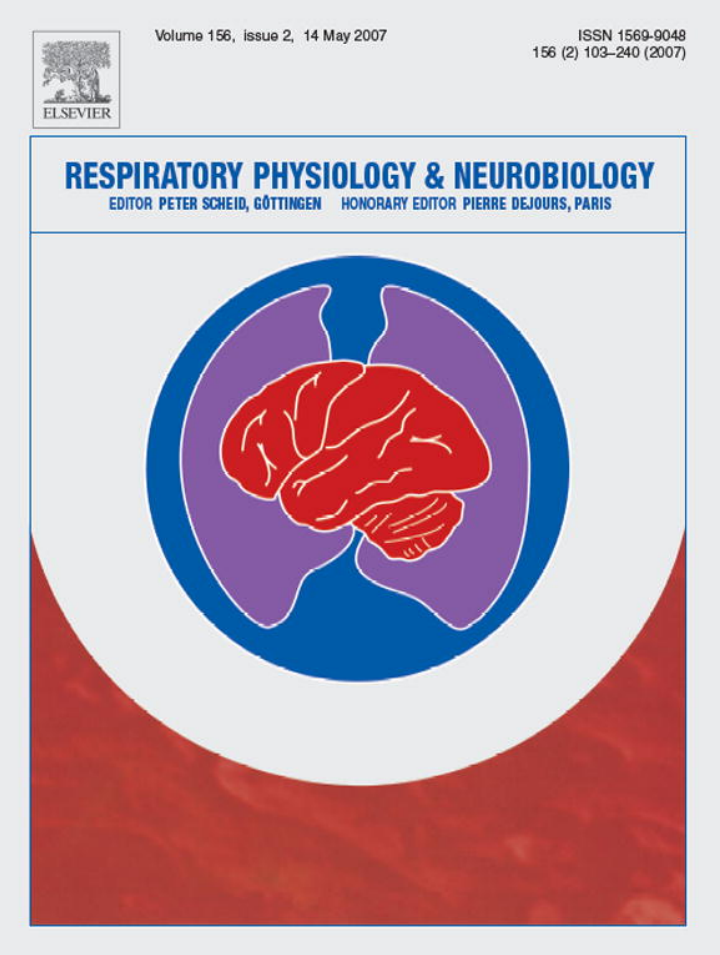

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ScienceDirect
Respiratory Physiology & Neurobiology 156 (2007) 220–228
RESPIRATORY PHYSIOLOGY & NEUROBIOLOGY
www.elsevier.com/locate/resphysiol

Control of rectal gland secretion by blood acid–base status in the intact dogfish shark (*Squalus acanthias*)

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Accepted 14 September 2006

Abstract

In order to address the possible role of blood acid–base status in controlling the rectal gland, dogfish were fitted with indwelling arterial catheters for blood sampling and rectal gland catheters for secretion collection. In intact, unanaesthetized animals, isosmotic volume loading with 500 mmol L^{-1} NaCl at a rate of 15 mL kg^{-1} h^{-1} produced a brisk, stable rectal gland secretion flow of about 4 mL kg^{-1} h^{-1}. Secretion composition (500 mmol L^{-1} Na^+ and Cl^-; 5 mmol L^{-1} K^+; <1 mmol L^{-1} Ca^{2+}, Mg^{2+}, SO_4^{2-}, or phosphate) was almost identical to that of the infusate with a pH of about 7.2, HCO_3^- mmol L^{-1} < 1 mmol L^{-1} and a P_{CO_2} (1 Torr) close to Pa_{CO_2}. Experimental treatments superimposed on the infusion caused the expected disturbances in systemic acid–base status: respiratory acidosis by exposure to high environmental P_{CO_2}, metabolic acidosis by infusion of HCl, and metabolic alkalosis by infusion of $NaHCO_3$. Secretion flow decreased markedly with acidosis and increased with alkalosis, in a linear relationship with extracellular pH. Secretion composition did not change, apart from alterations in its acid–base status, and made negligible contribution to overall acid–base balance. An adaptive control of rectal gland secretion by systemic acid–base status is postulated—stimulation by the "alkaline tide" accompanying the volume load of feeding and inhibition by the metabolic acidosis accompanying the volume contraction of exercise.

Keywords: NaCl excretion; Volume loading; Metabolic alkalosis; Metabolic acidosis; Respiratory acidosis; Shark

1. Introduction

Smith (1931) concluded that the urea-based osmoregulatory strategy of marine elasmobranchs must involve an extra-renal mechanism for the excretion of Cl^- at high concentration, but it was not until 30 years later that Burger, in a remarkable series of *in vivo* cannulation experiments, identified the organ involved as the rectal gland of the posterior intestine (Burger and Hess, 1960; Burger, 1962). These studies demonstrated that the rectal gland of the dogfish shark (*Squalus acanthias*) secreted an almost pure NaCl solution at a concentration of approximately 500 mmol L^{-1}, almost twice the NaCl level in blood plasma, but close to isosmotic with seawater and the urea-rich plasma. While composition was constant, secretion flow was intermittent and highly variable from time to time, presumably reflecting changing ionoregulatory demands and/or changing systemic physiology. Since that time, the gland has been developed as a powerful *in vitro* model for understanding the mechanism, intracellular signalling, and neuroendocrine control of NaCl secretion, using perfused whole gland preparations, perfused rectal gland tubules, cultured tubular epithelial cells, and membrane vesicles (reviewed by Epstein et al., 1983; Shuttleworth, 1988; Riordan et al., 1994; Silva et al., 1990, 1996, 1997; Hazon et al., 2003). However, surprisingly, there have been no further true *in vivo* studies on rectal gland function or its control in the last 40 years, presumably because of the difficulty of maintaining patent rectal gland catheters in intact, unrestrained sharks. Instead, a number of workers have profitably employed *in situ* preparations in pithed, artificially ventilated dogfish in which the spinal cord and

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doi:10.1016/j.resp.2006.09.003

brainstem were destroyed, so that the animals were incapable of movement. Perhaps the most important findings of such studies are that rectal gland secretion is primarily activated by volume expansion, and that a blood-borne humoral factor is involved (Solomon et al., 1984a,b, 1985).

The current model assumes that oral intake of seawater or salty food during feeding results in volume expansion, and the resulting mobilization of C-type natriuretic peptide (CNP) from the heart stimulates increased rectal gland blood flow and secretion. The latter may involve both direct mechanisms such as activation of protein kinase C and guanyl cyclase signalling pathways (Silva et al., 1999), and at least in *S. acanthias*, an indirect action by which CNP causes the release of vasoactive intestinal polypeptide (VIP) from nerve endings in the rectal gland, and VIP in turn activates the adenyl cyclase pathway (Stoff et al., 1979, 1988; Silva et al., 1987). A cascade of events (reviewed by Silva et al., 1997) is initiated by the various intracellular signals, resulting in the equimolar secretion of Cl^- by a transcellular pathway and Na^+ by a paracellular pathway, both ultimately powered by basolateral Na^+,K^+ ATPase activity. Isosmotic water flux is entrained by the net NaCl transport.

