Is the Alkaline Tide a Signal to Activate Metabolic or Ionoregulatory Enzymes in the Dogfish Shark (*Squalus acanthias*)?

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ABSTRACT

Experimental metabolic alkalosis is known to stimulate whole-animal urea production and active ion secretion by the rectal gland in the dogfish shark. Furthermore, recent evidence indicates that a marked alkaline tide (systemic metabolic alkalosis) follows feeding in this species and that the activities of the enzymes of the ornithine-urea cycle (OUC) for urea synthesis in skeletal muscle and liver and of energy metabolism and ion transport in the rectal gland are increased at this time. We therefore evaluated whether alkalosis and/or NaCl/volume loading (which also occurs with feeding) could serve as a signal for activation of these enzymes independent of nutrient loading. Fasted dogfish were infused for 20 h with either 500 mmol L^{-1} $NaHCO_3$ (alkalosis + volume expansion) or 500 mmol L^{-1} NaCl (volume expansion alone), both isosmotic to dogfish plasma, at a rate of 3 mL kg^{-1} h^{-1}. $NaHCO_3$ infusion progressively raised arterial pH to 8.28 (control = 7.85) and plasma $[HCO_3^-]$ to 20.8 mmol L^{-1} (control = 4.5 mmol L^{-1}) at 20 h, with unchanged arterial P_{CO_2}, whereas NaCl/volume loading had no effect on blood acid-base status. Rectal gland Na^+,K^+-ATPase activity was increased 50% by NaCl loading and more than 100% by $NaHCO_3$ loading, indicating stimulatory effects of both volume expansion and alkalosis. Rectal gland lactate dehydrogenase activity was elevated 25% by both treatments, indicating volume expansion effects only, whereas neither treatment increased the activities of the aerobic enzymes citrate synthase, NADP-isocitrate dehydrogenase, or the ketone body–utilizing enzyme β-hydroxybutyrate dehydrogenase in the rectal gland or liver. The activity of ornithine-citrulline transcarbamoylase in skeletal muscle was doubled by $NaHCO_3$ infusion, but neither treatment altered the activities of other OUC-related enzymes (glutamine synthetase, carbamoylphosphate synthetase III). We conclude that both the alkaline tide and salt loading/volume expansion act as signals to activate some but not all of the elevated metabolic pathways and ionoregulatory mechanisms needed during processing of a meal.

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Introduction

After feeding, the ureotelic dogfish shark (*Squalus acanthias*) exhibits an intense activation of some of the key enzymes of the ornithine-urea cycle (OUC) in both skeletal muscle and liver (Kajimura et al. 2006). This serves to trap N in the valuable osmolyte urea; blood urea levels rise, but there is no increase in urea excretion (Kajimura et al. 2006, 2008). At the same time as this increased urea synthesis, there is also a marked activation of the enzymes of aerobic respiration, glycolysis, and ketone body utilization, especially in the rectal gland (Walsh et al. 2006). This small, digitiform organ in the posterior intestine was first identified as the major route of extrabranchial NaCl excretion by Burger and Hess (1960) and Burger (1962). Although this process has never been directly demonstrated, it seems very likely that after feeding, the gland excretes the excess NaCl load ingested with the meal. Certainly, feeding is followed by a marked activation of Na^+,K^+-ATPase activity in the rectal gland (MacKenzie et al. 2002; Walsh et al. 2006), in addition to activities of the other enzymes noted above.

Recently, we have shown that a classic "alkaline tide" (Wang et al. 2001; Niv and Fraser 2002), or metabolic alkalosis, also occurs in the bloodstream after a meal in the shark, presumably reflecting elevated HCl secretion by the gastric mucosa (Wood et al. 2005, 2007*b*). Interestingly, metabolic alkalosis created by $NaHCO_3$ infusion alone (in the absence of any nutrient loading) causes an apparent stimulation of urea production in this species, manifested as moderately increased urea excretion with unchanged blood urea levels (Wood et al. 1995). Could the alkaline tide serve as a signal to activate required postprandial metabolic processes? Our idea is not simply that increased substrate (i.e., HCO_3^-) availability is driving increased urea production, because we already know that ammonia is more potent

in this regard (Wood et al. 1995). Rather, we propose that metabolic alkalosis may act as a cue to increase the activity of the OUC in preparation for the increased ureagenesis that is associated with nutrient processing after a meal. Furthermore, metabolic alkalosis created by $NaHCO_3$ infusion also stimulates NaCl excretion by the rectal gland in vivo (Wood et al. 2007*c*), and the phenomenon can be duplicated in the isolated, perfused rectal gland in vitro (Shuttleworth et al. 2006), suggesting that the effect is direct rather than neurally or hormonally mediated. We therefore hypothesized that the alkaline tide may serve as a metabolic signal to activate the enzymes of the OUC in muscle and liver and/or the metabolic and salt-secretory enzymes of the rectal gland at a time when increased N trapping and NaCl excretion are required. This study therefore tests these hypotheses, employing a well-established $NaHCO_3$ infusion protocol (Wood et al. 1995, 2007*c*; Gilmour et al. 2001) to create metabolic alkalosis, together with equimolar NaCl loading and non-infused treatments, to separate the effects of alkalosis from those due to salt loading or volume expansion alone. The latter, for example, is well known to be involved in triggering rectal gland secretion (Solomon et al. 1984*a*, 1984*b*, 1985). The activities of key enzymes of the OUC, energy metabolism, ketone body utilization, and Na^+,K^+-ATPase activity were measured, together with selected plasma osmolytes and metabolites. The results support some elements of our hypotheses while tending to discount others.

