These studies complement our previous experiments on the mechanism of chloride transport by the perfused rectal gland of the spiny dogfish. Evidence for the dependence of active secretion on Na-K-ATPase is strengthened by the fact that removal of potassium from the perfusion medium stops ion movement across the rectal gland. Secretion is dependent not only on the presence of sodium but also on that of chloride in the perfusate. Lithium cannot replace sodium and reversibly inhibits chloride secretion. Anions such as nitrate and acetate cannot replace chloride or do so to an extremely limited extent. They inhibit sodium secretion in a linear fashion and show clear dependence of sodium transport on chloride. Bromide can partially replace chloride in this system. Chloride secretion by the shark rectal gland is consistent with the presence of a linked sodium and chloride carrier that transports chloride into the cell against an electrochemical gradient. Na-K-ATPase is essential to maintain a gradient powering the downhill movement of sodium into the cell. The carrier that moves both sodium and chloride appears to be very selective for these ions.

THE OXYGEN COST OF CHLORIDE TRANSPORT IN THE RECTAL GLAND OF THE SPINY DOGFISH *Squalus acanthias*

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The isolated perfused rectal gland of the spiny dogfish, *Squalus acanthias*, offers many advantages for the study of the relationship between oxygen consumption and electrolyte transport. The artery and vein are cannulated and readily accessible for oxygen measurement. The rate of rectal gland secretion can be varied at will over a range that is tenfold that of basal secretion by stimulation with cAMP and theophylline. It remains viable for long periods of time and is perfused with an artificial medium lacking erythrocytes. We have previously shown that chloride secretion by the rectal gland is linearly related to oxygen consumption (Rosa et al. Bull. MDIBL 16:87, 1976). This relation was further studied in the present series of experiments.

Spiny dogfish weighing 2 to 7 Kg were taken by hook and line from Frenchman Bay, Maine and kept in marine live cars until killed, usually within three days. The dogfish were killed by segmental transections of the cord and the rectal glands removed by an abdominal incision. The rectal gland artery, vein and duct were catheterized with PE90 tubing. The glands were then placed in a plexiglass and aluminum chamber kept at 15° to 17° with running sea water. The glands were perfused by gravity at a pressure of 4 mm Hg with an artificial perfusion medium containing in mM: Na 280; K 5; Cl 290; HCO_3 8; phosphate 1; Ca 2.5; Mg 3; sulfate 0.5; urea 350; glucose 5; pH 7.6 when gassed with 99% O_2 1% CO_2. Collections of arterial perfusate were obtained through a self-sealing rubber connector close to the arterial cannula. Rectal gland vein collections were taken anaerobically from the venous catheter. Rectal gland fluid was collected in 1.5 ml conical centrifuge tubes. Perfusate flow through the gland was measured directly by collecting all venous effluent in a graduated cylinder. Oxygen tension determinations were done using a polarographic oxygen electrode equipped with a constant temperature cell maintained at the same temperature as the perfused gland. Chloride in perfusate and rectal gland fluid was measured by amperometric titration with a Buchler-Cotlove chloridometer. Sodium and potassium measurements were done in an IL 143 flame photometer. Results are expressed as mean ± SE.

Figure 1 shows the relation between oxygen consumption and chloride secretion in 278 observations in 91 perfused rectal glands during resting unstimulated periods, represented by the + sign, and after varying degrees of stimulation with theophylline and dibutyryl cyclic AMP, shown as the open symbols. There is no correlation between the rate of chloride secretion and oxygen consumption in the resting state as illustrated by a correlation coefficient of 0.008. The line of best fit is a horizontal line exactly parallel to the

