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Using omeprazole to link the components of the post-prandial alkaline tide in the spiny dogfish, *Squalus acanthias*

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SUMMARY

After a meal, dogfish exhibit a metabolic alkalosis in the bloodstream and a marked excretion of basic equivalents across the gills to the external seawater. We used the H^+, K^+-ATPase pump inhibitor omeprazole to determine whether these post-prandial alkaline tide events were linked to secretion of H^+ (accompanied by Cl^-) in the stomach. Sharks were fitted with indwelling stomach tubes for pretreatment with omeprazole (five doses of 5 mg omeprazole per kilogram over 48 h) or comparable volumes of vehicle (saline containing 2% DMSO) and for sampling of gastric chyme. Fish were then fed an involuntary meal by means of the stomach tube consisting of minced flatfish muscle (2% of body mass) suspended in saline (4% of body mass total volume). Omeprazole pre-treatment delayed the post-prandial acidification of the gastric chyme, slowed the rise in Cl^- concentration of the chyme and altered the patterns of other ions, indicating inhibition of H^+ and accompanying Cl^- secretion. Omeprazole also greatly attenuated the rise in arterial pH and bicarbonate concentrations and reduced the net excretion of basic equivalents to the water by 56% over 48 h. Arterial blood CO_2 pressure (Pa_{CO_2}) and plasma ions were not substantially altered. These results indicate that elevated gastric H^+ secretion (as HCl) in the digestive process is the major cause of the systemic metabolic alkalosis and the accompanying rise in base excretion across the gills that constitute the alkaline tide in the dogfish.

Key words: feeding, shark, gastric acid secretion, H^+, K^+-ATPase, chyme composition, metabolic alkalosis, branchial base excretion.

INTRODUCTION

Although originally referring to a surge of pH in human urine after a meal (Bence-Jones, 1845; Roberts, 1859), the term 'alkaline tide' later evolved to represent the general phenomenon of post-prandial metabolic alkalosis occurring in the bloodstream of most vertebrates (Brunton, 1933). It is now well accepted, at least in mammals, that the alkalosis is caused by activation of the gastric parietal cells to secrete H^+ ions into the lumen of the stomach in order to facilitate digestion, thereby adding an equivalent amount of HCO_3^- ions to the extracellular fluid (Niv and Fraser, 2002). The H^+ and HCO_3^- are produced intracellularly by the CO_2 hydration reaction catalysed by carbonic anhydrase. Extracellular Cl^- is exchanged for the HCO_3^- and secreted into the lumen in approximately equimolar amounts to H^+ – so, effectively, HCl is secreted. Recently we have shown that the elasmobranch *Squalus acanthias* exhibits a marked metabolic alkalosis after involuntary feeding (Wood et al., 2005), an even larger increase in plasma [HCO_3^-] after voluntary feeding [blood pH measurements were not possible in these unrestrained animals (Wood et al., 2007b)] and a large excretion of basic equivalents to the external water after the latter protocol (Wood et al., 2007a). The interpretation offered was that these responses were all components of the alkaline tide, driven initially by increased H^+ secretion (as HCl) by the gastric mucosa.

However, at present, there is no evidence directly linking these putative alkaline tide events to increased secretion of gastric acid in the dogfish shark, and other explanations might apply. For example, it is possible that the rise in plasma [HCO_3^-] might come from an entirely different source, such as the increased oxidation of keto-acids (Ballantyne, 1997), thereby supplying the HCO_3^- needed for the increased ureagenesis that is known to occur after a meal (Kajimura et al., 2006). Plasma β-hydroxybutyrate levels drop precipitously, and β-hydroxybutyrate dehydrogenase activities increase markedly in many shark tissues at this time (Walsh et al., 2006). This shift might also drive increased excretion of metabolic base across the gills. Furthermore, although it is well established that the elasmobranch stomach is capable of equimolar H^+ and Cl^- secretion (Babkin et al., 1935; Hogben, 1959; Hogben, 1967; Rehm, 1962; Kidder, 1976; Kidder, 1991) and expresses a putative H^+, K^+-ATPase (Smolka et al., 1994; Choe et al., 2004), the cells involved are oxyntincopeptic cells that synthesize both enzymes and acid (Rebolledo and Vial, 1979) – rather different from the pure H^+- and Cl^--secreting parietal cells of mammals. It is unclear whether the acid secretion rate actually increases after a meal because the pH of the gastric fluid actually increases rather than decreases at this time in many elasmobranchs (Babkin et al., 1935; Menon and Kewalramani, 1959; Papastamatiou and Lowe, 2004; Papastamatiou and Lowe, 2005), including *Squalus acanthias* (Wood et al., 2007b). This alkalinisation apparently occurs because of the buffering action of the food. Recent reports in teleosts further complicate the picture. Several species apparently show no alkaline tide phenomena after a meal (Taylor and Grosell, 2006b; Taylor et al., 2007), whereas the rainbow trout exhibits a blood alkalosis that might (Bucking and Wood, 2008) or might not (Cooper and Wilson, 2008) be accompanied by elevated base excretion to the water.

With this background in mind, we sought to establish a causal link between increased H^+ secretion (as HCl) into the stomach,

metabolic alkalosis in the bloodstream and elevated excretion of base to the environmental water in response to feeding in the dogfish shark. The tool we adopted was intra-gastric pretreatment of sharks with the specific H^+, K^+-ATPase inhibitor omeprazole (Fellenius et al., 1981; Sachs et al., 1995; Huang and Hunt, 2001), a very similar approach to that used by Andersen and colleagues (Andersen et al., 2003) and Andrade and colleagues (Andrade et al., 2004) to dissect successfully the ventilatory components of the alkaline tide in the toad *Bufo marinus* and the snake *Boa constrictor*, respectively. In order to measure blood acid–base status and base excretion rates to the water, it was necessary to cannulate and confine the animals, which prevented voluntary feeding. We therefore exploited the involuntary feeding protocol of Wood and colleagues (Wood et al., 2005) that involves prior implantation of an indwelling stomach tube. An added advantage of this approach is that this tube facilitated both the necessary pre-treatment with omeprazole, and the sampling of gastric contents, with minimal disturbance to the animals. Our specific hypotheses were that omeprazole pre-treatment would reduce the acidification of gastric chyme following a meal and that, in turn, this would reduce both the alkalinization of the blood and the excretion of basic equivalents to the external water. Plasma and chyme ions and ammonia levels were also measured, revealing other correlates of the digestive process.