The gland has a high content of carbonic anhydrase (Maren, 1967; Lacy, 1983) though its function remains unclear. There are several reports using isolated-perfused preparations that show secretion is inhibited by low perfusate pH (Siegel et al., 1975; Silva et al., 1992), but that blockade of carbonic anhydrase has no effect (Siegel et al., 1975; Silva et al., 1977; Swenson and Maren, 1984). Effects in the intact animal are unknown, but the most detailed investigation to date employed an *in situ* gland preparation in pithed, artificially ventilated dogfish (Swenson and Maren, 1984). This study reported that severe but not moderate systemic acidosis inhibited secretion, whereas responses to carbonic anhydrase inhibitors were inconsistent. The few reports on the acid–base composition of rectal gland fluid are rather variable (Burger and Hess, 1960; Siegel et al., 1975; Swenson and Maren, 1984), but the latter authors discounted any significant role for the gland in systemic acid–base balance.

Our renewed interest in the possible acid–base control of gland function has been fuelled by recent reports demonstrating that systemic acid–base status can vary considerably in intact dogfish, including metabolic acidosis following exercise (Richards et al., 2003) and metabolic alkalosis ("alkaline tide") following feeding (Wood et al., 2005). Feeding is also followed by a marked activation of enzymatic activity in the gland, including Na^+,K^+ ATPase activity (Mackenzie et al., 2002; Walsh et al., 2006). Therefore in the present study, we returned to the original *in vivo* cannulation approach (Burger and Hess, 1960; Burger, 1962) to evaluate whether changes in acid–base status could serve as a controlling mechanism for gland function *in vivo*. Intact, unanaesthetized dogfish were subjected to volume expansion under several experimental acid–base treatments. Rectal gland secretion rates were recorded and detailed analyses of secretion composition and acid–base status were performed. The results demonstrate that systemic acid–base status has a profound effect on rectal gland function in intact, unanaesthetized dogfish in a manner which may have adaptive significance.

2. Materials and methods

2.1. Experimental animals and catheterization

Pacific spiny dogfish (*S. acanthias* L., 1.0–4.0 kg) were collected by angling or trawling (as bycatch from commercial shrimp fishing) in Barkley Sound, British Columbia, Canada over the course of three summers. At Bamfield Marine Sciences Centre, they were held in a large outdoor tanks (3000 L) served with running seawater at the experimental temperature ($11 \pm 1\,^{\circ}C$), salinity ($29 \pm 2‰$), and pH (7.9 ± 0.15) for a period of at least 1 week and up to 4 weeks prior to experimentation. While food (herring) was offered, the fish would not feed. 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.

Each dogfish was anaesthetized with MS-222, weighed, placed on an operating table, and fitted with an indwelling mesenteric artery catheter (Clay-Adams PE50 polyethylene tubing) for infusion and blood sampling, by the method of Graham et al. (1990). The catheter was filled with heparinised dogfish saline (sodium heparin, 50 i.u. mL^{-1}, saline recipe as in Pärt et al. (1998), but with the omission of PVP-40 and albumin). For rectal gland catheterization, a small incision was made in the body wall just anterior to the vent, and the lower intestine and rectal gland were exposed and gently retracted. The secretory duct, which runs in parallel to the large rectal gland vein in the intestinal wall, was identified, and catheterized via a small slit made in the serosal membrane. The catheter (120 cm long, typically PE50, or PE90 in larger animals, and filled with 500 mmol L^{-1} NaCl) was fire-polished at one end. The fire-polished tip was inserted approximately 1 cm into the secretory duct, taking care not to penetrate the gland itself, and then carefully tied in place with silk suture using shallow stitches. At this point it was particularly important to avoid tying off or puncturing the parallel rectal gland vein. Several further stitches were applied to firmly ligate the catheter as it ran anteriorly along the serosal surface of the intestinal wall, and then the lower intestine was returned to its original position, and the incision was closed with silk sutures. Additional sutures, a sleeve of larger PE tubing, and dental dam plus cyanoacrylate glue (3-M Vetbond) were used to anchor the catheter externally to the ventral skin. The animal was then revived and placed in an individual plexiglass chamber served with aeration and a constant flow (>0.5 L min^{-1}) of fresh seawater. Care was taken to ensure that the catheter always drained approximately 3 cm below the water surface. Animals were allowed to recover for at least 36 h before experiments commenced.

2.2. Infusion experiments

Rectal gland secretion flow proved to be negligible in these unfed resting dogfish. Therefore it was necessary to establish an infusion protocol which would create a brisk, stable secretory flow from the gland, against which the effects of acid–base manipulations could be tested. The goal was

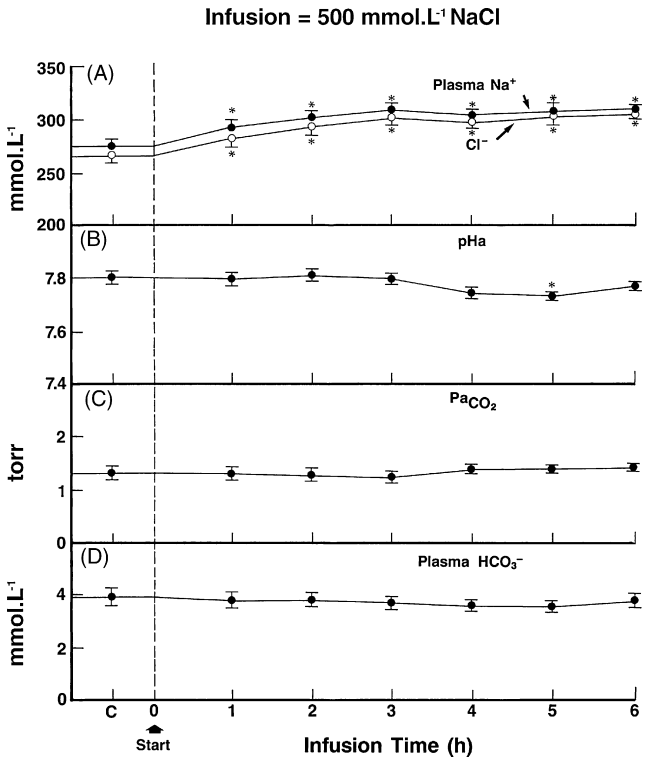

Fig. 1. The influence of infusion with 500 mmol L^{-1} NaCl at 15 mL kg^{-1} h^{-1} in the control treatment on: (A) plasma Na^+ and Cl^- concentrations; (B) arterial pH; (C) arterial CO_2 tension; (D) arterial plasma bicarbonate concentration. Means ± 1 S.E.M. ($N = 7$). Asterisks indicate means significantly different ($P < 0.05$) from pre-infusion values ("*C*").

