secretion in the rectal gland, TAP was used in an effort to selectively block this route. The effect is seen only at millimolar concentrations of TAP in the intestine and is thought to be due to competitive inhibition of sodium transport. In the rectal gland, TAP, at concentrations varying from 10^{-7} to 10^{-3} M and a pH of 6.3 evoked little or no inhibition in the rate of sodium, chloride or volume excretion in 5 rectal glands.

Substituted stilbenes, originally used as fluorescent markers of amino groups on the surface of cell membranes, have been shown to block anion transport in red cells, intestine and turtle bladder. It was therefore of interest to see whether one of these compounds 4-acetamido-4'-isothiocyano-2,2'-disulfonic stilbene (SITS) inhibited chloride transport by the rectal gland. Figure 5 shows the effect of SITS on the rate of volume, chloride and sodium secretion at concentrations ranging from 10^{-4} to 5×10^{-3} M. A 30 ± 6% inhibition is seen at 10^{-3} M SITS n=7, 38 ± 14% at 2×10^{-3} M n=4 and practically complete 92 ± 4%, at 5×10^{-3} M n=2. The inhibitory effect of SITS is reversible in the rectal gland even after 5×10^{-3} M.

This report complements previous work from our laboratory on the mechanism of chloride secretion in the rectal gland. The transport process appears to be quite specific for chloride since only bromide partially substitutes for chloride. The movement of bromide across the rectal gland epithelium appears to share the same pathway with chloride since furosemide which inhibits chloride transport also inhibits bromide transport. Ethacrynic acid has an effect similar to fruosemide on the inhibition of chloride transport in many tissues, an association that is confirmed for the rectal gland by the present experiments. Triaminopirimidine, a compound that has been shown to inhibit the paracellul ar movement of sodium in the intestine shows only limited effects in the rectal gland. This limited response may be due to the low concentrations of the drug used in these studies since in mammalian intestine a minimal concentration of 20 mM is required to produce an inhibitory effect on sodium movement. SITS is a powerful inhibitor of anion transport in the mammalian red cell, intestine and turtle bladder. In the rectal gland it shows an inhibitory effect at about the same concentrations that inhibit anion transport in these tissues. It is possible that an anion channel blocked by this compound in the rectal gland is similar to the one in the turtle bladder and red cell. It is also possible that due to the high affinity of this compound for amino groups, it binds indiscriminately to many plasma membrane proteins without selectively inhibiting the anion transport channel.

We conclude from these studies that chloride appears to be transported via a highly selective carrier coupled to sodium in agreement with the model previously postulated.

NA-K-ATPASE AND RECTAL GLAND SECRETION IN *Squalus acanthias*

Patricio Silva, Terry Baranano, Jonathan Epstein, Jeffrey Stoff and Franklin H. Epstein
Department of Medicine and Thorndike Laboratory of Harvard Medical School at Beth Israel Hospital, Boston, Massachusetts

NaKATPase is involved in the transepithelial transport of chloride in the rectal gland. Evidence for such participation is the inhibition of rectal gland secretion by ouabain and by removal of potassium from the extracellular space. A model has been postulated in which NaKATPase is responsible for the maintenance of a steep sodium gradient that facilitates the movement of chloride linked with that of sodium across the basolateral membrane of the rectal gland cells. In this model the activity of NaKATPase is crucial for the maintenance of transepithelial transport. Enzyme activity might therefore control chloride secretion by the rectal gland. This control could be exerted by a direct effect of hormonal agents or their second messenger cAMP on the membrane enzyme, or by intracellular electrolyte changes brought about by the hormonal activation of transport.

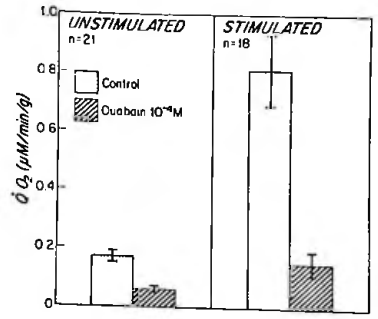

Figure 1. Effect of ouabain 10^{-4} M on oxygen consumption in unstimulated and stimulated perfused rectal glands. Ouabain reduced oxygen consumption from 0.17 ± 0.02 to 0.06 ± 0.01 μM/min/g (n=21) in unstimulated glands and from 0.81 ± 0.12 to 0.15 ± 0.04 (n=18) in stimulated glands. Ouabain inhibitable oxygen consumption is sixfold greater after stimulation.