MATERIALS AND METHODS

Experimental animals

Dogfish sharks (*Squalus acanthias* Linnaeus, 1.3–2.9 kg, *N*=60) of both sexes were collected by trawl or angling in Barkley Sound, British Columbia, Canada in June and July 2007. Animals were cared for in accord with the principles of the Canadian Council for Animal Care, and protocols were approved by institutional animal-care committees. Before experimentation, the fish were held for several weeks as part of a large group (~100 animals) in a 151,000 l circular indoor tank served with running seawater at the experimental temperature (12±1°C), salinity (30±2 ppt) and pH (7.90±0.15). The animals were able to feed naturally in this large tank. They were fed freshly thawed whole hake (*Merluccius productus*, from which the heads had been removed) at a ration of about 5% of body mass, every fourth day. Fish were transferred in batches of 10 to smaller 1500 l tanks, where they were fasted for one week before surgery, a period sufficient to virtually clear the gastro-intestinal tract (Wood et al., 2007b).

Surgical procedures were identical to those described by Wood and colleagues (Wood et al., 2005). In brief, each dogfish was anaesthetised with MS-222 (0.2 g l^{-1}), weighed, placed on an operating table and fitted with indwelling catheters. In all three experimental series, dogfish received stomach tubes, and, in series 2, caudal artery catheters were also inserted for repetitive blood sampling. Stomach tubes consisted of flexible polyethylene tubing (0.32 cm internal diameter, heat polished at the stomach end) and were individually fitted to each fish via the esophagus, terminating several centimetres anterior to the pylorus. The tube exited by means of a small puncture wound through the jaw muscle at the side of the mouth and was firmly ligated with a silk suture along the upper jaw, terminating in an upward projection of about 3 cm anterior to the eye. Before insertion, the tube was filled with 500 mmol l^{-1} NaCl and sealed with a plug at the anterior end. Caudal artery catheters (Clay-Adams polyethylene PE50) were implanted through a small hole in the haemal canal by means of a 5 cm incision through the muscle of the caudal peduncle, as described by DeBoeck and colleagues (DeBoeck et al., 2001). The catheters were filled with heparinized dogfish saline [lithium heparin, 50 i.u. ml^{-1}; saline recipe as described in Wood and colleagues (Wood et al., 1994), but with the urea level raised to 400 mmol l^{-1}]. Wounds were dusted with powdered oxytetracycline to avoid infection and tightly closed with silk ligatures.

After surgery, the dogfish were revived in anaesthetic-free water and transferred to covered polyurethane-coated wooden fish boxes (Wood et al., 1995). The boxes were 105 cm in length, 16.5 cm in width and 25 cm in height, with a flow-through of 1 litre per minute. Perimeter aeration over the complete length of the box ensured good mixing during flux measurements. The boxes were bathed in an external running seawater bath to maintain temperature (11–12°C) when flow-through was suspended for the flux measurements. A recovery period of at least 36 h was allowed before experiments were started.

Pre-treatment with omeprazole

Omeprazole (Sigma, St Louis, MO, USA) was dissolved in DMSO and then diluted with 500 mmol l^{-1} NaCl to yield a final omeprazole concentration of 0.5 mg ml^{-1} in a vehicle of 2% DMSO+500 mmol l^{-1} NaCl. This solution was administered at approximately 12-h intervals five times over 48 h by means of the stomach catheter at a dose of 5 mg omeprazole per kilogram in 10 ml vehicle per kilogram at each infusion. Therefore, the total dose received was 25 mg omeprazole per kilogram. Control animals received the same volume of vehicle alone.

Experimental feeding

A food slurry that could be infused by means of the stomach tubes was prepared as described by Wood and colleagues (Wood et al., 2005). In brief, filets of white muscle from freshly caught flatfish (*Hippoglossoides elassodon* and *Parophrys vetulus*) were ground to a fine paste in a Waring food blender, then stored frozen at –20°C in small aliquots until used. The meal administered consisted of 2% of the dogfish's body mass of the flounder muscle paste mixed 50:50 with an equal volume of 140 mmol l^{-1} NaCl (isosmotic to the teleost food) to create a smooth slurry. The total volume infused was therefore 4% of the body mass, administered as a bolus down the stomach tube over a period of approximately 5 min. This meal was given approximately 1 h after the fifth infusion of omeprazole or vehicle.

Experimental series

Series 1

This series focused on repetitive sampling of the chyme in dogfish that had been pre-treated with either omeprazole (*N*=11; 1.88±0.07 kg) or vehicle (*N*=14; 2.24±0.11 kg). Samples were taken at 12 h, 24 h, 36 h and 48 h after the meal, but it was not possible to obtain samples from all animals at all time-points. In general, it became more difficult to obtain chyme samples as time progressed, so the 48-h data set were supplemented with samples taken at this time from the dogfish of series 2 and 3, immediately after completion of those experiments. At each sampling time, the plug was removed from the stomach tube, and stomach chyme was aspirated by applying suction with a 50-ml syringe. Sufficient volume was withdrawn to clear the dead-volume of the tube before the actual chyme sample (1–3 ml) was taken. The pH of the sample was measured immediately, and then the sample was frozen at –80°C for later analysis.

Series 2

This series focused on repetitive sampling of blood so as to track changes in arterial blood gases, acid–base status and plasma ions

and ammonia in dogfish that had been pre-treated with either omeprazole (N=8; 2.01±0.05 kg) or vehicle (N=9; 2.37±0.13 kg). Samples were taken at 0 h (control, which was approximately 30 min after the fifth infusion with omeprazole or vehicle, and 30 min before the meal), and at 2 h, 4 h, 6 h, 9 h, 18 h, 24 h and 48 h after the meal. At each sample time, blood samples (600 µl) were withdrawn by means of the catheters into ice-cold gas-tight Hamilton syringes. A subsample was spun at 9000 ***g*** for 30 s in a sealed tube to separate plasma, which was then aliquoted for immediate analysis of total CO_2 or storage at –80°C for later analyses of plasma ions and ammonia. The remainder of the blood sample was processed for immediate analysis of arterial pH and oxygen tension. Blood recovered from the electrodes was mixed with the red cell pellet, made up to the original volume with non-heparinized saline and re-infused by means of the arterial catheter to prevent experimentally induced anaemia.