to volume-load and NaCl-load the animal while creating minimal osmotic or acid–base disturbance. A survey (blood samples by blind caudal puncture) revealed the following plasma composition in this population of dogfish ($N = 16$): osmolality 893 ± 2 mOsm kg^{-1}, Na^+ 278 ± 3 mmol L^{-1}, Cl^- 275 ± 4 mmol L^{-1}, K^+ 3.22 ± 0.14 mmol L^{-1} and urea 379 ± 11 mmol L^{-1}. Therefore an infusate of 500 mmol L^{-1} NaCl, which has an osmolality (930 mOsm kg^{-1}) just slightly higher than that of blood plasma, was employed to establish baseline control conditions. After a number of preliminary trials, an infusion rate of 15 mL kg^{-1} h^{-1} was chosen, which created a brisk secretion flow which reached a plateau by 1.5 h and remained stable up to 6 h (see Section 3, Fig. 1). Experimental treatments were initiated at 3 h, using the 1.5–3.0 h period as the pre-treatment control. While experiments were terminated at 6 h, collection of rectal gland secretion continued up to 24 h to ensure that the preparation was still working. Routinely >70% of the total infused volume and NaCl loads were recovered in the collected rectal gland secretion over this time period. In order to conserve experimental animals, most dogfish were used for at least two and up to four experimental treatments, which were presented in random order, with an intervening recovery period of at least 48 h. Several control experiments repeated at the beginning and end of the series demonstrated that there was no deterioration of rectal gland performance over these multiple treatments.

2.3. Experimental series

The following experimental series were performed:

2.3.1. Control treatment

In this series ($N = 7$), dogfish were infused with 500 mmol L^{-1} NaCl at a rate of 15 mL kg^{-1} h^{-1} (range 13.5–16.5 mL kg^{-1} h^{-1}) for 6 h using a Gilson Minipuls peristaltic pump. The infusion reservoir was placed in a seawater bath to prevent thermal shock. Rectal gland secretion flow was collected over successive 0.5 h periods (12 in total), quantified gravimetrically, analysed immediately for acid–base status (pH and total CO_2) and frozen at −20 °C for later ionic analysis. Blood samples (0.6 mL) were drawn from the arterial catheter prior to the start of infusion, and then at 1, 2, 3, 4, 5, and 6 h. Blood samples were analysed immediately for arterial blood gases and pH, and a separate portion was immediately centrifuged (2 min at 9000 × *g*), then the plasma was aliquoted and frozen (−20 °C) for later ion measurements. The red cell pellet was re-suspended in dogfish saline, combined with blood recovered from the electrodes, and the original volume re-infused, so the impact of blood sampling on hematocrit was minimal.

2.3.2. High P_{CO_2} (respiratory acidosis) treatment

In this series ($N = 5$), the infusion protocol was identical to that of the control series, and the experiment continued with 500 mmol L^{-1} NaCl infusion up to 6 h. However, a high P_{CO_2} treatment was initiated at 3 h by changing the gassing via the airstone in the dogfish box from air to a 2% CO_2 mixture. This created a progressively increasing water P_{CO_2} which reached about 15 Torr by 6 h. In order to facilitate the timing of the experimental treatment, the blood samples during the control period were taken at 0.75, 1.75, and 2.75 h, while those during the experimental period were taken at 4, 5, and 6 h.

2.3.3. HCl (metabolic acidosis) treatments

In these two series, the blood sampling protocols were identical to that of the respiratory acidosis series. However at 3 h, the infusate composition was changed from 500 mmol L^{-1} NaCl to either 50 mmol L^{-1} HCl + 475 mmol L^{-1} NaCl ($N = 5$) or 100 mmol L^{-1} HCl + 450 mmol L^{-1} NaCl ($N = 6$). The partial adjustment of NaCl concentrations was chosen to ensure constancy of perfusate osmolality.

2.3.4. $NaHCO_3$ (metabolic alkalosis) treatment

In this series ($N = 7$), the protocol was identical to that of the metabolic acidosis series, except that at 3 h, the infusate was changed from 500 mmol L^{-1} NaCl to 100 mmol L^{-1} $NaHCO_3^-$ + 450 mmol L^{-1} NaCl. Again the partial adjustment of NaCl concentrations was chosen to ensure constancy of perfusate osmolality.

2.4. Analytical techniques

Blood samples were withdrawn via the arterial catheter into ice-cold gas-tight Hamilton syringes. Arterial blood pH (pHa)

and oxygen tension (Pa_{O_2}) were measured using Radiometer micro-electrodes kept at the experimental temperature with water jackets. Electrode outputs were displayed on Radiometer pHM 71 and pHM 72 acid–base analysers. True plasma CO_2 was measured on plasma obtained from blood samples centrifuged in sealed tubes. In some experiments, the measurements were made using a Corning 965 total CO_2 analyser and in others the method of Cameron (1971); the two yielded identical results, though the former was more rapid. The same analytical methods (pH, total CO_2) were used for rectal gland secretions. Because of the low buffer capacity of the secretions, it was necessary to flush the pH electrode capillary three times with the sample over a two minute period followed by a further 2 min of equilibration to achieve stable, reproducible values. Carbon dioxide tensions (P_{CO_2}) and bicarbonate concentrations ($[HCO_3^-]$) were calculated using the solubility of carbon dioxide (α_{CO_2}), the apparent pK (pK_{app}) for dogfish at the experimental temperature, and rearrangements of the Henderson–Hasselbalch equation according to Boutilier et al. (1984). Cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) in blood plasma and rectal gland secretions were measured by atomic absorption spectrophotometry (Varian 1275 AA). Chloride was measured by coulometric titration (Radiometer CMT-10) and osmolality by vapour pressure osmometry (Wescor 5100C). Total phosphate, and SO_4^{2-} in rectal gland secretions (also NO_3^-, NO_2^-, Br^-) were measured by HPLC (IC-Pak anion exchange column, Waters 510 pump, Waters 430 conductivity detector). Urea in the preliminary plasma composition survey was measured by the diacetyl monoxime method (Rahmatullah and Boyde, 1980).

