ACID-BASE EQUILIBRIA OF THE RECTAL GLAND OF SQUALUS ACANTHIAS: EFFECTS OF CARBONIC ANHYDRASE INHIBITION

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This report describes our further studies on the role of carbonic anhydrase in the rectal gland of S. acanthias. We have shown that marked inhibition of stimulated rectal gland secretion in vivo occurs with severe respiratory and metabolic acidosis, and inhibition of carbonic anhydrase (Swenson et al., Bull MDIBL 21:68, 1981). Since secretion could be blocked by extracellular acidosis, we proposed that carbonic anhydrase inhibition may result in an equivalent intracellular acidosis. Throughout the animal and plant kingdoms, the enzyme usually subserves H^+ or HCO_3^-. We have now made metabolic, hemodynamic and acid-base measurements in order to define the function of carbonic anhydrase in the rectal gland.

METHODS--Five female dogfish weighing 4.5-5.5 kg were anesthetized by pithing and placed in small boxes with flowing seawater (T=16°C). PE-90 polyethylene catheters were placed in the dorsal aorta, urinary papilla and rectal gland duct. Rectal gland secretion was stimulated by arterial infusion of an elasmobranch Ringer's solution at 30 ml/hr. By 2 hours a steady state rate of secretion was achieved which could be maintained indefinitely by continued infusion. At this point a three inch midline ventral incision was made proximally from a point one inch cephalad of the cloaca to expose the rectal gland vein and then the incision was held closed by several hemostats. After 2 hours of infusion, fish received 1 cc of 1 N NaCl or 30 mg/kg methazolamide or 1 mg/kg benzolamide in the arterial line. Throughout the experiment blood pressure, arterial pO_2, total CO_2, pCO_2 and pH; and rectal gland pO_2, total CO_2, pCO_2 and pH, flow rate and titratable acid were measured. Special care was taken to insure strict anerobic sampling. Two hours after drug or control saline injection 3000 units of heparin was given and a PE-90 catheter was advanced and secured in the rectal gland vein for duplicate measurements of venous pCO_2, pO_2, pH, total CO_2 and blood flow. Following these samplings and measurements, the rectal gland was resected, placed in an equal volume of 0.01 N NaOH and frozen for measurement of total CO_2. We also measured in vivo benzolamide concentrations in plasma, red cell and rectal gland two hours after the 1 mg/kg dose, and in tissue slices incubated at 16°C with an oxygenated Ringer's solution containing 5 mM glucose and 0.5 μM benzolamide.

Arterial, venous and rectal gland fluid pO_2 and pH were measured on a blood gas analyzer, equilibrated with standard gases and pH solutions at 16°C. Arterial, venous and rectal gland fluid total CO_2 were determined manometrically on a microgasometer. The pCO_2 of these samples was calculated by the Henderson-Hassalbalch equation using pK of 6.1 (Maren, Bull MDIBL 11:63, 1971) and a factor of 0.045 for the CO_2/HCO_3^- system. Rectal gland tissue total CO_2 was measured by use of the Conway diffusion method. Benzolamide levels and rectal gland enzyme concentration, activity and inhibition were measured by the micromethod of Maren (J. Pharma. Exp. Therap. 130:26, 1960) and calculated according to Easson and Stedman (Proc. R. Soc. Lond. Series B 121:142, 1936). Acid was measured by titration of 1 ml of rectal gland fluid to pH 7.7 with 0.1 N NaOH.

RESULTS AND DISCUSSION--Table 1 shows the volume stimulated control and carbonic anhydrase inhibited fish. The CO_2 and pH values of control gland fluid agree with those cited by Maren (In Sharks, Skates and Rays, ed. by Gilbert et al. Johns Hopkins Press, 1967) showing it to be a slightly acidic fluid essentially equilibrated with venous pCO_2 and without measurable buffer capacity (i.e., titratable acid <0.1 mM). The effects of full carbonic anhydrase inhibition by high dose metazolamide on acid base equilibria were striking, despite a 42% reduction in metabolic activity as measured by CO_2 output and O_2 consumption. The pH of fluid fell from 6.7 to 6.4 and pCO_2 rose from 7 to 26 mmHg and the HCO_3^- from 1.2 to 2.4 mM. The gland tissue total CO_2 fell from 18 to 11 millimoles/kg. The CO_2 values in venous blood showed qualitatively the same changes as rectal gland fluid. The arterial pCO_2