Material and Methods

Experimental Animals

Experiments were performed on 23 male dogfish sharks (*Squalus acanthias*; 1.4–2.7 kg) that had been collected by trawl or by angling in Barkley Sound, British Columbia, in July and August 2005. 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. The fish were initially held for several weeks in a large group tank (200,000 L), where they were fed every third or fourth day with freshly thawed whole hake (*Merluccius productus*) at a ration of ~3% of body mass. After the final feeding, they were transferred to separate 1,500-L tanks (8–10 fish per tank) for 13 d of fasting. The tanks were served with running seawater at the experimental temperature (11° ± 1°C), salinity (32 ± 1 ppt), and pH (7.90 ± 0.15).

There were three treatment groups: controls (not cannulated or infused, $N = 8$), NaCl infused ($N = 7$), and $NaHCO_3$ infused ($N = 8$). The latter two groups were fitted with indwelling caudal artery catheters while under MS-222 (0.2 g L^{-1}) anesthesia on an operating table. These cannulae consisted of polyethylene PE50 tubing implanted through a small hole in the hemal canal via a 5-cm incision in the muscle of the caudal peduncle, as described by DeBoeck et al. (2001). The catheters were filled with 500 mM NaCl heparinized at 50 IU mL^{-1}. Wounds were dusted with powdered oxytetracycline to avoid infection, tightly closed with silk ligatures, and sealed with a sheet of rubber dental dam that was glued to the skin with tissue cement (3M Vetbond). After revival by irrigation of the gills with anesthetic-free water, the dogfish were transferred to covered 40-L wooden fish boxes that were coated with polyurethane, as used in an earlier study (Wood et al. 1995). The boxes were supplied with perimeter aeration and served with a vigorous flow of seawater (1 L min^{-1}) at experimental temperature. A recovery period of 36 h was allowed before experiments were started. Control dogfish were held in the same boxes for this period. The total fasting period of 15 d ensured that digestion of the final meal was complete (Wood et al. 2007*b*).

Experimental Procedures

Experimental dogfish were infused with either 500 mM $NaHCO_3$ (volume expansion + alkalosis) or 500 mM NaCl (salt loading/volume expansion alone) for 20 h at a nominal rate of 3 mL kg^{-1} h^{-1}, using individual channels of a Gilson Minipuls peristaltic pump, as described by Wood et al. (1995). These solutions were approximately isosmotic to dogfish blood plasma (Table 1), although the measured osmolality of the 500 mM $NaHCO_3$ solution (845 mOsm kg^{-1}) was about 8% less than that of the 500 mM NaCl solution (923 mOsm kg^{-1}) because of the lower osmotic activity of bicarbonate salts. Actual infusion rates over the period were monitored gravimetrically and were 3.264 ± 0.230 mL kg^{-1} h^{-1} for NaCl infusion and 3.384 ± 0.176 mL kg^{-1} h^{-1} for $NaHCO_3$ infusion, so the loading rates were 1,632 ± 115 mmol NaCl kg^{-1} h^{-1} and 1,692 ± 88 mmol $NaHCO_3$ kg^{-1} h^{-1}. Control animals were not infused. Blood samples (300 μL) for measurement of acid-base status were drawn anaerobically from the catheters before infusion and at 5, 12, and 20 h. Immediately after the final sample, the dogfish were killed with an overdose of MS-222, and a large blood sample (10 mL) was drawn by caudal puncture into a heparinized syringe. Plasma was obtained by centrifugation at 9,000 *g* for 2 min, and tissue samples of the liver, white muscle, and the rectal gland were quickly excised. All samples were immediately flash frozen with liquid N_2 and stored at −80°C for later analyses.

Analytical Techniques

Arterial blood pH was measured using a Radiometer (Copenhagen) combination electrode in a custom-built chamber kept at the experimental temperature with a water jacket. True plasma CO_2 was measured by the method of Cameron (1971) on plasma obtained from blood samples centrifuged in sealed tubes. Outputs of the electrodes (GK2401C for pH; E5046 for Pco_2 in the Cameron chamber) were displayed on Radiometer pHM 71 and pHM 72 acid-base analyzers. Arterial carbon dioxide tensions (Pco_2) and bicarbonate concentrations ($[HCO_3^-]$) were calculated using the solubility of carbon dioxide (α_{CO_2}), the apparent pK for dogfish plasma, and rearrangements of the Henderson-Hasselbalch equation according to Boutilier et al. (1984).

Plasma from terminal blood samples was analyzed using the

Table 1: Plasma osmolytes and metabolites in control (noninfused) and infused dogfish sharks

	Control ($N = 8$)	NaCl Infused ($N = 7$)	$NaHCO_3$ Infused ($N = 8$)
Na^+ (mmol L^{-1})	268.6 ± 2.9	284.7 ± 4.9*	281.1 ± 4.9*
K^+ (mmol L^{-1})	3.8 ± .1	3.7 ± .1	3.5 ± .1
Cl^- (mmol L^{-1})	251.1 ± 2.8	281.0 ± 4.3*	259.8 ± 4.3
Osmolality (mOsm kg^{-1})	917.1 ± 5.3	910.6 ± 4.2	909.0 ± 9.6
Urea (mmol L^{-1})	417.2 ± 13.4	408.5 ± 11.7	392.7 ± 5.7
Ammonia (μmol L^{-1})	7.2 ± 3.4	17.4 ± 8.6	14.3 ± 5.5
Glucose (mmol L^{-1})	6.3 ± .4	4.7 ± .6	5.4 ± .6
Free amino acid N (mmol L^{-1})	7.4 ± .4	5.7 ± .4*	5.4 ± .2*
β-hydroxybutyrate (mmol L^{-1})	10.7 ± 2.0	3.9 ± 1.0*	5.8 ± 1.9

Note. Infusion treatments consisted of either 500 mmol L^{-1} NaCl or 500 mmol L^{-1} $NaHCO_3$ at a nominal rate of 3 mL kg^{-1} h^{-1} for 20 h. Data are reported as means ± 1 SEM.