2.5. Statistics

Data have been reported as means ± 1 S.E.M. (N). A paired experimental design was used in the infusion experiments, so Dunnett's paired multiple comparison test was used to compare treatment values back to reference measurements in the same animals. In the control series, blood parameters during infusion were compared back to the pre-infusion measurement. In the experimental series, values measured after initiation of experimental treatments (i.e. post 3 h) were compared with the preceding control measurements (the mean of the 1.5–3.0 h values for rectal gland secretion parameters, and the mean of the 1.75 and 2.75 h measurements for blood parameters). Specific differences between treatments were evaluated using Student's unpaired two-tailed t-test, with the Bonferroni correction applied when multiple comparisons were made. Regression lines were fitted by the least squares method, and the significance of the correlation coefficient assessed. A significance level of 0.05 was used throughout.

3. Results

3.1. Control treatment

Infusion of dogfish with 500 mmol L^{-1} NaCl at 15 mL kg^{-1} h^{-1} in the control treatment caused about 30 mmol L^{-1} increases in plasma Na^+ and Cl^- levels, which were significant by 1 h, and stable from 2 h onwards (Fig. 1A). Other ions (K^+, Ca^{2+}, and Mg^{2+}) were measured in blood plasma only at 0, 3, and 6 h. For all three, concentrations fell significantly by about 20% from 0 to 3 h, presumably due to dilution, but showed no further change at 6 h, stabilizing at the values shown in Table 1. Arterial blood acid–base status was unaffected by the control infusion, apart from a marginally significant decrease in pHa at 5 h (Fig. 1B). Pa_{CO_2} (Fig. 1C) and plasma HCO_3^- (Fig. 1D) remained unchanged. Pa_{O_2} also remained stable at 90–100 Torr (data not shown).

This control infusion caused rapid activation of the secretion rate of the rectal gland, which was negligible prior to this stimulation. Secretion flow and Cl^- output reached plateau values of about 4 mL kg^{-1} h^{-1} and 2 mmol kg^{-1} h^{-1}, respectively, by 1.5 h, which remained stable through 6 h of infusion (Fig. 2A). The composition of major ions (Na^+, Cl^-, K^+) in this secretion was also constant after 1.5 h (Table 2). The rectal gland produced a fluid almost identical to the original infusate in terms of NaCl concentration (i.e. close to 500 mM L^{-1}) with a low concentration of K^+ (4–6 mmol L^{-1}). Other ions (Ca^{2+}, Mg^{2+}, SO_4^{2-}, phosphate) were measured only on the 2.5–3.0 and 5.5–6.0 h samples; they similarly remained constant at the very low values (0.1–1.0 mmol L^{-1}) shown in Table 3. NO_3^-, NO_2^-, and Br^- were also detected in trace amounts (<0.1 mmol L^{-1}) but did not change with experimental treatments (data not shown).

Thus Na^+, Cl^-, and K^+ concentrations in rectal gland secretion (Tables 2 and 3) were all significantly higher than in blood plasma (Table 1), while the reverse was true for Ca^{2+} and Mg^{2+} concentrations. In view of the constancy of Na^+ and Cl^- levels

Table 1
Plasma ions at the end of 6 h infusion (15 mL kg^{-1} h^{-1}) in the control and experimental treatments

	Control (N = 7)	High P_{CO_2} (N = 5)	HCl (high) (N = 6)	$NaHCO_3$ (N = 7)
Na^+ (mmol L^{-1})	312 ± 8	312 ± 12	294 ± 7*	321 ± 6*
Cl^- (mmol L^{-1})	313 ± 16	309 ± 10	310 ± 7	310 ± 7
K^+ (mmol L^{-1})	2.3 ± 0.2	2.2 ± 0.4	2.6 ± 0.3	2.4 ± 0.4
Ca^{2+} (mmol L^{-1})	4.7 ± 0.2	4.8 ± 0.2	5.3 ± 0.2	4.5 ± 0.3
Mg^{2+} (mmol L^{-1})	2.8 ± 0.2	2.8 ± 0.1	3.1 ± 0.2	2.8 ± 0.2

All dogfish received the same control treatment up to 3 h; the experimental treatments were implemented at 3–6 h. Means ± 1 S.E.M. There were no significant differences (P > 0.05) from the respective values in the control treatment. The only treatment values which differed significantly (P < 0.05) from one another are marked with asterisks. Plasma Na^+ was also significantly elevated relative to the 3 h value (not shown) in the $NaHCO_3$ treatment; there were no other significant differences from respective 3 h values (end of control period, not shown) in the same treatment group.

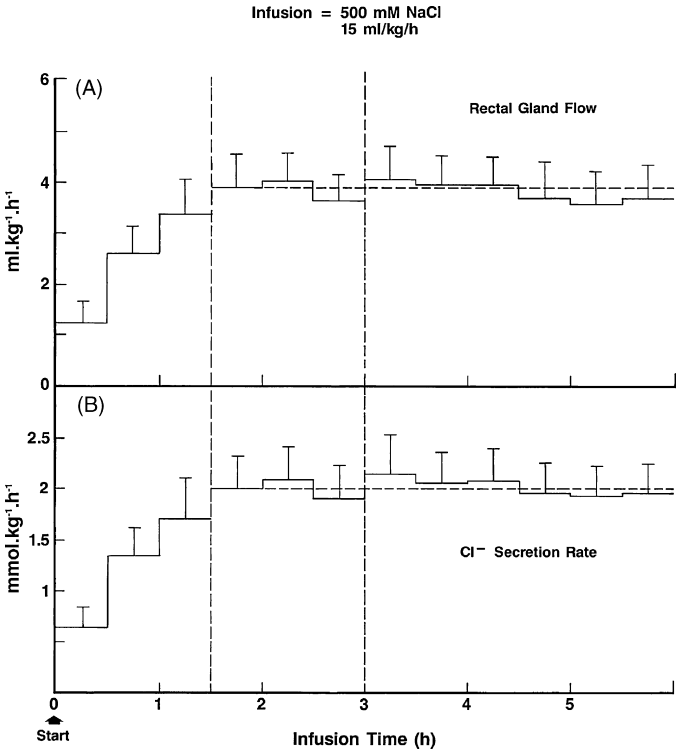

Fig. 2. The influence of infusion with 500 mmol L^{-1} NaCl at 15 mL kg^{-1} h^{-1} in the control treatment on: (A) rectal gland flow rate; (B) rectal gland Cl^- secretion rate. Means ± 1 S.E.M. ($N = 7$). Vertical dashed lines bracket the 1.5–3.0 period where flow rate and Cl^- secretion rate had stabilized. This was used as the reference period in subsequent experimental treatments. Horizontal lines indicate mean values during this period. There were no significant differences ($P > 0.05$) in the rates at 3–6 h (experimental period) from these reference control rates.

in the secretion, a situation which held true in all the experimental treatments as well (Table 3), rectal gland secretion rate has been portrayed in subsequent Figures as net Cl^- secretion rate. Net fluid and Na^+ secretion rates exhibited identical responses in all treatments (data not shown).