Series 3

This series focused on changes in the net fluxes of acidic or basic equivalents with the external water. Fluxes were measured during a 12-h pre-feeding control period that started approximately 30 min after the fourth infusion of either omeprazole (N=9; 1.79±0.08 kg) or vehicle (N=9; 2.38±0.11 kg) and ended approximately 30 min before the fifth infusion. Subsequent flux measurements were performed over intervals of approximately 0–12 h, 12–24 h, 24–36 h and 36–48 h after the meal. At the start of a flux period, the water inflow to the box was stopped, and the volume set to a known level (approximately 35 l, after subtraction of dogfish mass). At the end of each 12-h interval, the box was thoroughly flushed with fresh seawater by lowering the water level to the point where the dorsal fin of the animal was just exposed, then filling to the top, a procedure that was repeated three times before the volume was reset to 35 l. Water samples were taken at the start and end of a period and measured for titration alkalinity and total ammonia.

Analytical techniques

Arterial blood oxygen tension (Pa_{O_2}) and pH (pHa) in series 2 were measured using Radiometer–Copenhagen electrodes (Copenhagen, Denmark) kept at the experimental temperature with water jackets; outputs were displayed on Radiometer–Copenhagen pHM 71 or 72 blood-gas analysers. Chyme pH, food slurry pH and seawater pH (series 1) were determined on the same system. True plasma CO_2 was measured using a Corning 965 CO_2 analyzer (London, UK) Arterial blood carbon dioxide tension (Pa_{CO_2}) and plasma bicarbonate concentration ([HCO_3^-]a) were calculated using the solubility of carbon dioxide (α_{CO_2}), the apparent pK (pK_{app}) for dogfish plasma and rearrangements of the Henderson–Hasselbalch equation, as described by Boutilier and colleagues (Boutilier et al., 1984).

Total ammonia was measured enzymatically (L-glutamate dehydrogenase, Raichem Ammonia Reagent, Product No. 85446) (Mondzac et al., 1965) on the first thaw of frozen plasma (series 2) and on the supernatant taken from the first thaw of frozen chyme and food samples (series 1). All ions were measured on digests of whole chyme and food samples. These samples (1–3 g) were initially dried to a constant mass at 65°C to determine water content and then digested with 1 ml of 1 mol l^{-1} HNO_3 at 65°C for 48 h in sealed tubes. Cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) in plasma, digests of chyme and food samples and ambient seawater were analysed by flame atomic absorption spectrophotometry (Varian SpectrAA-220FS, Mulgrave, Australia) after appropriate dilution. Chloride in plasma, seawater and digests was measured by coulometric titration (Radiometer–Copenhagen CMT-10) without dilution.

In series 3, titratable alkalinity was determined by titration of 10 ml water samples to pH 4.0, using a Radiometer–Copenhagen GK2401C combination electrode, and a Gilmont microburette (Great Neck, New York, NY, USA) to dispense standardized acid (0.04 mol l^{-1} HCl). The total ammonia concentration in water was measured by the indophenol blue method (Ivancic and Degobbis, 1984). Fluxes were calculated from changes in concentration, factored by total volume in the chamber, time and dogfish mass and expressed as µmol kg^{-1} h^{-1}. Net acid–base flux was measured as the difference between the flux of titratable alkalinity and the flux of total ammonia to the external water (McDonald and Wood, 1981). A positive difference represents net base (i.e. HCO_3^- equivalent) flux, and a negative difference represents net acid (i.e. H^+) equivalent flux.

Statistics

Data have been expressed as means ±1 s.e.m. (N), where N=number of fish. In a few cases, data were log-transformed before analysis to equalize variances. In series 1, one-way analysis of variance followed by Tukey's test was applied to detect specific differences within a treatment group (omeprazole-treated or vehicle-treated); means not sharing the same case letters are significantly different. In addition, Bonferroni tests were used to evaluate whether the composition of chyme at each time was significantly different from that of the original meal (indicated by a triangle) or the external seawater (indicated by a dagger). In series 2 and 3, repeated measures analysis of variance followed by Dunnett's paired multiple comparison test was employed to detect specific differences within a treatment group (omeprazole-treated or vehicle-treated), relative to the pre-feeding value, as indicated by asterisks. Student's t-tests (unpaired) were applied to detect specific differences between omeprazole-treated and vehicle-treated groups at the same sampling time, indicated by + signs. A significance level of 0.05 was used throughout.

RESULTS

Series 1: effects of omeprazole on chyme chemistry

Omeprazole pre-treatment had no significant effect on the water content of the chyme at any sampling time (Table 1). Chyme water content tended to fall over time in both treatments, although the decline was significant only in the vehicle group. Notably, in both groups, chyme water content at the first sampling time (12 h) was significantly greater than in the infused meal, indicating the addition of fluid from another source. For this reason, the chemistry of the

Table 1. Water content of the gastric chyme relative to that of the meal in dogfish pretreated with omeprazole or vehicle (means±s.e.m.)

	Vehicle pre-treatment (ml g^{-1})	Omeprazole pre-treatment (ml g^{-1})
Meal	0.9089±0.0004 (N=3)	
12 h chyme	0.9464±0.0103^{*,A} (N=7)	0.9414±0.0027^{*,a} (N=7)
24 h chyme	0.9372±0.0086^{*,A,B} (N=9)	0.9255±0.0080^{a} (N=7)
36 h chyme	0.9296±0.0123^{A,B} (N=5)	0.9369±0.0084^{a} (N=11)
48 h chyme	0.9188±0.0064^{B} (N=15)	0.9278±0.0052^{a} (N=21)

Within a pre-treatment group, means sharing letters of the same case are not significantly different (P<0.05). There were no significant differences (P<0.05) in chyme values at the same times between the two pre-treatment groups. *Significant difference (P<0.05) between water content of the chyme and that of the original meal.