TABLE 1

	pO_2 mm Hg	pH	pCO_2 mm Hg	HCO_3^- mM	Flow ml/hr	$\dot{V}^*_{CO_2}$ µmol/hr	$\dot{V}^{**}_{O_2}$ µmol/hr	Cl^- mM
CONTROL (n=5)								
Gland Fluid	3	6.70	7	1.2	18	--	--	520
Arterial	118	7.72	4	6.7	--	--	--	--
Venous	5	7.60	6	9.1	132	--	--	--
Gland	--	--	--	18.0†	--	315	312	--
METHAZOLAMIDE+ (n=5)								
Gland Fluid	3	6.40	26	2.4	10.5	--	--	500
Arterial	116	7.55	7	9.0	--	--	--	--
Venous	9	7.45	10	11.1	78	--	--	--
Gland	--	--	--	11.0†	--	180	175	--
BENZOLAMIDE+ (n=2)								
Gland Fluid	3	6.50	17	1.8	14	--	--	--
Arterial	120	7.60	6	9.5	--	--	--	--

+ 2 hours after drug administration.

* Calculated as $[([CO_2]_V-[CO_2]_A)\cdot\text{blood flow}] + [(CO_2)_{RGF}\cdot\text{rectal gland fluid flow}]$.

** Calculated as $[(Cl)_{RGF} \cdot \text{Gland Fluid flow} : 30]$. See Silva et al., J. Memb Biol 53:215, 1980.

† m moles/kg gland

and HCO_3^- rose with inhibition and the pH fell, reflecting the respiratory acidosis secondary to inhibition of gill and erythrocyte carbonic anhydrase (Swenson, Hildesley and Maren, this volume).

Benzolamide (1 mg/kg) was also used, because at this dose red cell carbonic anhydrase is not inhibited (Swenson et al., vide supra) and we wanted selective inhibition in the rectal gland. The carbonic anhydrase concentration of the rectal gland is 6 µmoles/kg; benzolamide incubated with the gland, or injected into the whole animal, gave on the average (n=4) the same concentration. For a K_i of 10^{-8} M against the enzyme and the observed plasma concentration of 1 µM, we calculated 99% inhibition at the gland, assuming that drug in plasma is in equilibrium with tissue enzyme. The drug measurements in the gland, as given above, confirm this idea.

The lesser effect of benzolamide (Table 1) compared to methazolamide is probably explained by a lesser degree of inhibition at the much lower dose level. However, the benzolamide data seem to rule out the red cell as mediator of the effect.

Both drugs, at these dosages, inhibit gill enzyme and lead to respiratory acidosis (Swenson, vide supra). However we feel that this is not the basis for their pharmacological effect on the rectal gland, since their local effect (i.e., in gland fluid) in elevating pCO_2 is much greater than seen in the blood (Table 1).

These data suggest that rectal gland carbonic anhydrase promotes a proper acid base milieu for stimulated fluid secretion. Inhibition of the enzyme results in profound intracellular acidosis as evidenced both by an elevation of gland fluid pCO_2 and reduction of total CO_2 of the gland. In the normal animal the pCO_2 of rectal gland fluid is indistinguishable from that of end capillary and venous pCO_2, but with inhibition a gradient of 16 mmHg develops between fluid and venous blood. We believe that rectal gland fluid pCO_2 probably equals intracellular pCO_2, since this fluid resides longer in contact with the cells and has no buffering capacity. During inhibition with methazolamide, the high end capillary pCO_2 (reflecting a raised intracellular pCO_2) is not seen in venous blood, because concurrent red cell inhibition prevents instantaneous equilibration between pCO_2 and HCO_3^-. One may estimate the intracellular pH (pHi) by using the total CO_2 of gland and equating fluid pCO_2 with intracellular pCO_2. Such a calculation gives values of pHi of 7.85 in the normal and 7.07 during inhibition. We showed (Swenson et al., Bull. MDIBL 21:68, 1981) that an acute respiratory acidosis (pH_a=7.05 P_aCO_2=30) and presumably and equivalent intracellular acidosis imposed by ventilation with hypercapnic seawater caused a 60% reduction in fluid secretion. These measurements and calculations are consistent with the concept that rectal gland carbonic anhydrase facilitates transport of carbon dioxide between sites of metabolism and capillary blood over small pCO_2 gradients. The enzyme promotes rapid interconversion of CO_2 and HCO_3^-, allowing a greater fraction of metabolically generated CO_2 to diffuse as HCO_3^- through the cell. This prevents large elevations in pCO_2 and hydrogen ion concentration. The finding that total gland CO_2 fell with inhibition was unexpected and evades easy explanation. It may reflect decreased CO_2 production attendant on a fall in secretion, or may represent inhibition of a process (dependent on carbonic anhydrase) whereby the cell is able to excrete acid (presumably into blood) to counter the acidifying effects of metabolism.