The acid–base status of the rectal gland secretion (Table 2) also stabilized by 1.5 h of control infusion, with P_{CO_2} levels only slightly (but significantly) lower than the Pa_{CO_2} of arterial blood (Fig. 1C), whereas pH was about 0.5 units lower than pHa (7.3 versus 7.8; Fig. 1B) and HCO_3^- was only about 20% of arterial plasma concentrations (Fig. 1D).

3.2. High P_{CO_2} treatment

Gassing the water with an elevated CO_2 in air mixture (2% CO_2) from 3 h onwards also caused a progressively developing respiratory acidosis and an inhibition of rectal gland secretion which stabilized at a 52% decrease by 4 h (Fig. 3). Pa_{CO_2} had increased to 8.5 Torr by 4 h, and 14 Torr by 6 h (Fig. 4C), resulting in a 0.6 unit fall in pHa (Fig. 3B). However pHa more or less stabilized after 4 h as plasma HCO_3^- increased (Fig. 3D), indicating partial metabolic compensation. Pa_{O_2} also increased significantly (data not shown). There were no changes in electrolyte concentrations in either the blood plasma (Table 1) or the rectal gland fluid (Table 3), but the acid–base status of the latter was markedly affected.

3.3. HCl treatment

The addition of 50 mmol L^{-1} HCl to the infusate at 3 h caused a small decrease in pHa (~0.16 unit) and rectal gland output (~22% by 6 h), neither of which were statistically significant (data not shown). However 100 mmol L^{-1} HCl elicited marked effects: a classic metabolic acidosis and a progressive decrease in rectal gland secretion rate which was significant by 4.0–4.5 h, and had reached 50% by 6 h (Fig. 4). Arterial pHa fell by 0.6–0.7 units by 6 h (Fig. 4B), a decline comparable to that with high P_{CO_2} exposure (Fig. 3B), and plasma HCO_3^- concentrations decreased by 75% (Fig. 4D). Pa_{CO_2} rose by about 60%, an effect which was significant only at 5 h (Fig. 4C), so there was also a slight respiratory component to the acidosis. There were no changes in Pa_{O_2} (data not shown), plasma ions (Table 1) or secretion fluid ionic concentrations and acid–base status (Table 3) associated with HCl infusion.

3.4. $NaHCO_3$ treatment

The addition of 100 mmol L^{-1} $NaHCO_3$ to the infusate at 3 h caused a progressively developing metabolic alkalosis and

Table 2
Concentrations of major ions (Na^+, Cl^-, K^+) and acid–base status (pH, P_{CO_2}, HCO_3^-) in rectal gland secretion in dogfish infused with 500 mmol L^{-1} NaCl at 15 mL kg^{-1} h^{-1} for 6 h in the control treatment

	Na^+ (mmol L^{-1})	Cl^- (mmol L^{-1})	K^+ (mmol L^{-1})	pH	P_{CO_2} (Torr)	HCO_3^- (mmol L^{-1})
0.0–0.5 h	499 ± 4	516 ± 5	5.8 ± 0.7	7.43 ± 0.07	0.95 ± 0.21	0.96 ± 0.25
0.5–1.0 h	504 ± 2	520 ± 3	5.8 ± 0.6	7.21 ± 0.08	0.98 ± 0.20	0.62 ± 0.12
1.0–1.5 h	489 ± 14	502 ± 16	5.2 ± 0.6	7.23 ± 0.07	0.95 ± 0.15	0.67 ± 0.10
1.5–2.0 h	514 ± 5	527 ± 7	5.6 ± 0.6	7.26 ± 0.09	0.84 ± 0.15	0.58 ± 0.06
2.0–2.5 h	516 ± 3	532 ± 3	4.8 ± 0.5	7.26 ± 0.07	0.77 ± 0.11	0.61 ± 0.11
2.5–3.0 h	515 ± 3	528 ± 5	4.8 ± 0.6	7.33 ± 0.07	0.62 ± 0.08	0.57 ± 0.11
3.0–3.5 h	514 ± 4	527 ± 5	4.9 ± 0.6	7.19 ± 0.07	0.97 ± 0.17	0.58 ± 0.08
3.5–4.0 h	508 ± 8	526 ± 8	5.0 ± 0.6	7.26 ± 0.08	0.92 ± 0.17	0.65 ± 0.12
4.0–4.5 h	514 ± 4	528 ± 5	4.8 ± 0.6	7.16 ± 0.09	1.11 ± 0.16	0.67 ± 0.12
4.5–5.0 h	515 ± 2	529 ± 5	4.9 ± 0.6	7.14 ± 0.08	1.06 ± 0.18	0.68 ± 0.20
5.0–5.5 h	508 ± 3	524 ± 5	4.7 ± 0.6	7.21 ± 0.08	1.12 ± 0.14	0.79 ± 0.18
5.5–6.0 h	517 ± 3	527 ± 6	4.5 ± 0.5	7.30 ± 0.07	0.90 ± 0.15	0.77 ± 0.18

There were no significant differences ($P > 0.05$) in any parameter from 1.5 h onwards.

Table 3
Concentrations of ions and acid–base status in rectal gland secretion at the end of 6 h infusion (15 mL kg^{-1} h^{-1}) in the control and experimental treatments

	Control (N = 7)	High P_{CO_2} (N = 5)	HCl (high) (N = 6)	$NaHCO_3$ (N = 7)
Na^+ (mmol L^{-1})	517 ± 3	495 ± 9	482 ± 13	510 ± 8
Cl^- (mmol L^{-1})	527 ± 6	509 ± 12	501 ± 16	521 ± 7
K^+ (mmol L^{-1})	4.5 ± 0.5	5.4 ± 1.5	5.1 ± 0.3	4.5 ± 0.4
Ca^{2+} (mmol L^{-1})	0.54 ± 0.16	0.48 ± 0.12	0.60 ± 0.12	0.37 ± 0.13
Mg^{2+} (mmol L^{-1})	0.12 ± 0.06	0.11 ± 0.07	0.07 ± 0.06	0.17 ± 0.14
SO_4^{2-} (mmol L^{-1})	0.10 ± 0.01	0.19 ± 0.02	0.11 ± 0.01	0.13 ± 0.09
Phosphate (mmol L^{-1})	0.11 ± 0.01	0.09 ± 0.01	0.21 ± 0.08	0.16 ± 0.02
pH	7.30 ± 0.07	6.88 ± 0.11*,#	7.19 ± 0.11	7.27 ± 0.19
P_{CO_2} (Torr)	0.90 ± 0.15	8.06 ± 1.00*,#	0.95 ± 0.24	0.92 ± 0.15
HCO_3^- (mmol L^{-1})	0.77 ± 0.18	2.53 ± 0.57*,#	0.65 ± 0.23	0.70 ± 0.11