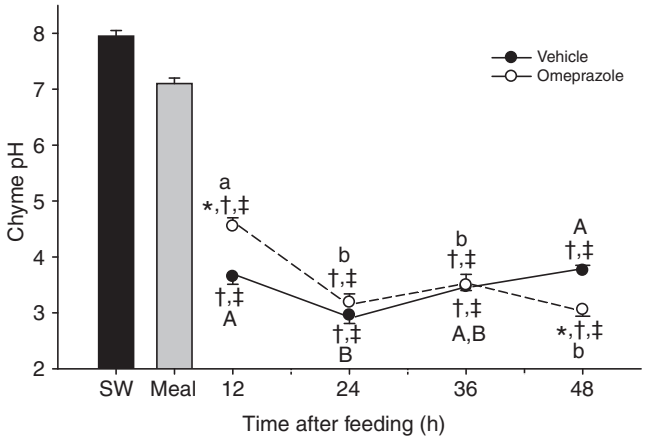

Fig. 1. Changes in the pH of gastric chyme in series 1 following an involuntary meal administered by stomach tube at 0 h in dogfish pre-treated with omeprazole (open circles) or vehicle (filled circles), relative to the pH values of the external seawater and the meal. *Significant difference between the two treatment groups at the same sample time; †significant difference from the external seawater (SW); ‡significant difference from the original meal; all at $P<0.05$. Within a treatment group, means sharing letters of the same case are not significantly different from one another. Means ±1 s.e.m. (omeprazole: N=11, 7, 7 and 21 at 12 h, 24 h, 36 h and 48 h, respectively; vehicle: N=9, 7, 5 and 15 at 12 h, 24 h, 36 h and 48 h, respectively).

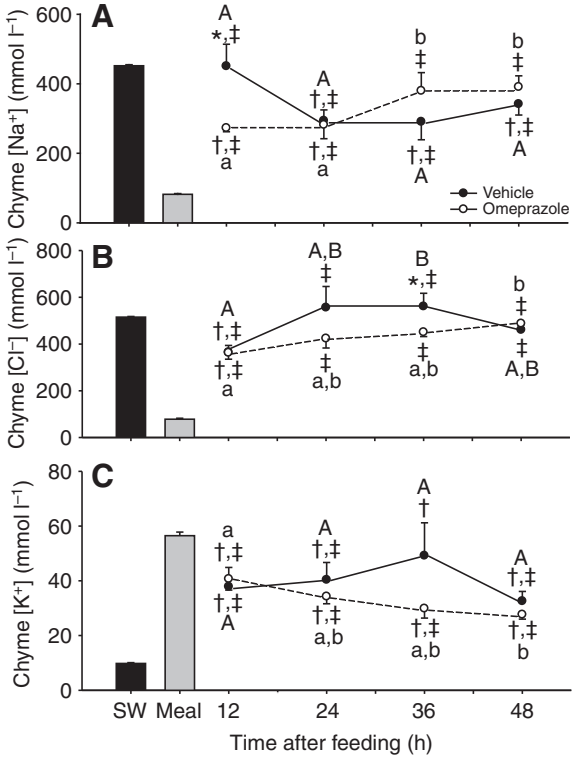

Fig. 2. Changes in (A) sodium concentration, (B) chloride concentration and (C) potassium concentration of gastric chyme in series 1 following an involuntary meal administered by stomach tube at 0 h in dogfish pre-treated with omeprazole (open circles) or vehicle (filled circles), relative to the concentrations in the external seawater (SW) and the meal. *Significant difference between the two treatments groups at the same sample time; †significant difference from the SW; ‡significant difference from the original meal; all at $P<0.05$. Within a treatment group, means sharing letters of the same case are not significantly different from one another. Means ±1 s.e.m. (N numbers as in legend of Fig. 1).

chyme has been compared with that of both the original meal and the external seawater in Figs 1–3.

Pre-treatment with omeprazole delayed the post-prandial acidification of the administered meal (Fig. 1). The pH of the gastric chyme at the first sampling time (12 h) was 4.55 with omeprazole pre-treatment, almost one pH unit higher than the 3.65 in the vehicle pre-treatment, a highly significant difference. These values can be compared with the much higher circumneutral pH values of the infused food slurry (7.10) and external seawater (7.95). The chyme pH continued to fall significantly in both groups, reaching about 3.00 at 24 h, but there were no longer any significant differences between treatments at either 24 h or 36 h. By 48 h, chyme pH had increased in the vehicle group to 3.76 but was significantly lower in the omeprazole group at 3.06.

These differences in the time-course of acidification were accompanied by several differences in the ionic composition of the chyme (Fig. 2). At 12 h, chyme [Na^+] was significantly lower in the omeprazole pre-treatment than in the vehicle pre-treatment (Fig. 2A). This difference disappeared at 24 h, but thereafter chyme [Na^+] increased significantly in the omeprazole pre-treatment, whereas there was no significant change through 48 h in the vehicle pre-treatment. Notably, all these chyme Na^+ concentrations were at least threefold higher than in the originally ingested meal. However, in the vehicle-treated group, the 12 h chyme [Na^+] was not significantly different from that in ambient seawater, although subsequent values were significantly lower. In the omeprazole pre-treated group, only the 12 h and 24 h Na^+ concentrations were significantly lower than those in the external seawater.

Chyme Cl^- concentrations followed a very different pattern (Fig. 2B). At 12 h, chyme [Cl^-] values were identical in the two treatments at levels that again were much higher (>4-fold) than in the meal but significantly lower than in the seawater. Thereafter, chyme Cl^- concentrations increased significantly in both groups but diverged, with substantially higher values in the vehicle pre-treatment than in the omeprazole pre-treatment, suggestive of a difference in Cl^- secretion. This difference became significant at 36 h but had disappeared by 48 h.