* $P < 0.05$ with respect to the control group. There were no significant differences between the $NaHCO_3$-infused and NaCl-infused groups.

following methods. Plasma [ammonia] was measured enzymatically (L-glutamate dehydrogenase; Raichem ammonia reagent, product 85446, San Diego, CA; Mondzac et al. 1965) on the first thaw of frozen plasma. Plasma total free amino acid levels (FAA-N) were measured using the ninhydrin assay (Moore 1968), with subtraction of previously measured ammonia-N concentration because of the partial detection of ammonia by the ninhydrin method (Kajimura et al. 2004). The correction was small, because plasma ammonia-N concentrations were less than 1% of FAA-N concentrations. Plasma [urea] was measured with the diacetyl monoxime method (Rahmatullah and Boyde 1980). Plasma [glucose] was determined with hexokinase reagent (Thermotrace kit 1542, Noble Park, Australia). Plasma [β-hydroxybutyrate] was measured enzymatically (β-hydroxybutyrate dehydrogenase) by the method of McMurray et al. (1984) with Stanbio Laboratory kit 2440 (Boerne, TX). Plasma [Cl^-] was measured by coulometric titration (Radiometer CMT-10), and osmolality was measured by vapor pressure osmometry (Wescor 5100C, Logan, UT). Plasma [Na^+] and [K^+] were determined by flame atomic absorption spectrophotometry (Varian AA-220, Mulgrave, Australia).

For analysis of the activities of most enzymes, tissues were homogenized on ice in 5–10 volumes of homogenization buffer (20 mmol L^{-1} K_2HPO_4, 10 mmol L^{-1} HEPES, 0.5 mmol L^{-1} EDTA, 1 mmol L^{-1} dithiothreitol, 50% glycerol adjusted with NaOH to pH 7.5 at 24°C) using a Brinkmann Polytron. Homogenates were centrifuged at 8,000 *g* for 20 min at 4°C in a Jouan CR412 (St. Herblain, France) centrifuge. The supernatant, or a 1 : 9 or 1 : 99 dilution in homogenization buffer, was used directly for assaying the activity of β-hydroxybutyrate dehydrogenase (BHBDH, EC 1.1.140), lactate dehydrogenase (LDH, EC 1.1.1.27), NADP-isocitrate dehydrogenase (ICDH, EC 1.1.1.41), citrate synthase (CS, EC 2.3.3.1), glutamine synthetase (GS, EC 6.3.1.2, assayed via the formation of γ-glutamyl-hydroxamate), ornithine-citrulline transcarbamoylase (OTC, EC 2.1.3.3), and carbamoylphosphate synthetase III (CPS III, EC 6.3.5.5) by previously described methods (Mommsen et al. 1980; Mommsen and Walsh 1989; Barber and Walsh 1993; Walsh et al. 1994). For the CPS III assay, we employed the colorimetric production of a citrulline CPS assay (Mommsen and Walsh 1989), using 1.7 mM uridine triphosphate (UTP), 20 mM ATP, 25 mM $MgCl_2$, 5 mM $NaHCO_3$, 5 mM *N*-acetylglutamate, 2 mM dithiothreitol, 5 mM ornithine, 20 mM glutamine, 25 mM phosphoenolpyruvate, 6 U mL^{-1} pyruvate kinase, 1 U mL^{-1} OTC, and 50 mM HEPES (pH 8.0).

For determination of Na^+,K^+-ATPase activity (EC 3.6.1.37), rectal gland tissue was homogenized on ice in SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) containing 0.1% Na^+-deoxycholate and centrifuged at 5,000 *g* for 30 s at 4°C. Supernatants were immediately frozen in liquid nitrogen and stored at −80°C until analyzed. ATPase activity (McCormick 1993) was determined in the presence or absence of 0.5 mM ouabain and normalized to total protein content (measured using the bicinchoninic acid method; Sigma-Aldrich, St. Louis, MO). Ouabain-sensitive ATPase activity was expressed as μmol ADP mg $protein^{-1}$ h^{-1}.

Statistical Analysis

Data have been expressed as means ± 1 SEM (*N*). Student's unpaired two-tailed *t*-tests were employed to detect significant differences ($P < 0.05$) between treatments; the Bonferroni correction for multiple comparisons was applied. Repetitive measurements of acid-base status on the same animals were made in the infusion experiments, so Dunnett's paired multiple-comparison test was used to compare treatment values with preinfusion measurements in the same animals.

Results

$NaHCO_3$ infusion caused a progressive metabolic alkalosis in the bloodstream, with arterial pH reaching 8.28 and plasma

[HCO_3^-] reaching 20.8 mmol L^{-1} by 20 h (Fig. 1). Arterial P_{CO_2} did not change significantly (2.12 ± 0.26 Torr [8] at 20 h vs. a preinfusion level of 1.53 ± 0.36 Torr [8]; data not shown). In contrast, NaCl infusion had no significant effect on blood acid-base status, with arterial pH staying around 7.85 and plasma [HCO_3^-] around 4.5 mmol L^{-1} (Fig. 1).