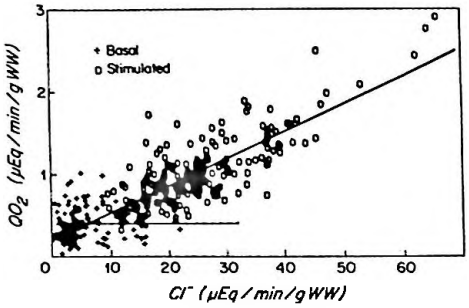

Figure 1. Relation between chloride transport and oxygen consumption in perfused rectal glands during resting unstimulated periods represented by a + sign and after varying degrees of stimulation with theophylline and dibutyryl cyclic AMP shown in the open symbols. The line of best fit for the unstimulated periods crosses the vertical line at .41 ± 0.3 μM of O_2/min/g and its slope is not different from 0. The regression line for the stimulated periods has an intercept of .2 ± .06 which is different from 0 and the intercept of the unstimulated periods and has a slope of .033 ± .002 indicating that the ratio of Cl/O_2 is 30.

horizontal axis denoting no change in the rate of oxygen consumption even at the highest rate of chloride secretion observed in resting conditions. When the glands are stimulated, however, the rate of oxygen consumption rises in a linear relation to the rate of chloride secretion as shown by a highly significant slope of .033 ±.002 mM/mEq (slope ± SD) $p < .001$ and a correlation coefficient of .78. The average ratio of Cl^- transported to O_2 consumed was 30 mEq/mM. The regression line crosses the vertical axis at a point of .2 ± .06 M of O_2/min/g, a value significantly different from zero. This suggests that in the rectal gland there is a "basal" component of oxygen consumption not related to transport when determined by the classical extrapolation method. This "basal" oxygen consumption is much lower than the one calculated using the same method from the line obtained from the resting, nonstimulated glands (.41 ± .03 in the resting vs. .20 ± .06 in the stimulated gland $p < .01$). This suggests that oxygen utilized for both transport and non-transport needs in the unstimulated gland can be diverted to the work of electrolyte transport when the gland is stimulated.

One of the advantages of the isolated perfused rectal gland preparation is that its rate of secretion can be varied over a wide range by stimulating it with theophylline and dibutyryl cyclic AMP. Oxygen consumption at different rates of chloride secretion is shown graphically in Figure 2. The resting unstimulated rectal

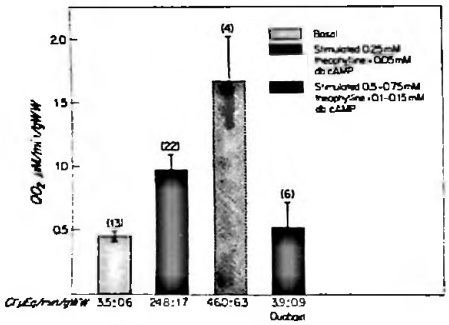

Figure 2. Oxygen consumption in the rectal gland at different rates of chloride secretion and effect of ouabain on oxygen consumption in the stimulated rectal gland. As chloride secretion rises after stimulation with theophylline and dibutyryl cyclic AMP oxygen consumption rises in a parallel fashion ouabain decreased both oxygen consumption and chloride secretion to basal levels. Bars represent ± S.E.M. (n).

gland has an oxygen consumption of .45 ± .04 μM of O_2/min/g at an average rate of chloride secretion of 3.5 ± .06 μEq/min/g. When the rate of chloride secretion is stimulated with 0.25 mM theophylline and 0.5 mM dibutyryl cyclic AMP oxygen consumption rises to .97 ± .12 and chloride secretion to 24.8 ± 1.7. Further stimulation of the gland with .5 to .75 mM theophylline and 0.1 to 0.15 mM dibutyryl cyclic AMP increases oxygen consumption to 1.67 ± .35 and chloride secretion to 46.0 ± 6.32.