In contrast to [Na^+] and [Cl^-], chyme K^+ concentrations at 12 h in both groups were about fourfold higher than in the ambient seawater but significantly lower than in the original meal (Fig. 2C). Thereafter, there were no significant differences between pre-treatments, although chyme [K^+] fell significantly by 48 h in the omeprazole group but not in the vehicle group,

For chyme Mg^{2+} (Fig. 3A), Ca^{2+} (Fig. 3B) and total ammonia concentrations (Fig. 3C), there were no significant differences or substantial divergences in time-dependent trends between the two groups. Notably, chyme [Mg^{2+}] values were midway between the levels in seawater and those in the ingested meal and did not change significantly over time. Chyme [Ca^{2+}] values were much closer to those in seawater, whereas levels in the ingested meal were negligible. [Ca^{2+}] increased significantly over time in the vehicle group but remained stable in the omeprazole group. Although total ammonia concentrations in both seawater and the meal were negligible, there appeared to be considerable generation of ammonia in the chyme, with concentrations of ~1.5 mmol l^{-1} at 12 h, increasing to as high as 3.0 mmol l^{-1} by 48 h in the vehicle pre-treatment group. Chyme ammonia concentrations in the omeprazole pre-treatment were not significantly lower at this time.

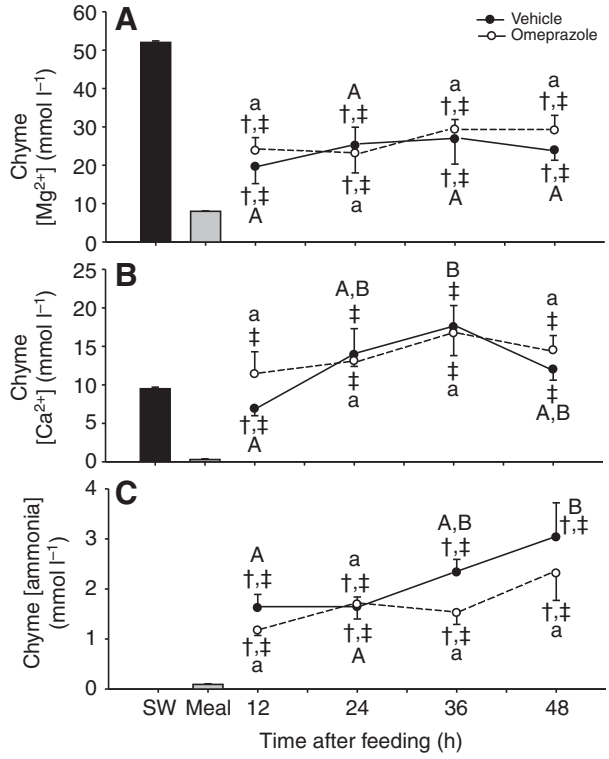

Fig. 3. Changes in (A) magnesium concentration, (B) calcium concentration and (C) total ammonia concentration of gastric chyme in series 1 following an involuntary meal administered by stomach tube at 0 h in dogfish pre-treated with omeprazole (open circles) or vehicle (filled circles), relative to the concentrations in the external seawater (SW) and the meal. *Significant difference between the two treatments groups at the same sample time; †significant difference from the SW; ‡significant difference from the original meal; all at $P<0.05$. Within a treatment group, means sharing letters of the same case are not significantly different from one another. Means ±1 s.e.m. (*N* numbers as in legend of Fig. 1).

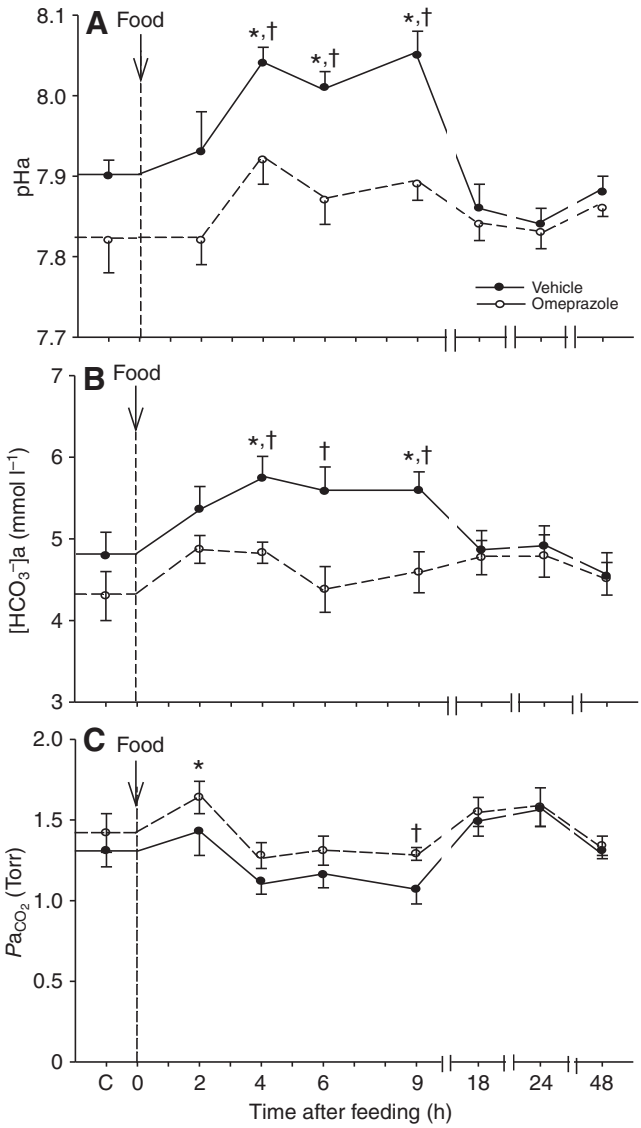

Fig. 4. Changes in (A) arterial pH (pHa), (B) arterial plasma bicarbonate concentration [HCO_3^-] and (C) arterial partial pressure of carbon dioxide (Pa_{CO_2}) following an involuntary meal administered by stomach tube at 0 h in dogfish pre-treated with omeprazole (open circles) or vehicle (filled circles) in series 2. *Significant difference from the pre-feeding control value in the respective treatment group; †significant difference between the two treatment groups at the same sample time; both at $P<0.05$. Means ±1 s.e.m. (omeprazole: $N=8$; vehicle $N=9$ at each time).