Both $NaHCO_3$ and NaCl infusion caused modest but significant increases (5%–6%) in plasma [Na^+], whereas plasma osmolality did not change (Table 1). As might be expected, plasma [Cl^-] also increased (by 12%) in response to NaCl loading but did not change in response to $NaHCO_3$ loading. Plasma [ammonia], [urea], [K^+], and [glucose] did not change significantly, although the latter three all tended to decline, likely reflecting the dilution effect of infusion. However, both free amino acid–N levels (25% declines) and β-hydroxybutyrate levels (45%–65% declines) decreased to a much greater extent in response to the infusions. The effects of NaCl versus $NaHCO_3$ loading appeared similar, although the decrease in [β-hydroxybutyrate] caused by the latter was not significant (Table 1).

Both of the experimental treatments caused significant increases in the Na^+,K^+-ATPase activity of the rectal gland (Fig. 2*A*), but the increase associated with $NaHCO_3$ infusion was more than twice as large as that associated with NaCl infusion, a significant difference. Na^+,K^+-ATPase activity in the $NaHCO_3$-loaded fish was slightly more than double that in the controls.

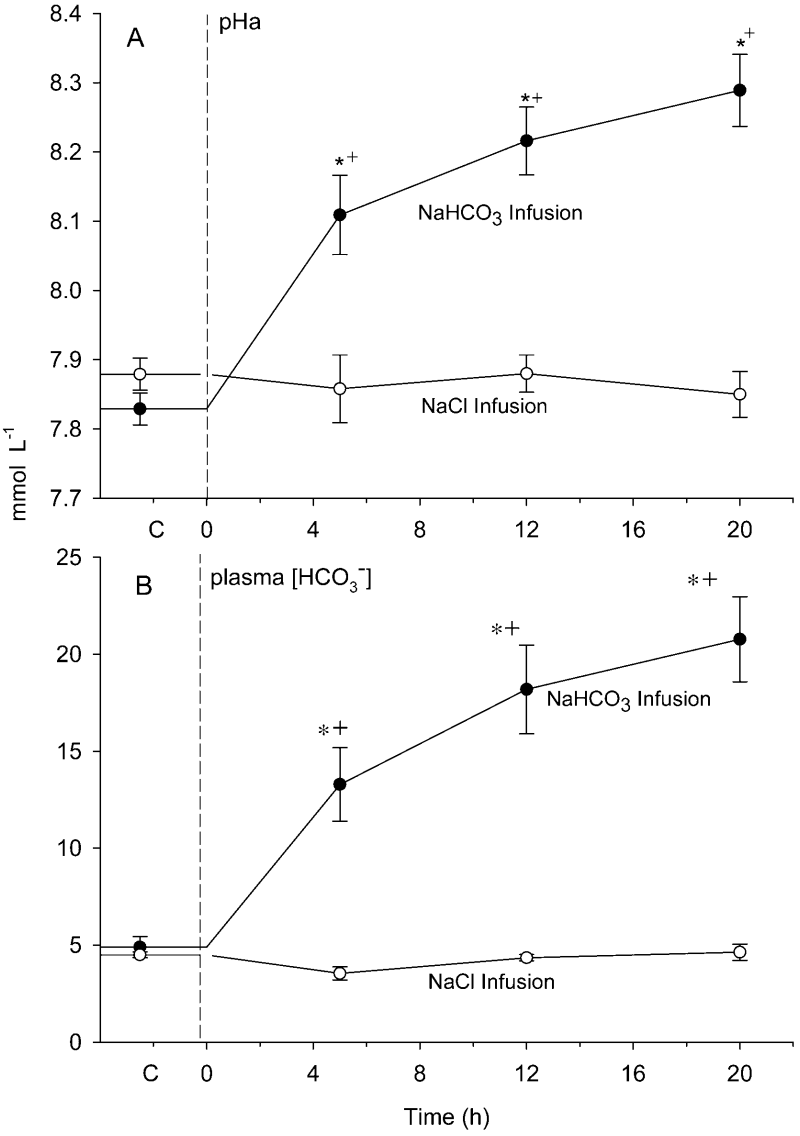

Figure 1. Influence of infusion for 20 h with either 500 mmol L^{-1} $NaHCO_3$ ($N = 8$) or 500 mmol L^{-1} NaCl ($N = 7$) at a rate of 3 mL kg^{-1} h^{-1} on (*A*) arterial blood plasma pH and (*B*) plasma bicarbonate concentration ([HCO_3^-]) in the dogfish shark. Means ± 1 SEM. Asterisks indicate significant differences ($P \leq 0.05$) between the two groups at the same time. Plus signs indicate significant differences ($P \leq 0.05$) within a treatment relative to the preinfusion control value "C."