Evidence for the activation of NaKATPase by stimulation of the perfused rectal gland with theophylline and cyclic AMP is provided by two lines of evidence. The first involves the measurement of ouabain-inhibitable oxygen consumption in unstimulated and stimulated rectal glands. Figure 1 shows that the decrease in oxygen consumption induced by a maximal concentration of ouabain in unstimulated perfused rectal glands is 0.10 ± 0.02 μM/g/min, after stimulation the change in $\dot{Q}O_2$ induced by ouabain is 0.61 ± 0.09 (mean ± SEM). Thus, ouabain-inhibitable oxygen consumption increases sixfold after stimulation of rectal gland secretion. Since ouabain inhibits consumption that is mediated by NaKATPase, this suggests that the activity of the enzyme has increased six times.

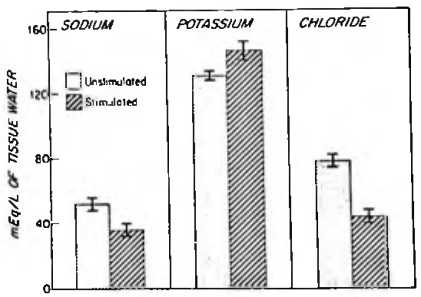

Figure 2. Intracellular concentration of sodium, potassium and chloride in unstimulated and stimulated perfused rectal glands. With stimulation, intracellular concentrations of sodium and potassium change reciprocally. Sodium falls while potassium rises. Chloride concentration falls by 40%. Bars represent mean ± SEM.

A second line of evidence arises from the changes in intracellular electrolytes observed in the presence and absence of stimulation. Figure 2 shows the intracellular electrolytes in unstimulated and stimulated perfused rectal glands. Intracellular sodium content decreases after stimulation while that of potassium rises. These reciprocal changes are consistent with activation of the NaKATPase present in the basolateral membrane of the rectal gland cells.

Measurements of NaKATPase in different portions of rectal gland cut in either longitudinal or coronal sections show that the activity of NaKATPase is distributed uniformly throughout the gland. Measurements of the enzyme activity in any portion of the gland adequately represent the whole gland. The activity of the enzyme was similar whether or not deoxycholate was present in the homogenizing solution (37.8 ± 4.5(3) μM Pi/mg of protein/hr with DOC and 35.1 ± 0.4(3) without DOC, mean ± SEM, number in parenthesis). In all subsequent experiments deoxycholate was not added to the homogenizing solution and units of NaKATPase activity are the same.

NaKATPase activity in perfused glands was similar to that of nonperfused glands assayed immediately after removing them from the fish (38.5 ± 3.0(4) in nonperfused glands vs 40.2 ± 1.9(6) in perfused glands, mean ± SEM). In addition, it was the same whether perfused with or without cyclic AMP and theophylline (40.2 ± 1.9(6) unstimulated vs 38.1 ± 1.9(7) stimulated). Furthermore, *in vitro* addition of cyclic AMP and theophylline to homogenates of rectal gland did not alter the activity of NaKATPase.

In an effort to determine whether stimulation of the perfused gland with cyclic AMP and theophylline resulted in activation of NaKATPase present in the cell membrane, unstimulated glands were perfused with ouabain 10^{-4} M for a total of thirty minutes in order to block all sites available for ouabain binding in the unstimulated state. The perfusate was then changed to a solution lacking ouabain, the gland was perfused for additional twenty minutes and tissue was sampled for NaKATPase activity. Following this twenty minutes of "washing," the glands were stimulated with either theophylline 0.25 mM alone or theophylline 0.25 mM and cyclic AMP 0.05 mM. At the end of the experiment, the gland was again sampled for NaKATPase activity. No change in either chloride secretion or NaKATPase was observed after stimulation of ouabainized glands (4.2 ± 1.7 before ouabain vs 3.7 ± 1.5 after ouabain).