Series 2: effects of omeprazole on blood acid–base status and plasma ions

Dogfish pre-treated with vehicle exhibited a post-prandial alkaline tide in the arterial bloodstream (Fig. 4) very similar to that reported previously for animals fed in an identical manner by means of a stomach tube but without any pre-treatment (Wood et al., 2005). Arterial pHa rose by ~0.15 units (Fig. 4A), and plasma [HCO_3^-]a by ~1 mmol l^{-1} (Fig. 4B), effects that were significant relative to pre-feeding control values at 4 h through 9 h but that had attenuated by 18 h. Pre-treatment with omeprazole had no effect on pre-feeding acid–base status but largely abolished these responses to feeding. Although there were minor increases in pHa (Fig. 4A) and plasma [HCO_3^-]a (Fig. 4B) in the omeprazole group, they were not significant relative to pre-feeding control values. Furthermore, both parameters were significantly lower in the omeprazole pre-treatment relative to the vehicle pre-treatment at 4 h through 9 h (Fig. 4A,B). Changes in Pa_{CO_2} were very small, but, in the omeprazole group, Pa_{CO_2} increased slightly relative to the pre-feeding control at 2 h and was significantly higher than in the vehicle group at 9 h (Fig. 4C). Pa_{O_2} values were very similar in the two groups and showed no significant changes after feeding (data not shown), averaging 105±7 Torr or 14.0±0.9 kPa ($N=17$) overall.

Plasma ions and total ammonia concentrations were measured at every sampling time in series 2, but changes were minimal relative to pre-feeding control values in both groups, as were differences between pre-treatments. Therefore, only mean values are reported: [Na^+]=273±5 mmol l^{-1}, [Cl^-]=261±2 mmol l^{-1}, [K^+]=3.27±0.12 mmol l^{-1}, [Mg^{2+}]=2.59±0.19 mmol l^{-1}, [Ca^{2+}]=5.15±0.17 mmol l^{-1} and total ammonia=32.4±11.1 μmol l^{-1} ($N=17$). The only significant difference between the groups was a lower plasma [K^+] at 6 h in the omeprazole pre-treatment [3.08±0.07 ($N=8$) *vs* 3.57±0.15 ($N=9$) mmol l^{-1}], and the only significant difference relative to pre-feeding values was a rise in plasma [Cl^-] at 24 h [264±2 ($N=8$) mmol l^{-1}] and 48 h [265±2 ($N=8$) mmol l^{-1}] relative to the pre-feeding control level [257±2 ($N=8$) mmol l^{-1}] in the omeprazole pre-treatment group. Note that plasma ion concentrations were very different therefore from gastric chyme

concentrations (c.f. Figs 2 and 3), and there was a pronounced gradient between millimolar levels of total ammonia in chyme versus micromolar levels in blood plasma.

Series 3: effects of omeprazole on net fluxes of basic equivalents with the external water

Dogfish pre-treated with vehicle excreted small amounts of acid (i.e. negative flux of basic equivalents) to the external water before feeding (Fig. 5A). After feeding, this changed to a significant positive flux of basic equivalents within the first 12 h, a trend that peaked at ~340 μmol kg^{-1} h^{-1} at 12–24 h and continued through 48 h. In animals pre-treated with omeprazole, the pre-feeding acid–base flux was close to zero but was not significantly different from that in the vehicle group (Fig. 5B). However, after feeding, the net flux of basic equivalents to the external water was greatly attenuated by the omeprazole pre-treatment. None of the post-prandial flux rates was significantly different from the pre-feeding rate, and there was no clear peak. Net basic equivalent fluxes to the external water were significantly lower than in the vehicle pre-treatment at 12–24 h. Over 48 h, net basic excretion to the water, as calculated by integration under the curves, was reduced by 56% from 12,351±1922 μmol kg^{-1} (N=9) in the vehicle group to 5405±1090 μmol kg^{-1} (N=9) in the omeprazole group, a highly significant difference. These responses were entirely due to changes in the titratable alkalinity components; there were no significant differences in the ammonia components, which averaged 21±5 μmol kg^{-1} h^{-1} (N=18) overall (data not shown).

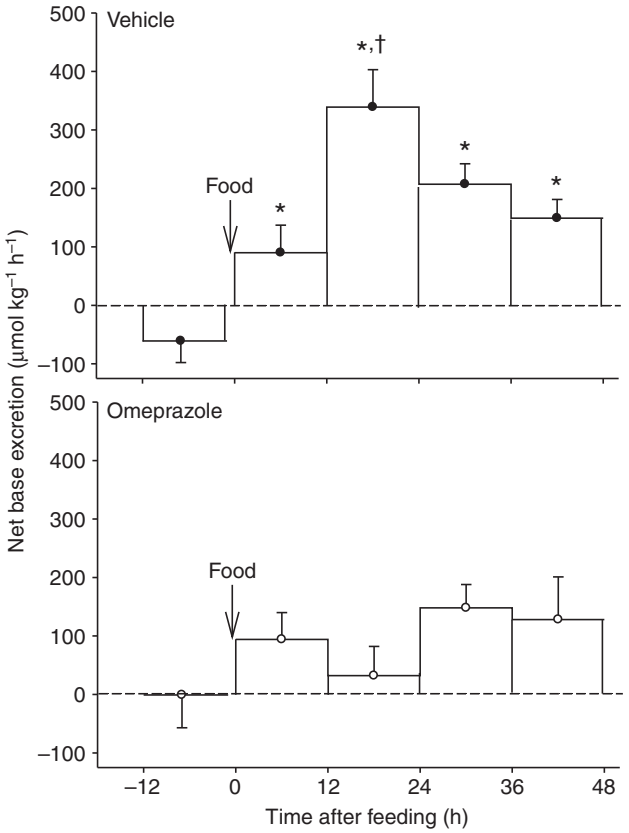

Fig. 5. Changes in the net excretion rates of basic equivalents to the external seawater following an involuntary meal administered by stomach tube at 0 h in dogfish pre-treated with omeprazole (open circles) or vehicle (filled circles) in series 3. Positive values indicate net base excretion, negative values indicate net acid excretion. *Significant difference from the pre-feeding control value in the respective treatment group; †significant difference between the two treatment groups at the same sample time; both at P<0.05. Means ±1 s.e.m. (omeprazole: N=9; vehicle N=9 at each time).