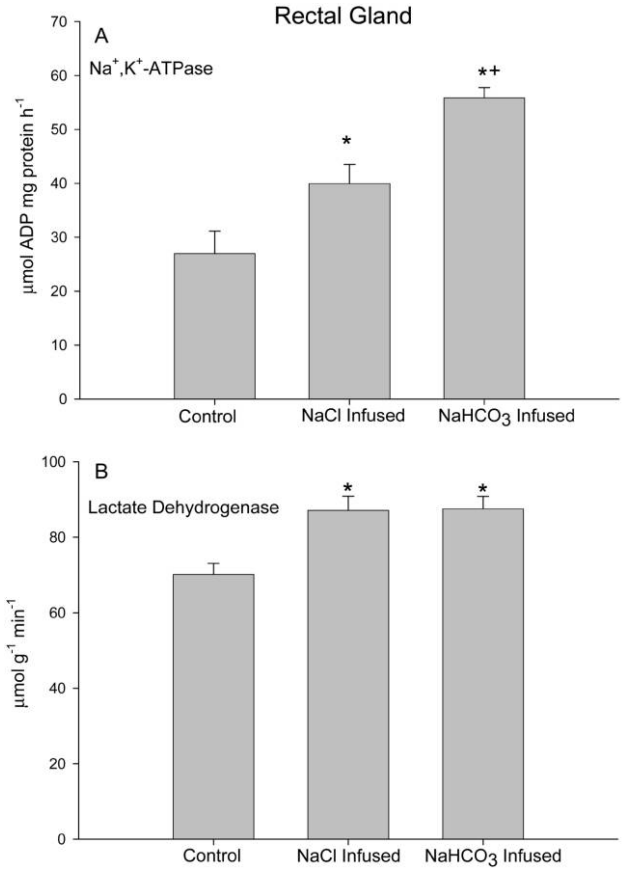

Figure 2. Activities of (*A*) Na^+,K^+-ATPase and (*B*) lactate dehydrogenase in the rectal glands of dogfish infused for 20 h with either 500 mmol L^{-1} $NaHCO_3$ ($N = 8$) or 500 mmol L^{-1} NaCl ($N = 7$) at a rate of 3 mL kg^{-1} h^{-1}, relative to those of noninfused control animals ($N = 8$). Asterisks indicate significant differences ($P \leq 0.05$) relative to the control group. The plus sign indicates a significant difference ($P \leq 0.05$) relative to the NaCl-infused group.

LDH activity of the rectal gland was elevated significantly by both infusion treatments (Fig. 2*B*). There was no differential effect of $NaHCO_3$ loading; both treatments caused 25% increases relative to noninfused control animals.

These stimulatory effects of infusion were not reflected in the activities of aerobic enzymes of the gland. CS activity was the same in the three treatments (Fig. 3*A*), whereas ICDH activity tended to fall, an effect that was significant only for the $NaHCO_3$ group, which exhibited a 20% decrease relative to the control group (Fig. 3*B*). ICDH was also measured in the liver and was unaffected by either of the experimental treatments (Table 2). Similarly, BHBDH was unaffected in both the rectal gland and the liver (Table 2). For both ICDH and BHBDH, activities were two- to threefold higher in the rectal gland than in the liver.

Two key enzymes of the OUC (CPS III and OTC), together with the important N-fixing enzyme GS, were also measured in the liver and in white muscle (Fig. 4). Neither GS (Fig. 4*A*) nor CPS III (Fig. 4*B*) exhibited any change in response to either of the infusion treatments, but OTC activity in white muscle doubled in response to $NaHCO_3$ infusion (Fig. 4*C*). OTC activity in the liver was unaffected, and there were no responses in the OTC activity of either tissue to NaCl loading. For both OTC and GS, activity was 10–20-fold higher in the liver than in muscle, while for CPS III, activity was similar in the two tissues.

Discussion

In general, the concentrations of all plasma components (Table 1) and the activities of all enzymes (Figs. 2–4; Table 2) in the fasted dogfish of this study were very similar to those we reported for fasted dogfish that had been collected a year earlier from the same area (Kajimura et al. 2006; Walsh et al. 2006). The one exception was rectal gland Na^+,K^+-ATPase activity (Fig. 2*A*), which was about fourfold higher in our study. This probably reflects the fact that a different homogenization buffer system (McCormick 1993) was employed specifically for Na^+,K^+-ATPase activity in our study, in contrast to the general

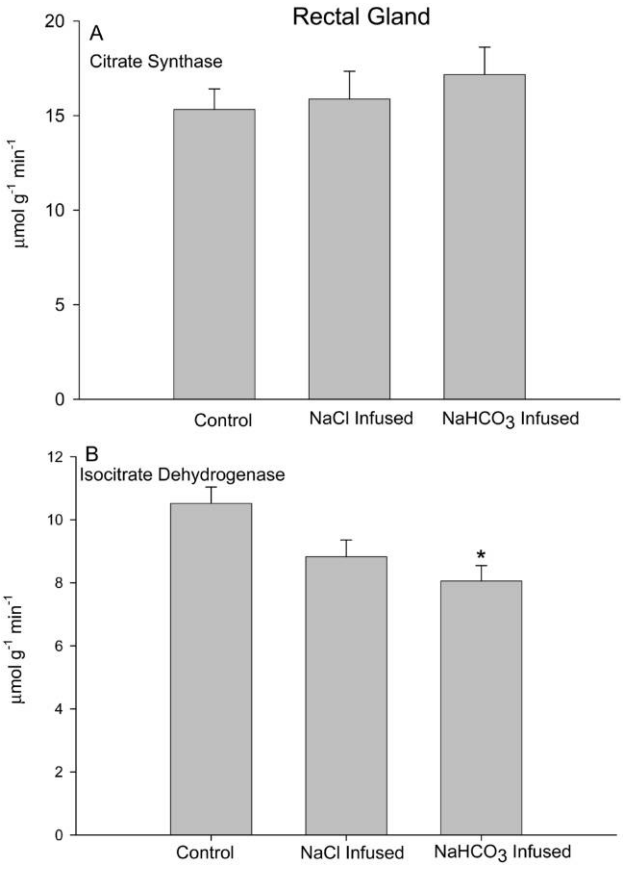

Figure 3. Activities of (*A*) citrate synthase (CS) and (*B*) isocitrate dehydrogenase (ICDH) in the rectal glands of dogfish infused for 20 h with either 500 mmol L^{-1} $NaHCO_3$ ($N = 8$) or 500 mmol L^{-1} NaCl ($N = 7$) at a rate of 3 mL kg^{-1} h^{-1}, relative to those of noninfused control animals ($N = 8$). The asterisk indicates a significant difference ($P \leq 0.05$) relative to the control group.

