are involved in mediating ventilatory regulation of pHa during the postprandial period, but it indicates that the overall modality of the chemoreceptors controlling ventilation, at fast and during digestion, is pHa and not P_{aCO_2}. The ventilatory response to

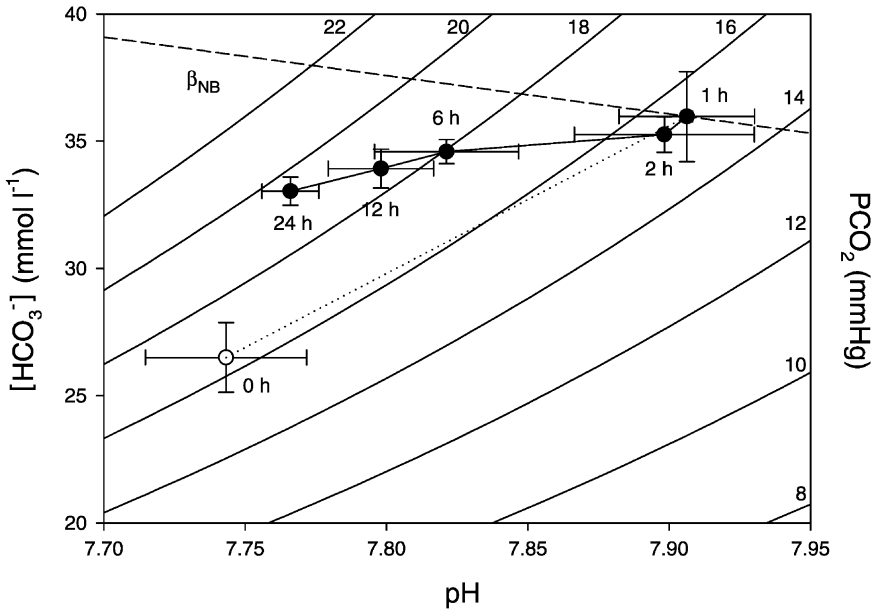

Fig. 4. Davenport diagram showing plasma [HCO_3^-] and arterial pH of fasting *Bufo marinus* before (open symbols) and after (closed symbols) a vascular injection of bicarbonate (6.9 ± 0.04 mmol kg^{-1}). The Davenport diagram includes two in vitro non-bicarbonate buffer lines (dotted lines, β_{NB}) determined by Andersen et al. (2001), and isobars for the partial pressure of CO_2 in arterial blood (P_{aCO_2}, curved lines). Data are presented as mean ± 1 S.E.M. ($N=6$).

hypercapnia (the combination of increased P_{CO_2} and reduced pH) of *B. marinus* is primarily driven by central chemoreceptors in the medulla (Branco et al., 1992, 1993; Smatresk and Smits, 1991). If central receptors are responsible for the postprandial response, it is required that metabolic acid–base disturbances are transmitted from plasma to the cerebrospinal fluid (CSF). In mammals, the blood brain barrier separating blood from CSF, is rather impermeable to ions while changes in P_{CO_2} are readily transmitted between the two compartments, (e.g. Fencl, 1986). The relative permeability of the blood brain barrier to ions and CO_2 has not been characterised in ectothermic vertebrates, but it is likely that the slow rate for the development of the alkaline tide allows for the metabolic alkalosis to be transmitted from the blood to the CSF. This may even be the case in mammals, since the small alkaline tide is associated with small respiratory compensations (Higgins, 1914; Erdt, 1915; Van Slyke et al., 1917; Ou and Tenney, 1974). A slow exchange of acid–base

Table 2
Effects of a bicarbonate injection (6.9 ± 0.04 mmol kg^{-1}) on arterial blood gases and haematological parameters in toads (*Bufo marinus*)

	Time after bicarbonate injection (h)					
	Control	1	2	6	12	24
$[O_2]$ (mM)	3.20 ± 0.81	3.20 ± 0.91	2.86 ± 0.64	2.97 ± 0.89	3.33 ± 0.22	3.26 ± 0.57
P_{aO_2} (mM)	80.9 ± 7.6	81.3 ± 6.5	74.9 ± 4.3	80.0 ± 7.6	91.8 ± 10	80.2 ± 6.4
HbO_2sat	0.88 ± 0.03	0.88 ± 0.05	0.87 ± 0.03	0.89 ± 0.05	0.90 ± 0.04	0.89 ± 0.03
Hct	0.20 ± 0.05	0.19 ± 0.04	0.18 ± 0.04	0.18 ± 0.05	0.20 ± 0.04	0.20 ± 0.03
$[Hb_4]$ (mM)	0.88 ± 0.23	0.85 ± 0.21	0.79 ± 0.18	0.80 ± 0.22	0.87 ± 0.19	0.88 ± 0.14
MCHC (mM rbc)	4.4 ± 0.06	4.4 ± 0.10	4.5 ± 0.04	4.4 ± 0.11	4.3 ± 0.09	4.3 ± 0.09

Oxygen concentration ($[O_2]$), oxygen tension (P_{aO_2}), haemoglobin oxygen saturation (HbO_2sat), haematocrit (Hct), haemoglobin concentration ($[Hb_4]$), mean cellular haemoglobin concentration, plasma pH, plasma carbon dioxide (P_{aCO_2}) and bicarbonate concentration ([HCO_3^-]).

Values are mean ± 1 S.E.M ($N=6$)

relevant ions between plasma and CSF could also explain the rather slow and progressive ventilatory compensation to the alkalosis following infusion of bicarbonate in the present study. It may also be important to consider pH/P_{CO_2} sensitivity of the peripheral chemoreceptors.

Thus, while the mechanisms underlying the postprandial regulation of pHa remains to be understood in more detail, the present study adds strong support for the view that the relative hypoventilation during the elevated metabolic rate associated with digestion, reflects a regulation of arterial pH rather than a state dependent change in ventilatory control.

Acknowledgments

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