DISCUSSION

The influence of omeprazole on the responses to feeding: linking the components of the alkaline tide

In support of our original hypotheses, pre-treatment with omeprazole reduced the acidification of gastric chyme (Fig. 1), reduced the alkalinization of the blood (Fig. 4) and reduced the excretion of basic equivalents to the external water (Fig. 5) following a meal in *Squalus acanthias*. Omeprazole also attenuated the post-prandial rise in Cl^- concentration in the gastric chyme (Fig. 2B). These results therefore provide evidence that the metabolic alkalosis in the bloodstream and the excretion of basic equivalents to the external water are caused by the secretion of H^+ (accompanied by Cl^-) in the stomach in the process of digestion.

While all of these effects were statistically significant, it is apparent that, relative to the vehicle pre-treatment, omeprazole pre-treatment delayed or attenuated these responses, rather than eliminating them entirely. This is very likely because the omeprazole dosing was stopped just before the meal, and the inhibition of the gastric H^+, K^+-ATPase pumps gradually wore off over time. In preliminary trials, we continued to infuse omeprazole at 12 h intervals after the meal, but this resulted in vomiting, making the protocol unworkable. An alternative or additional explanation for the persistence of small, non-significant elevations in plasma [HCO_3^-] and base excretion to the water in omeprazole pre-treated fish is that these reflected a small portion of the alkaline tide that was due to increased oxidation of keto acids (Walsh et al., 2006) after the meal (see Introduction).

As a weak base with a pK_a of 4.0, omeprazole partitions into acidic compartments of parietal cells in mammals, where it is converted to a sulphenamide. Irreversible reaction of this moiety with two cysteine residues on the gastric H^+, K^+-ATPase leads to specific inhibition of H^+ secretion into the gastric lumen (Sachs et al., 1995; Huang and Hunt, 2001). Presumably, the same phenomena take place in the oxyntincopeptic cells of the shark, which synthesize both enzymes and acid (Hogben, 1967; Rebolledo and Vial, 1979). Gastric H^+, K^+-ATPase appears to be well conserved between elasmobranchs and mammals. In the stingray *Dasyatis sabina*, the gastric H^+, K^+-ATPase exhibits greater than 80% homology to the mammalian enzyme at the amino acid level (Choe et al., 2004) and strongly reacts with an antibody against pig H^+, K^+-ATPase (Smolka et al., 1994).

We are aware of no previous studies on the use of omeprazole in fish, but a higher dose of this drug (88 mg per kg total over 8 days versus 25 mg per kg over 2 days) was used successfully by Andrade and colleagues (Andrade et al., 2004) to virtually eliminate the metabolic alkalosis in the blood stream of the snake *Boa constrictor* following a meal. Andersen and colleagues (Andersen et al., 2003) used a much lower dose (0.3 mg per kg total over 5 days) to cause a comparable inhibition of the post-prandial alkalosis (with respiratory compensation) in the cane toad *Bufo marinus*. Chyme chemistry was not measured in either of these studies, but the large rise in metabolic rate that typically occurs after a meal in the snakes was delayed (Andrade et al., 2004), suggesting a delay in the digestive process, as in the present study. In humans, the standard maintenance dosage of omeprazole (under the trade names Losec and Prilosec) is 20–40 mg daily, or ~0.3–0.6 mg kg^{-1} d^{-1} for partial inhibition (~70% blockade) of gastric HCl secretion (Sachs et al., 1995). Oral

administration is more effective and less likely to cause nonspecific internal effects than systemic administration, so the same approach was used in the present study (i.e. intragastric administration by stomach tube). The inhibitory effect develops gradually because the sulphenamide derivative of omeprazole reacts only with actively secreting pumps inserted into the apical membranes of H^+ secretory cells and not with nascent pumps stored in vesicles (Sachs et al., 1995; Sachs, 1997; Huang and Hunt, 2001). The half-life of inhibition of acid secretory capacity in dogs is ~54 h (Äbelö et al., 2000), in almost exact agreement with the half-life of pump turnover of ~54 h measured in rats (Gedda et al., 1995). Therefore, when dosing stops, progressive recovery of H^+ (and Cl^-) secretion capacity occurs as fresh pump molecules are inserted into the membranes.

The regulation of acid–base status in response to feeding

The metabolic alkalosis in the arterial blood of sharks pre-treated with the vehicle only (Fig. 4) was virtually identical to that seen previously in dogfish fed in an identical manner by means of a stomach tube but without any pre-treatment (Wood et al., 2005), and the base excretion to the external water (Fig. 5) was actually greater than that reported earlier in naturally fed dogfish (Wood et al., 2007a). We can conclude, therefore, that DMSO has a negligible effect on the acid–base regulatory processes. After natural feeding, meals are much bigger (typically 5–6% of body mass of whole fish *vs* 2% of body mass of minced muscle), and increases in HCO_3^- in the blood plasma are much larger (Wood et al., 2007b) than after involuntary feeding (Wood et al., 2005) (Fig. 4). It was surprising, therefore, that total base excretion to the water over 48 h was slightly greater after the small involuntary meal of the present study (12,351 μmol kg^{-1}) (Fig. 5) than after a large voluntary meal (10,470 μmol kg^{-1}) (Wood et al., 2007a). Indeed, Cooper and Wilson (Cooper and Wilson, 2008), based on work with the rainbow trout *Oncorhynchus mykiss*, concluded that fish that feed voluntarily are more effective in regulating post-prandial acid–base disturbances than those that are fed involuntarily, although they detected no excretion of basic equivalents to the external water in either group, in contrast to the findings of Bucking and Wood (Bucking and Wood, 2008) in trout. Cooper and Wilson (Cooper and Wilson, 2008) hypothesized that the better regulatory capacity of voluntary-fed trout was due to a greater recycling of HCO_3^- into the intestine, although this was not measured. At present, it is unclear to what extent this occurs in elasmobranchs after feeding, but theoretical considerations and some measurements suggest it would be lower than in teleosts (Wilson et al., 2002; Taylor and Grosell, 2006a; Anderson et al., 2007). Clearly, further research is needed on the consequences of voluntary versus involuntary feeding in both teleosts and elasmobranchs, in light of the potential for additional central neuroendocrine regulation in voluntary feeding.