All dogfish received the same control treatment up to 3 h; the experimental treatments were implemented at 3–6 h. Means ± 1 S.E.M.
* Significantly different (P < 0.05) from respective value in the control treatment.
# Significantly different (P < 0.05) from respective 3 h value (end of control period, not shown) in the same treatment group.

about a 20% increase in rectal gland output (Fig. 5). The elevation in secretion rate was significant and stable from 4.0 to 4.5 h onwards (Fig. 5A) despite a continuing rise in pHa which reached 0.15 units by 4 h and 0.3 units by 6 h (Fig. 5B). This was associated with a three-fold increase in plasma HCO_3^- concentration (Fig. 5D). There were also small but significant increases in Pa_{CO_2} throughout the period of $NaHCO_3$ loading (Fig. 5C) indicating a small respiratory compensation for the dominant metabolic alkalosis. There were no changes in Pa_{O_2} (data not shown) or secretion fluid ionic concentrations and acid–base status (Table 3) associated with $NaHCO_3$ infusion. However plasma Na^+ level had increased slightly by 6 h, and was also significantly higher than in the 100 mmol L^{-1} HCl treatment at this time (Table 1). There were no other effects on plasma ions.

4. Discussion

4.1. Secretory performance of the rectal gland in vivo

To our knowledge, the present records of rectal gland secretion rate and composition in intact, unanaesthetized *S. acanthias* are the first since the classic studies of Burger and Hess (1960) and Burger (1962). The measurements are difficult, both because of the delicate surgery involved in cannulating the rectal gland duct without damaging the parallel rectal gland vein, and because of the tendency for dogfish to tangle the catheter and/or pull it out of its collection vial by continual turning in the holding box. In practice, we found it useless to try to restrict the movements of the dogfish. Rather it was more productive to allow the animal to turn as it wanted over the first 24–48 h following cannulation, after which it would usually settle into one position for days on end, often wedged into one corner of its box. The outflow of the rectal gland collection catheter and the inflow of the arterial infusion catheter could then be positioned appropriately.

The concentrations of major ions in the secretion (essentially 500 mmol L^{-1} NaCl with 5 mmol L^{-1} of K^+ and very little else) and its constancy in the face of experimental perturbations (Tables 2 and 3) are in close agreement with the original data of Burger and Hess (1960) and Burger (1962), as well as with data from *in situ* preparations (Solomon et al., 1984a) and isolated-perfused glands (reviewed by Epstein et al., 1983). Our preliminary experiments indicated that the volume loading rate used (15 mL kg^{-1} h^{-1}) produced close to maximum secretion flow rates of about 4 mL kg^{-1} h^{-1} (e.g. Fig. 2). The elevated secretion rates generally continued for up to 20 h after the 6 h experimental infusion was stopped, resulting in excretion of >70% of the total volume load. These rates may be compared with very similar values for highest occasional individual rates (about 4 mL kg^{-1} h^{-1}) reported by Burger (1962) in spontaneously secreting intact dogfish, whereas overall mean rates in unstimulated animals were only 0.47 mL kg^{-1} h^{-1}. *In situ* preparations in volume-loaded, pithed dogfish also secreted at about 2–4 mL kg^{-1} h^{-1} (Solomon et al., 1984a,b, 1985; Swenson and Maren, 1984) and comparable rates have been seen with *in vitro* perfused gland preparations subjected to maximal stimulation by VIP or CNP (Silva et al., 1999) or directly through the adenyl cyclase system (Swenson and Maren, 1984; Silva et al., 1996).

There are few previous data on the acid–base composition of rectal gland fluid. In collections from unstimulated intact dogfish, Burger and Hess (1960) reported pH's (~6.8) about 1.0 unit below pHa, while Swenson and Maren (1984) reported similarly low secretion pH (6.7) accompanied by low HCO_3^- (1.2 mmol L^{-1}) but greatly elevated P_{CO_2} (7 Torr) in their maximally stimulated *in situ* preparation. Siegel et al. (1975) recorded a higher secretion pH (7.3) but very high HCO_3^- (7 mmol L^{-1}) in a perfused rectal gland preparation which was not stimulated. The present data for maximally stimulated glands in intact animals are much more extensive (Tables 2 and 3), and differ from all these earlier reports: secretion HCO_3^- was very low (<1 mmol L^{-1}) whereas secretion P_{CO_2} (about 1 Torr) was only slightly below Pa_{CO_2}, resulting in a pH about 0.5 units below pHa. These values fluctuated with experimental acid–base disturbance (Table 3), but the patterns were preserved. Overall, they suggest that the secretion barrier has low permeability to HCO_3^- but that the CO_2 tension of the secreted fluid is in approximate equilibrium with arterial rather than venous P_{CO_2}. Note that the very high background concentrations of Na^+ and Cl^- in the secretion renders strong ion difference analysis impossible. We also attempted to measure the buffer capacity of the secretion fluid by both CO_2 and strong acid titration, and found it to be negligible (<0.5 slykes) by both methods, in accord with its mini-