Table 2: Enzyme activities (μmol g^{-1} min^{-1}) in the livers and rectal glands of control (noninfused) and infused dogfish sharks

	Control ($N = 8$)	NaCl Infused ($N = 7$)	$NaHCO_3$ Infused ($N = 8$)
Liver ICDH	4.33 ± .29	4.37 ± .87	4.50 ± .55
Liver BHBDH	1.31 ± .15	1.89 ± .53	1.94 ± .42
Rectal gland BHBDH	4.65 ± .17	4.58 ± .30	4.12 ± .31

Note. Infusion treatments consisted of either 500 mmol L^{-1} NaCl or 500 mmol L^{-1} $NaHCO_3$ at a nominal rate of 3 mL kg^{-1} h^{-1} for 20 h. Data are reported as means ± 1 SEM. ICDH = NADP-isocitrate dehydrogenase; BHBDH = β-hydroxybutyrate dehydrogenase. There were no significant differences ($P > 0.05$) among the three groups.

glycerol buffer system used for all other enzymes. For the infused dogfish, samples were taken after 20 h of treatment, because this was the time of maximal activation of most enzymes (Walsh et al. 2006) and greatest plasma urea levels after a meal in two previous studies (Kajimura et al. 2006, 2008).

In this study, we elected to load the animals with either 500 mM NaCl or 500 mM $NaHCO_3$. These solutions were chosen for several reasons. First, because one of our goals was to evaluate whether alkalosis activated the OUC enzymes for urea production, we wished to avoid simultaneous loading with urea, which would occur with infusion of standard shark saline as the background solution. Second, in selecting 500 mM NaCl as the reference treatment, we used a solution that is approximately isosmotic to shark blood plasma and at the same time essentially identical to the secretion produced by the rectal gland in vivo (Burger and Hess 1960; Burger 1962; Solomon et al. 1984*b*; Wood et al. 2007*c*). Third, extensive earlier studies by Burger (1962) and Solomon et al. (1984*a*, 1984*b*, 1985) have investigated the relative roles of salt loading, plasma electrolytes, plasma tonicity, and volume expansion in stimulating rectal gland secretion and have concluded that the last factor (expansion of intravascular fluid volume) is the stimulus of primary importance. Nevertheless, our experiments cannot separate the effects of salt loading from those of volume expansion, and it is possible that different results might have been seen if the two stimuli were given separately, as done by Solomon et al. (1985), although in vivo, they would likely occur simultaneously after feeding. In this regard, experiments employing infusion of standard shark saline with normal plasma levels of urea and NaCl may prove useful in the future.

The strongest evidence in support of our original hypothesis that the alkaline tide can serve as a signal for enzyme activation independent of nutrient loading was provided by the more than twofold elevation of Na^+,K^+-ATPase activity in the rectal gland of $NaHCO_3$-loaded animals (Fig. 2*A*). Recently, we reported that metabolic alkalosis stimulates the secretory output of NaCl by the rectal gland both in vivo (Wood et al. 2007*c*) and in vitro (Shuttleworth et al. 2006). However, these studies provided little information as to the mechanism of action, apart from evidence that the effect appeared to be mediated via extracellular pH rather than by extracellular [HCO_3^-] or intracellular acid-base parameters. The present results demonstrate that at least in part, the effect is attributable to an increase in the activity of the key transport enzyme, Na^+,K^+-ATPase. Notably, both in vitro (Shuttleworth et al. 2006) and in vivo (Wood et al. 2007*c*), the content of acid-base equivalents in the secretion of the rectal gland and its contribution to overall acid-base balance are both negligible.

It is noteworthy that NaCl loading also caused a significant increase in Na^+,K^+-ATPase activity, albeit less than half that associated with $NaHCO_3$ loading (Fig. 2*A*). This is not surprising, as isosmotic volume expansion appears to be a key factor in initiating activation of rectal gland secretion in vivo, with the primary signal being the intravascular volume increase rather than the NaCl load (Solomon et al. 1984*a*, 1984*b*, 1985). Indeed, isosmotic NaCl infusion is a standard experimental treatment used to create brisk levels of rectal gland secretion (Wood et al. 2007*c*). The current model (reviewed by Silva et al. [1997, 1999]) assumes that oral intake of seawater or salty food during feeding results in volume expansion and that the resulting mobilization of C-type natriuretic peptide from the heart stimulates increased rectal secretion by direct (e.g., protein kinase C and guanyl cyclase pathways) and indirect pathways (vasoactive intestinal polypeptide release, which in turn activates the adenyl cyclase pathway). The additional stimulatory effect of postfeeding alkalosis (Wood et al. 2005, 2007*a*, 2007*b*) on Na^+,K^+-ATPase activity must now be superimposed on this framework.