The present blood data confirm that there is no $P_{a}CO_2$ elevation (Fig. 4C) in the blood after feeding in the dogfish (Wood et al., 2005), and this pattern now appears to be true of teleosts also (Cooper and Wilson, 2008; Bucking and Wood, 2008). Owing to the low O_2 capacitance of water, fish do not have the luxury of restricting ventilation after a meal so as to retain respiratory CO_2, thereby effecting a partial respiratory compensation of the post-prandial metabolic alkalosis. This contrasts with the situation in most higher (air-breathing) vertebrates (Wang et al., 2001; Andrade et al., 2004). Instead, dogfish compensate by excreting the excess base across the gills. Tresguerres and colleagues (Tresguerres et al., 2007) demonstrated that the branchial mechanism of base excretion activated during the post-prandial alkaline tide in *Squalus acanthias* is the same as that earlier described in response to infusion with $NaHCO_3$ (Tresguerres et al., 2005; Tresguerres et al., 2006). In brief, this involves a microtubule-dependent translocation of existing vacuolar proton ATPase (V-H^+-ATPase) molecules from cytoplasmic storage vesicles to the basolateral membrane in a subpopulation of mitochondria-rich cells in the gills that are rich in carbonic anhydrase. The intracellular HCO_3^- ions left behind create an electrochemical gradient driving apical Cl^-–HCO_3^- exchange, such that base is secreted to the environment. The role of the kidney in acid–base regulation in elasmobranchs appears to be negligible (Hodler et al., 1955; King and Goldstein, 1983; Wood et al., 1995); nevertheless, it would be interesting to test whether the original 'alkaline tide' phenomenon reported in human urine (Bence-Jones, 1839; Roberts, 1859) could be detected in dogfish urine after a meal.

The chemistry of gastric chyme

The infused meal had a pH of 7.10 (Fig. 1), whereas, in fasted untreated dogfish, the small amount of gastric fluid has a pH of 1.77–2.05, which rises rapidly after a meal owing to the buffering action of the food (Wood et al., 2007b). Gastric fluid pH was not measured before the meal in either the vehicle-pre-treated- or omeprazole-pre-treated dogfish of the present study. Nevertheless, we assume that the chyme pH values measured at 12 h post-feeding (vehicle=3.65; omeprazole=4.55) reflect this buffering action of the food, with the omeprazole group starting from a higher gastric pH and/or exhibiting less new H^+ secretion into the chyme in the first 12 h. Based on autopsy of animals sacrificed at 48 h in the present study, chyme volume in the stomach was reduced at this time, but digestion was not complete in either pre-treatment group. This is in accord with our previous measurements after natural feeding (Wood et al., 2007b), where it took approximately 5 days for elimination of all chyme from the stomach and a return of gastric pH to the highly acidic values characteristic of fasting animals.

In mammals, as the rate of gastric secretion increases, the concentrations of $[H^+]$ and $[Cl^-]$ in gastric juice typically increase, whereas $[Na^+]$ falls, and $[K^+]$ changes very little because it is recycled as fast as it is absorbed (Davenport, 1982). These same basic patterns were seen in the chyme composition of vehicle-pre-treated dogfish, with $[H^+]$ increasing (i.e. pH falling) (Fig. 1), $[Cl^-]$ increasing (Fig. 2B), $[Na^+]$ falling (Fig. 2A) and $[K^+]$ (Fig. 2C) staying more-or-less constant from 12 h through 36 h post-feeding. These patterns were disrupted in the sharks pre-treated with omeprazole, such that $[H^+]$ (Fig. 1) and $[Cl^-]$ (Fig. 2B) increased more gradually, $[Na^+]$ was initially lower and rose rather than fell after 12 h (Fig. 2A), whereas $[K^+]$ fell slowly through 48 h. While these time-dependent trends are all consistent with omeprazole inhibition of the H^+ and accompanying Cl^- secretion mechanisms, it is not clear why chyme $[Na^+]$ was lower at 12 h in the omeprazole pre-treatment group, but it might reflect events occurring before the 12 h time-point. These events may include drinking of seawater.

Previously, Wood and colleagues (Wood et al., 2007b) studied the chemistry of the chyme in dogfish sacrificed at various times before and after natural feeding and concluded that seawater drinking actually occurs at a low level in fasted animals and continues at a comparable low rate after feeding. This is in accord with other recent studies on fasted animals (reviewed by Anderson et al., 2007) suggesting that drinking does occur at low levels in elasmobranchs, contrary to original belief (Smith, 1931; Smith, 1936). Thus, the increased water content of the chyme (relative to that of the original meal) at 12 h (Table 1) might have been due not only to secretion of gastric juice but also to seawater drinking, and the latter probably contributed to some degree to the much higher levels of $[Na^+]$ (Fig. 2A), $[Cl^-]$ (Fig. 2B), $[Mg^{2+}]$ (Fig. 3A) and $[Ca^{2+}]$

(Fig. 3B) in the chyme than in the original meal. In general [Na^+], [Cl^-], [K^+] and pH levels in gastric chyme of the present study were comparable to those seen after a natural meal (Wood et al., 2007b), whereas [Ca^{2+}] and [Mg^{2+}] levels were much lower. This undoubtedly reflects the virtual absence of bone in minced flounder muscle, in contrast to whole-fish meals.

We are aware of no previous measurements of total ammonia concentrations in fish chyme, but the present data (Fig. 3C) suggest that considerable amounts are generated in the digestive processes, raising levels far above those in the bloodstream. At the low pH present in the stomach, all ammonia would exist as NH_4^+, and toxicity should not be a problem. However, it would be interesting to know what happens when this chyme is neutralised in the intestine (Wood et al., 2007b). Perhaps the enzymes of the ornithine urea cycle known to be present in the intestinal wall (Kajimura et al., 2006) detoxify it to urea.

Conclusions

To summarise, the actions of omeprazole in the dogfish shark are generally consistent with those reported in higher vertebrates. These responses indicate that the systemic metabolic alkalosis and the massive excretion of basic equivalents to the external environment following a meal are largely caused by H^+ secretion (as HCl) in the stomach. Where sharks differ from higher vertebrates is in their inability to offset the systemic alkaline tide by CO_2 retention, but instead they rely on a powerful base excretion mechanism at the gills. Wood and colleagues (Wood et al., 2007a) calculated that, if this did not occur, blood pH would rise by at least 0.8 pH units after a meal, which undoubtedly would be fatal.

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