Chloride secretion by the rectal gland is depressed by inhibitors of Na-K-ATPase (Silva et al. Am. J. Physiol. 233:F298-F306, 1977). Figure 2 also shows the effect of inhibiting Na-K-ATPase by adding ouabain to the perfusion medium on oxygen consumption and chloride secretion by the isolated perfused rectal gland. In six stimulated glands ouabain decreased oxygen uptake from 1.17 ± .19 to .52 ± .19 M of O_2/min/g while

chloride secretion fell from 21.97 ± 2.73 to 3.86 ± .89 μEq/min/g. In two unstimulated glands, oxygen consumption fell from .30 ± .05 to 0.16 ± .02 after addition of 10^{-4} M ouabain while chloride secretion did not change (5.21 ± 1.33 prior to ouabain and 5.35 ± 1.11 after). Removing potassium from the perfusion medium also blocks Na-K-ATPase and decreases chloride secretion in the stimulated perfused rectal gland. In two stimulated glands oxygen consumption falls from 1.20 to .95 while chloride secretion drops from 15.56 to 4.83.

Chloride secretion in the rectal gland depends on the presence of sodium in the perfusate and can be reduced to zero by removing sodium from the solution perfusing the gland (Silva et al. Am. J. Physiol. 233:F298-F306, 1977). When the sodium in the perfusate of four stimulated glands is replaced by lithium oxygen consumption falls from 1.09 ± .04 to .64 ± .07 μM of O_2/min/g while chloride secretion falls from 21.68 ± 1.33 to 2.04 ± .57 μEq/min/g. The ratio Cl/O_2 also falls from 23.30 ± .88 to 3.34 ± 1.87.

Removing chloride from the perfusate also results in a decrease in the rate of rectal gland secretion and oxygen consumption. Figure 3 shows the effect of removing chloride from the perfusate and replacing it with nitrate. Oxygen consumption falls from 1.33 ± .13 to .40 ± .05 while sodium secretion falls from 31.3 ± 4.82 to 8.03 ± 1.75 n = 4. The ratio of Na/QO_2 did not change (23.53 ± 3.67 and 20.08 ± 2.16 respectively). Replacing chloride with acetate also results in inhibition of secretion and reduces oxygen oxygen consumption from 184 ± .1 to .32 ± .11 while sodium secretion drops from 23.10 ± 3.1 to 3.45 ± 1.1 n = 4 as shown in Figure 3.

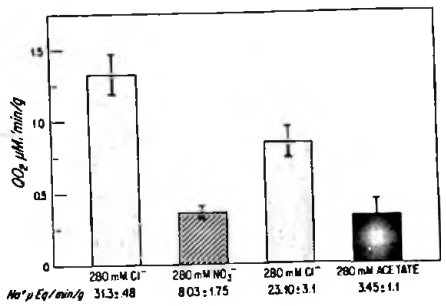

Figure 3. Substitution of chloride by either nitrate or acetate in the solution perfusing the rectal gland decreases the rate of secretion and also decreases the rate of oxygen utilization. Since chloride was omitted from the perfusate the rates of electrolyte secretion are shown for sodium. Bars represent means ± S.E.M.

The chemical gradient against which the rectal gland transports chloride can also be varied by experimental manipulation. Since fluid secreted by the rectal gland is isotonic with the perfusing solution and little urea is found in the secretion, changing the concentration of urea in the perfusate results in a change in the concentration of electrolytes in rectal gland fluid. In the absence of urea there is no chemical gradient for chloride across the gland. As urea concentration increases in the perfusate from 0 to 350, 550 and 700 mM, chloride concentration in the perfusate rises from 297 through 422, 531, and 570 mM, r = 0.95. The chemical gradient against which chloride is secreted is then a factor of the urea concentration of the perfusate. It was therefore of interest to determine whether oxygen consumption changed as the gradient for chloride secretion became steeper. Table 1 summarizes the results. No significant change in the amount of oxygen required to transport chloride into the rectal gland lumen was observed at any of the different gradients for chloride secretion that were studied.