In future studies, it will be of interest to test whether the effects of NaCl and $NaHCO_3$ loading are reflected in increased mRNA levels and greater synthesis of the enzyme or whether posttranscriptional activation of the existing enzyme is the explanation in *Squalus acanthias*. In the European dogfish *Scyliorhinus canicula*, an ever greater (50-fold) activation of rectal gland Na^+,K^+-ATPase activity after a meal of NaCl-laden food occurred in the absence of changes at the mRNA level, indicating posttranscriptional activation (MacKenzie et al. 2002).

It is very probable that other ionoregulatory tissues and enzymes may also be activated by postprandial alkalosis and/or salt/volume loading. The kidney appears to play no role in dynamic acid-base regulation (Hodler et al. 1955; King and Goldstein 1983; Wood et al. 1995) but does respond to volume loading with increased urine flow (Burger 1965; Solomon et al. 1984*b*). However, the gills respond to alkalosis. For example,

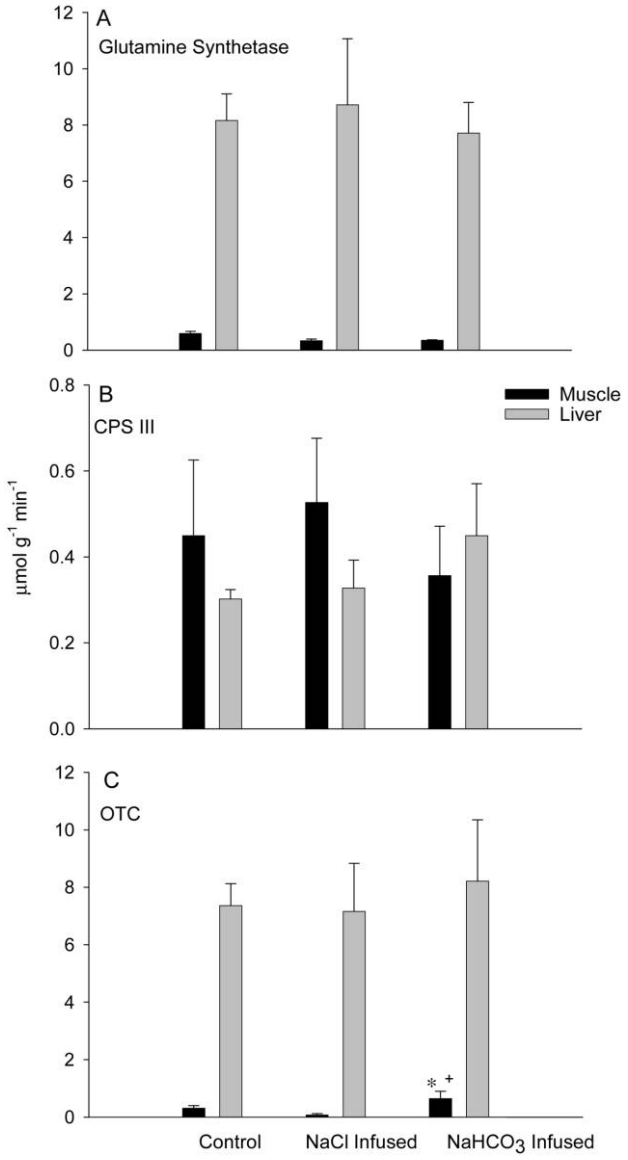

Figure 4. Activities of (*A*) glutamine synthetase, (*B*) carbamoylphosphate synthetase III (CPS III), and (*C*) ornithine-citrulline transcarbamoylase (OTC) in the skeletal muscle (*black bars*) and liver (*gray bars*) of dogfish infused for 20 h with either 500 mmol L^{-1} $NaHCO_3$ ($N = 8$) or 500 mmol L^{-1} NaCl ($N = 7$) at a rate of 3 mL kg^{-1} h^{-1}, relative to those of noninfused control animals ($N = 8$). The asterisk indicates a significant difference ($P \leq 0.05$) relative to the same tissue in the control group. The plus sign indicates a significant difference ($P \leq 0.05$) relative to the same tissue in the NaCl-infused group.

Tresguerres et al. (2005, 2006) have shown that metabolic alkalosis created by $NaHCO_3$ infusion stimulates a microtubule-dependent translocation of vacuolar proton ATPase (V-H^+-ATPase) molecules from cytoplasmic storage vesicles to the basolateral membrane in a subpopulation of mitochondrion-rich cells in the gills. With prolonged alkalosis, there is later synthesis of additional new V-H^+-ATPase molecules, as shown by western blotting. The basolateral V-H^+-ATPase activity appears to be fueled by the cytoplasmic carbonic anhydrase, and it returns H^+ ions to the blood. 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 (Wood et al. 1995). Recently, Tresguerres et al. (2007) reported that this same V-H^+-ATPase translocation, together with a twofold increase in V-H^+-ATPase abundance, occurs in gill samples taken from noninfused dogfish 24 h after a meal, which represents the time of greatest base excretion across the gills (Wood et al. 2007*a*).