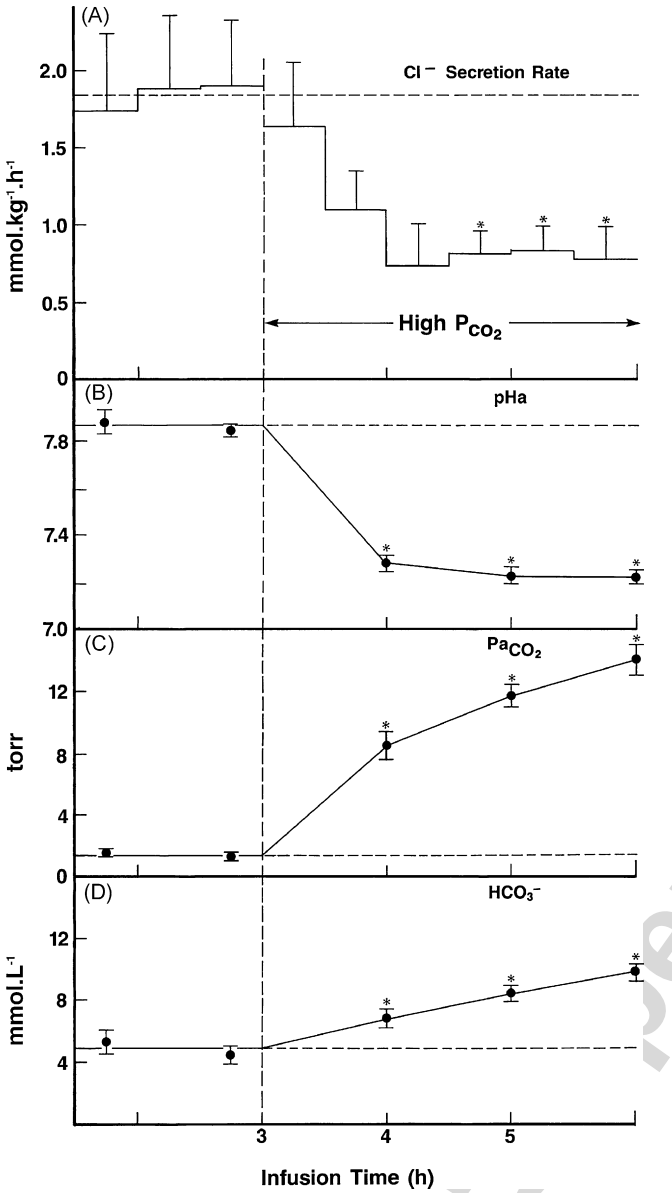

Fig. 3. The influence of exposure to high water P_{CO_2} (gassing with 2% CO_2) from 3 h onwards on: (A) rectal gland Cl^- secretion rate; (B) arterial pH; (C) arterial CO_2 tension; (D) arterial plasma bicarbonate concentration. Means ± 1 S.E.M. ($N = 5$). Horizontal lines indicate mean values during the pre-treatment reference period. Asterisks indicate values significantly different ($P < 0.05$) from the pre-treatment reference values.

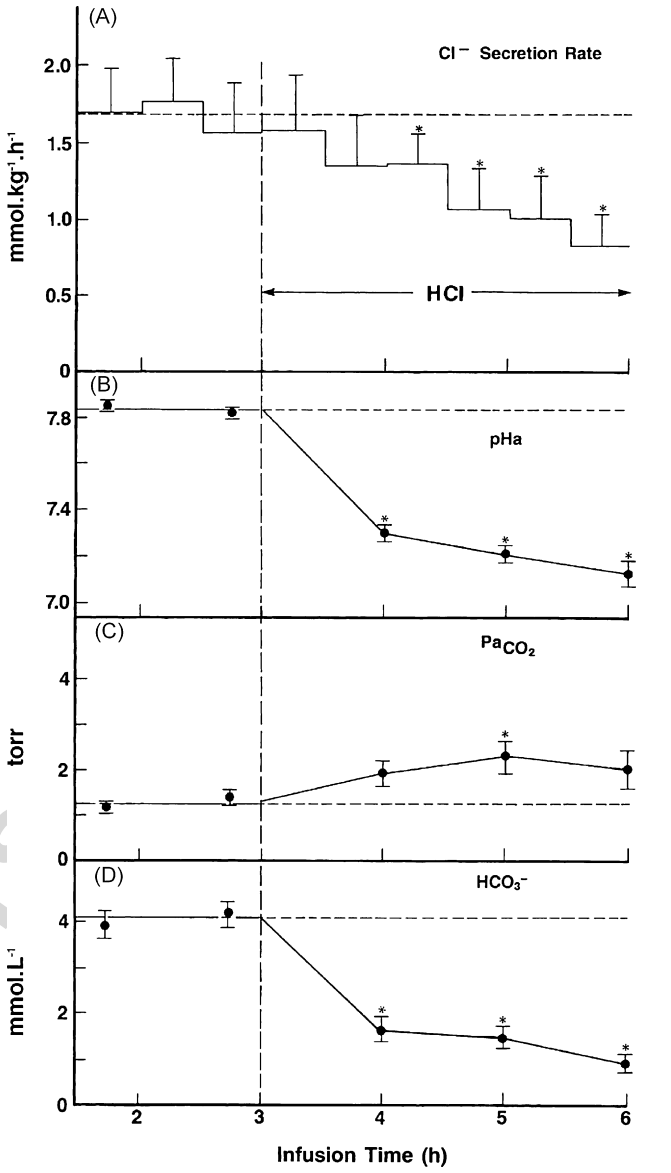

Fig. 4. The influence of addition of 100 mmol L^{-1} HCl to the infusate from 3 h onwards on: (A) rectal gland Cl^- secretion rate; (B) arterial pH; (C) arterial CO_2 tension; (D) arterial plasma bicarbonate concentration. Means ± 1 S.E.M. ($N = 6$). Horizontal lines indicate mean values during the pre-treatment reference period. Asterisks indicate values significantly different ($P < 0.05$) from the pre-treatment reference values.

mal phosphate content (Table 3). We are therefore in agreement with Swenson and Maren (1984) that the rectal gland plays a negligible role in systemic acid–base balance, simply because the buffer capacity and HCO_3^- content of its secretion are so small.

4.2. Acid–base control of rectal gland secretion

The present results demonstrate that the secretory function of the rectal gland in intact, unanaesthetized *S. acanthias* is strongly influenced by systemic acid–base status. This is the first study to show stimulation by alkalosis (Fig. 5) in any rectal gland preparation, and the first to show inhibition by acidosis (Figs. 3 and 4) in intact, unanaesthetized dogfish. Also unique to the present study is the finding that metabolic acidosis and respiratory acidosis of equal magnitude are approximately equipotent in causing inhibition (Figs. 3 and 4). Our results agree with earlier studies showing inhibition by acidosis in the perfused gland *in vitro* (Siegel et al., 1975; Silva et al., 1992) as well as with some of the data presented by Swenson and Maren (1984) on the *in situ* gland preparation in pithed, artificially ventilated dogfish.