In conclusion, the role of carbon anhydrase in the rectal gland appears to be in the dissimilation of metabolic CO_2. Enzyme inhibition generates high pCO_2 in the rectal gland fluid, and the resulting low intracellular pH reduces the normal rate of saline-induced secretion of NaCl. This work was supported by NIH grant HL-22258.

FURTHER STUDIES ON OUABAIN BINDING IN THE RECTAL GLAND

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Stimulation of rectal gland secretion by dibutyryl cAMP and theophylline increases the binding of ouabain by rectal gland cells, but the mechanism of the increase is not clear. We have previously shown that maneuvers that interfere with sodium chloride cotransport do not prevent this phenomenon in rectal gland slices (Bull MDIBL 21:103, 1981). Both bumetanide 10^{-5}M and removal of sodium or chloride or both were used to interfere with sodium and chloride cotransport. Stimulation of ouabain binding in the presence of bumetanide 10^{-5}M could be due to the use of insufficient amount of the inhibitor, therefore, the experiment was repeated using a concentration ten-fold larger. Binding was measured using the technique previously described (Bull MDIBL 21:103, 1981). As shown in Table 1,

Table I

Effect of bumetanide on the stimulation with dibutyryl cyclic AMP (10^{-3}M) and theophylline (10^{-3}M) of ouabain binding by rectal gland slices.

	10^{-9}M ouabain		10^{-8}M ouabain		mean % increase with stimulation
	Basal	Stimulated	Basal	Stimulated	
Control	14.3	26.9	154.4±16.4(3)	226.4±20.5(3)	58±11(4)***
Bumetanide 10^{-5}M	15.2	19.8	186.2±14.5(3)	278.3±32.1(3)	46±16(4)*
Control			113.3±10.9(6)	173.0±20.8(6)	55±12(6)***
Bumetanide 10^{-4}M			139.3±14.2(3)	200.7±35.5(6)	42±16(6)**
Solvent			144.3±19.8(4)	179.8±8.5(4)	30±13.4(4)*

Units are picomoles of ouabain bound per gram wet weight. Values are Mean ± SEM(n)
* $p < 0.05$
** $p < 0.025$
*** $P < 0.01$

theophylline and dibutyryl cyclic AMP increased ouabain binding even in the presence of bumetanide 10^{-4}M. Thus, the increased ouabain binding after stimulation with theophylline and dibutyryl cyclic AMP appears to be independent of the entry of sodium and chloride into the cell via the coupled sodium and chloride carrier system since high concentrations of bumetanide fail to alter the increase in binding at a time when coupled entry of NaCl should be slowed or stopped.

If the effect of dibutyryl cyclic AMP to increase ouabain binding were mediated through an increase in Na-K-ATPase activity secondary to the admission of sodium into the cell, other maneuvers that increase sodium influx should have the same effect. Monensin, an ionophore with a cation selectivity $Ag^+ > Na^+ > K^+ > Rb^+ > Cs^+ > Li^+ > NH_4^+$, at a concentration of 1.5×10^{-5}M, did not (control 129.4±7.7, monensin 144.5±16.5, solvent 137.4±6.0, $p < 0.1$). In two of these slice preparations monensin increased ouabain inhibitable oxygen consumption from an average of 29.4 to 36.7 micromoles of O_2/hr/g wet weight or 26±2%, confirming the action of monensin in these concentrations to activate Na-K-ATPase by enhancing sodium entry. Since monensin, which increases the entry of sodium into the cell as judged by the increase in ouabain inhibitable oxygen consumption, does