While there was no evidence that the infusion treatments activated enzymes of aerobic metabolism (see below), it is interesting that both NaCl and $NaHCO_3$ loading increased rectal gland LDH activity by about 25% (Fig. 2*A*), very comparable to the increase reported at 20 h after feeding by Walsh et al. (2006). Therefore, this effect appears to be attributable to salt loading/volume expansion accompanying the meal rather than to alkalosis per se. The activated rectal gland contains substantial amounts of lactate (7 mmol kg^{-1}; Shuttleworth et al. 2006) but does not secrete it (Silva et al. 1980; Shuttleworth et al. 2006), and it appears to be unable to use lactate as a metabolic fuel, showing instead an absolute glucose dependency, which in turn permits the additional utilization of β-hydroxybutyrate (Walsh et al. 2006). It therefore appears very likely that the relatively high LDH activity of the gland is probably not for the use of lactate but rather for the production of lactate and that this glycolytic requirement increases when secretion is activated. Furthermore, the activation of secretion by volume loading in the absence of fresh aerobic substrates from a meal in the treatments of our study may have favored glycolytic support. Indeed, this may explain why the respiratory quotient of the activated gland was above 1.0 under these conditions in vitro (Shuttleworth et al. 2006). In vivo, Solomon et al. (1984*a*) have reported that the rectal gland of *S. acanthias* extracts 95% of the oxygen from the blood passing through it, both at rest and when activated, so the venous reserve for aerobic metabolism is negligible. Any extra energy demand may be supplied by anaerobic metabolism.

The lack of increase in CS, ICDH, and BHBDH activity in either rectal gland or liver (Fig. 3; Table 2) in response to the infusion treatments indicates that neither volume expansion nor the alkaline tide play a role in the marked activation of these enzymes reported by Walsh et al. (2006) in both tissues after a meal. Other factors (e.g., nutrient signals, hormonal controls) must be involved. It is curious that rectal gland ICDH activity actually tended to fall with infusion (an effect that was significant for $NaHCO_3$ loading; Fig. 3*B*), because of all the metabolic enzymes measured by Walsh et al. (2006), ICDH showed the greatest increase (>10-fold) in activity after a meal. ICDH is a decarboxylating enzyme producing CO_2 (Walsh and Henry 1991), which would be hydrated and produce HCO_3^-, so one possible explanation is that the rise in HCO_3^- served as an end-product signal to downregulate enzyme activity. It is also curious that plasma β-hydroxybutyrate concentrations fell after both infusion treatments, yet BHBDH activities were not

increased (Table 2). It has long been known that the levels of circulating ketone bodies increase greatly during starvation in elasmobranchs (Zammit and Newsholme 1979; Kajimura et al. 2008), but only recently has it been shown that they fall rapidly (within 6 h) after a meal (Walsh et al. 2006). Our results suggest that the volume expansion or salt loading accompanying a meal may cause an anticipatory increase in metabolism such that the rapid postprandial clearance of ketone bodies from the circulation may in some way be triggered before BHBDH activation by other signals. The utilization of free amino acids may be similarly stimulated (Table 1); both ketone bodies and amino acids appear to be important metabolic fuels in most elasmobranch tissues (Ballantyne 1997).

With respect to urea synthesis by the OUC, Kajimura et al. (2006) concluded that white muscle, rather than the liver, was the major site of urea synthesis in *S. acanthias* based on mass-weighted enzyme activities, although activities at both sites increased after feeding. Kajimura et al. (2006) reported that levels of the two key OUC enzymes CPS III and OTC were below detection limits in the gills in both fasted animals and at various times postfeeding, so the gills were not assayed in our study. Only one enzyme at one site, OTC in white muscle, responded to $NaHCO_3$ loading, and none responded to NaCl loading (Fig. 4). The increase in white muscle OTC activity caused by alkalosis was identical to that observed at 20 h after feeding (Kajimura et al. 2006). In general, the rate-limiting enzyme in fish systems is thought to be either GS, which provides the initial ammonia trapping as glutamine, or CPS III, the first enzyme of the cycle, which causes HCO_3^- to react with the amide group on glutamine; OTC, the second enzyme in the cycle, is generally not thought to be rate limiting (Anderson 2001). However, it is notable that while CPS III levels are high, OTC levels in shark muscle are only about one-tenth of those in two other fish where urea production in muscle appears to be important, the ureotelic Magadi tilapia (*Alcolapia grahami*; Lindley et al. 1999) and the little skate (*Raja erinacea*; Steele et al. 2005). The discovery of full OUCs in fish muscle is relatively new, and the enzymes are not yet well characterized. However, in at least one example, enzyme characteristics appear to be rather different from those of the traditional OUC in the liver (Lindley et al. 1999). It is possible that OTC may indeed be rate limiting in shark muscle and that its activation by the postfeeding alkaline tide will facilitate increased urea production from the ingested N load. Indeed, a similar $NaHCO_3$ infusion protocol induced a moderate increase in urea excretion with unchanged blood urea levels in an earlier study on the dogfish shark (Wood et al. 1995). It is also possible that changes in OTC activity are related to its parallel role in arginine biosynthesis.

The ideas of Atkinson and colleagues (e.g., Atkinson and Camien 1982; Atkinson 1992) that ureagenesis in higher vertebrates is geared primarily to the needs of acid-base regulation (i.e., metabolic removal of HCO_3^-) rather than those of N excretion have proven controversial (e.g., Halperin et al. 1986; Marsh and Knepper 1992). When the hypothesis was tested in ureagenic fish that possess the OUC, such as the gulf toadfish (*Opsanus beta*; Walsh et al. 1989; Barber and Walsh 1993), the Magadi tilapia (*Alcolapia grahami*; Wood et al. 1994), and the dogfish shark (Wood et al. 1995), the conclusion in all three was that while ureagenesis was moderately responsive to HCO_3^- supply, it was highly responsive to N supply. In our $NaHCO_3$ infusion experiments, only the HCO_3^- signal was supplied, whereas after a real meal, the combination of the alkaline tide and a surge in N supply might provide a much stronger signal, as well as an increased supply of both substrates for greatly increased urea production. Certainly, this idea could be tested in future experiments where OUC activity is measured after both ammonia and HCO_3^- loading in combination.

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