ENERGY COST OF SECRETION AGAINST DIFFERENT CHEMICAL GRADIENTS

	DUCT FLUID [Cl⁻] mEq/L	Cl⁻ SECRETION	Cl/QO_2 RATIO
No Urea	297	42±17	28±8
350 mM	422	29±9	26±7
550 mM	531	28±9	25±6
700 mM	570	23±6	31±10

Table 1

These experiments illustrate several important aspects of the relation between oxygen consumption and chloride transport in the rectal gland. Oxygen consumption increases in a linear fashion with increases in the rate of chloride secretion, indicating that chloride secretion depends on aerobic metabolism. The oxygen consumption at low rates of chloride secretion before stimulation by cAMP, is not correlated with changes in chloride secretion implying that at these rates there is no direct coupling. Some portion of oxygen consumption in this basal state appears to be available for transport of chloride when secretion is stimulated. The molar ratio of sodium chloride transported to oxygen consumed in the stimulated gland is greater than 18/1 (a value predicted from the ratio 3 Na/1 ATP found in red blood cells and membrane vesicles), and varies from 20/1 to 30/1. Finally, the chemical gradient against which chloride is secreted does not affect the rate of oxygen consumption.

THE ROLE OF VASOACTIVE INTESTINAL PEPTIDE (VIP) IN THE REGULATION OF ACTIVE CHLORIDE SECRETION IN THE RECTAL GLAND OF *Squalus acanthias*

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The observation that the intracellular level of cyclic AMP modulates active chloride secretion in the rectal gland has stimulated a search for the endogenous factor(s) which evokes this response (Stoff et al. Bull. Mt. Desert Island Biol. Lab. 16:95-98, 1976). A number of humoral and neurohumoral agents were investigated which failed to alter chloride secretory rate in an in vitro isolated perfused gland model; these included: vasopressin and several oxytocin derivatives normally found in the posterior lobe of the pituitary of the dogfish (aspartocin, valitocin, vasotocin), norepinephrine, epinephrine, serotonin, substance P and calcitonin. Since the rectal gland is an appendage of the elasmobranch hind gut, a number of hormones which stimulate intestinal secretion have been studied as well; these include: pentagastrin and a structural related group of three peptide hormones, glucagon, secretin, and vasoactive intestinal peptide (VIP). Vasoactive intestinal peptide (10^{-6}-10^{-8} M) was the only hormone which stimulated chloride secretory rate in this tissue.

All experiments were performed with spiny dogfish of either sex captured by hook and line from Frenchman Bay, Maine. Glands were removed and perfused in vitro as previously described (Stoff et al. J. Exptl. Zool. 199:443-448, 1977). All hormones studied were dissolved in the perfusion media and added to the perfusion reservoir or infused as a single bolus over a one minute time interval into the arterial line. Duct fluid was collected at timed intervals and measured volumetrically. Chloride was measured by amperometric titration. Partial pressure of oxygen in arterial and venous samples obtained under anaerobic conditions was measured by a polarographic oxygen electrode maintained at 15°C.

Two groups of four unanesthetized fish underwent either intravascular volume expansion (50 ml of 1 M NaCl) or intragastro-intestinal volume expansion (100 ml of 1 M NaCl). Blood samples were taken from the dorsal aorta at 0, 5, 15, 30 and 60 minutes. Plasma was assayed for VIP by radioimmunoassay, sodium by flame photometry and osmolality by freezing point depression. A third group of four fish were sacrificed and nine different organs were removed, frozen in liquid nitrogen and stored at -20°C for one month. Tissue was then thawed, homogenized in 0.3 M phosphate buffer (pH 7.4), centrifuged at 15,000 RPM for 30 minutes and the supernatant assayed directly by radioimmunoassay for VIP.

In the nonstimulated state the rectal gland secretory rate gradually declines to 10.3 ± 4.67 µl/min/g wet weight. Under these conditions the calculated chloride secretory rate averaged 4.95 ± 2.64 µEq/min/g wet weight and the oxygen consumption 0.34 ± 0.22 µM/min/g wet weight (n=15) (Table 1). These values are quite similar to previous data reported by our laboratory (Silva et al. Am. J. Physiol. 233:F298-F306, 1977)