The acid–base perturbations used here are within the ranges occurring naturally. For example, the metabolic acidosis observed after exhaustive exercise (depression of pHa by 0.35 pH units; Richards et al., 2003) was midway between those caused by the low doses (−0.16 units) and high doses

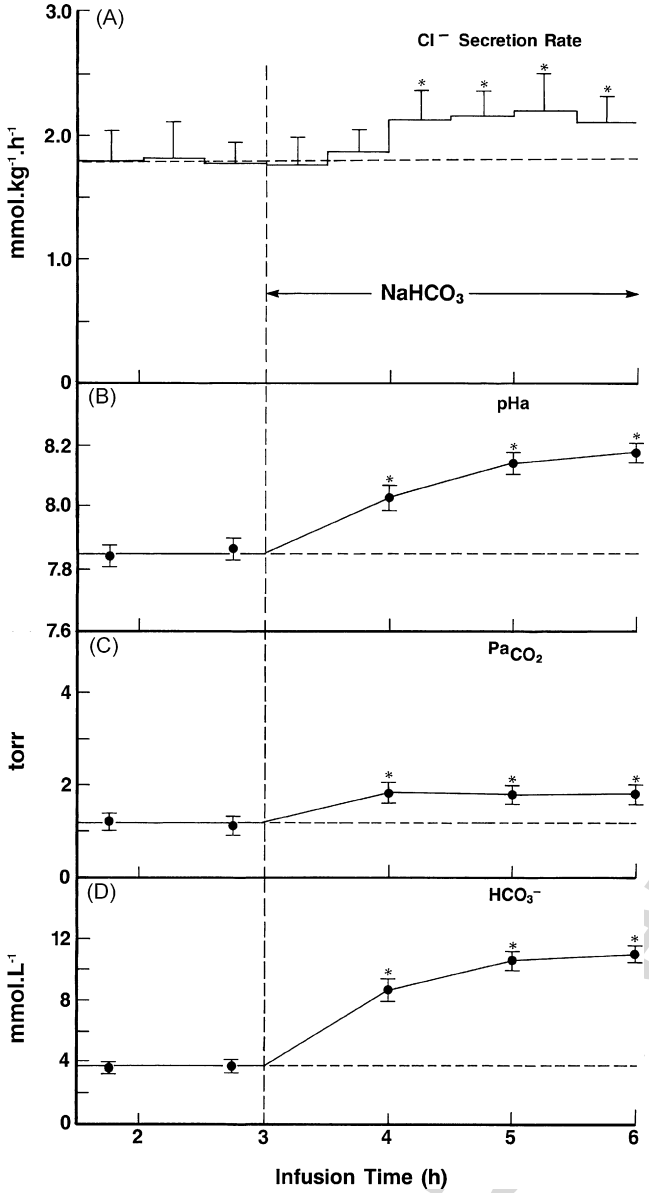

Fig. 5. The influence of addition of 100 mmol L^{-1} $NaHCO_3$ to the infusate from 3 h onwards on: (A) rectal gland Cl^- secretion rate; (B) arterial pH; (C) arterial CO_2 tension; (D) arterial plasma bicarbonate concentration. Means ± 1 S.E.M. ($N = 7$). Horizontal lines indicate mean values during the pre-treatment reference period. Asterisks indicate values significantly different ($P < 0.05$) from the pre-treatment reference values.

(−0.65 units) of HCl infusion, which resulted in 22% and 50% inhibitions, respectively, of rectal gland output. The metabolic alkalosis (elevation of pHa by 0.25 units; Wood et al., 2005) caused by the "alkaline tide" following feeding was comparable to that caused by $NaHCO_3$ infusion (+0.15–0.30 units), which resulted in a 20% elevation of rectal gland output. We suggest that these responses make sense in the context of the intact animal, because feeding will likely cause volume loading due to the ingestion of seawater and/or salty food, necessitating activation of rectal gland secretion (Mackenzie et al., 2002). Conversely, severe exercise will likely result in volume contraction associated with a shift of extracellular fluid into white muscle due to the osmotic gradient created by the high intracellular lactate load

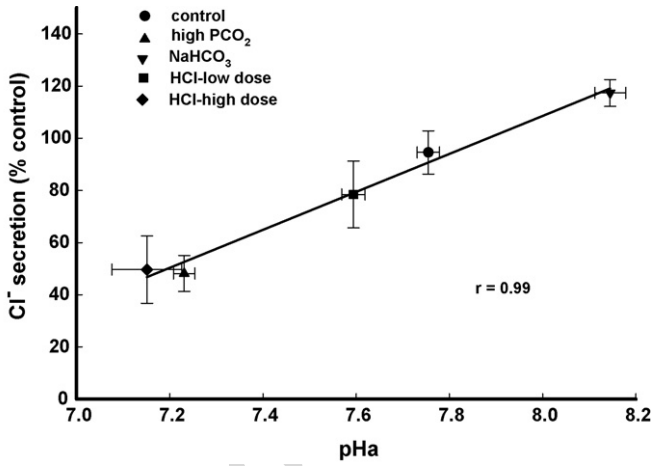

Fig. 6. The relationship between arterial pHa (X) and Cl^- secretion rate (Y) of the rectal gland at 6 h, relative to the reference value at 1.5–3.0 h, based on mean data for each of the experimental groups. Means ± 1 S.E.M. ($N = 5$–7 for each treatment). The equation of the regression line is: $Y = 72.75X - 473$ ($r = 0.99$, $P < 0.001$).

(Piiper et al., 1972; Holeton and Heisler, 1983; Richards et al., 2003). Inhibition of rectal gland output at this time would be adaptive.

An interesting question is whether the volume-loading used in the present study, which likely accompanies the post-feeding "alkaline tide" (Wood et al., 2005) and salt loading (Mackenzie et al., 2002) under natural circumstances, is synergistic or permissive to the effects of the alkalosis itself, or whether the effects are independent. If alkalosis alone were to activate rectal gland secretion, a direct regulatory mechanism would be indicated. In principle, it should be possible to answer this in future experiments by examining rectal gland secretion from non-volume-loaded dogfish subjected to acid–base manipulations, but whether this will be technically feasible remains to be seen.

Systemic acid–base status could alter gland performance at any point in the transduction pathway from volume detection through neuroendocrine communication, intracellular signalling and membrane level events in the rectal gland cells. However, the present data agree well with perfused gland studies where only extracellular pH was changed (Siegel et al., 1975; Silva et al., 1992), and all the experimental means appear to be in excellent agreement with a single regression line relating % change in rectal gland output to pHa (Fig. 6) regardless of the treatment used to change pHa. This suggests that extracellular pH, rather than HCO_3^-, P_{CO_2}, or intracellular acid–base status may be the master variable. It is problematical whether intracellular carbonic anhydrase (Maren, 1967; Lacy, 1983) plays a role in this acid–base responsiveness because carbonic anhydrase inhibition in perfused preparations was reported to have no effect on secretion rate (Siegel et al., 1975; Silva et al., 1977; Swenson and Maren, 1984). These are topics which are difficult to pursue in the intact animal because changes in systemic physiology may overshadow or confound changes mediated at the level of the rectal gland itself, a problem noted by Swenson and Maren (1984) in using drugs in their pithed, artificially ventilated preparation.

Therefore, having established the validity of acid–base control of rectal gland secretory rate *in vivo*, we have elected to explore these topics in greater depth by study *in vitro* (Shuttleworth et al., 2006).

Acknowledgements

Supported by an NSERC Discovery Grant to CMW. CMW is supported by the Canada Research Chair Program. We thank the director and staff of Bamfield Marine Sciences Centre for their help.

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