Ouabain binding to NaKATPase was next explored in perfused rectal glands before and after stimulation with theophylline 0.25 mM and cyclic AMP 0.05 mM. The glands were perfused with 10^{-4} M ouabain plus ^{3}H-ouabain added in tracer amounts for a total perfusion time of thirty minutes. The glands were then perfused for another forty minutes without ouabain and the tissue was sampled every ten minutes for a total of three times after discontinuing the ouabain. Tissue and perfusate were assayed for radioactivity and the amount of ouabain bound to rectal gland tissue was expressed as nM of ouabain bound per mg of protein or per g wet weight. Results show that after twenty minutes of washing the amount of ouabain bound reaches a constant level. The values after thirty minutes of washing are reported. Table 1 summarizes these results. No increase in ouabain binding was observed after stimulation; instead there was an unexpected decrease in ouabain binding in stimulated glands as compared with unstimulated glands.

Table 1

Ouabain Binding in Perfused Rectal Glands

	nM Ouabain/g	nM Ouabain/mg of Protein
Unstimulated	32.2 ± 1.8 (7)	0.32 ± 0.02 (4)
Stimulated	24.6 ± 1.2 (6)	0.26 ± 0.01 (6)

It is instructive to compare the theoretical transport capacity of rectal gland NaKATPase with the ionic movements actually observed in the stimulated gland. The average rate of chloride secretion in 251 collection periods in eighty stimulated perfused glands was 1440 ± 41 µEq/hr/g with a median of 1356 and a range reaching 3960. In our proposed model for chloride excretion by the rectal gland (Am. J. Physiol. 233:F298-F306, 1977), if a neutral carrier is responsible for the cotransport of NaCl into the cell Na:Cl-1, then for each sodium removed from the interior of the cell a chloride moves across the cell into the lumen. If this relation is correct, the ratio of Na efflux to ATP hydrolyzed would also apply to chloride secreted. This ratio for the red cell has been found to be 3 Na per ATP hydrolyzed. In vesicles with rectal gland NaKATPase the observed ratio is 1.5. Using these two ratios the transport capacity of the rectal gland has been calculated (Table 2).

When the activity of rectal gland NaKATPase, measured *in vitro* at 37°C under optimum conditions of Na^+ and K^+ concentration, is corrected for the ambient temperature and the actual concentrations of extracellular K^+ and intracellular Na^+ in the perfused gland at 20°C, it is apparent that the observed rate of Cl^- secretion exceeds the calculated transport capacity of NaKATPase.

We conclude from these experiments that in order to account for all the chloride transport via NaKATPase, the movement of chloride along with sodium into the cell has to be in the ratio of two or

TABLE II

NaKATPase transporting capacity in the rectal gland

Assay conditions			NaKATPase activity	Transport capacity* Na+ efflux = Cl^- secretion	
T °C	Na	K(mM)	µM Pi/mg of protein/hr	µEq/hr/g	
				a	b
37	100	20	38.1 ± 1.9(7)	12000	6000
37	50	5	21.0 ± 1.3(4)	6800	3400
20	100	20	6	1800	900
20	50	5	3?	900	450

*Calculated values based on: 1) 108 ± 3(28) mg of protein per gram of rectal gland
2) a. 3 Na removed per ATP hydrolyzed (red cells)
b. 1.5 Na removed per ATP hydrolyzed (rectal gland vesicles)

Average Cl^- secretion in the rectal gland = 1440 ± 41(250)

more Cl^- per Na+, clearly an electrogenic mechanism. If such is not the case, additional chloride carriers or pump have to be postulated.

EFFECT OF VASOACTIVE INTESTINAL PEPTIDE, SOMATOSTATIN AND THEOPHYLLINE ON ACTIVE CHLORIDE TRANSPORT AND CYCLIC AMP METABOLISM IN THE RECTAL GLAND OF *Squalus acanthias*

Jeffrey S. Stoff, Robert Rosa, Ralph Hallac, Diane Leone, Patricio Silva and Franklin H. Epstein
Dept. of Medicine and Thorndike Laboratory of Harvard Medical School at Beth Israel Hospital, Boston, Massachusetts

The rectal gland of the dogfish shark secretes a hypertonic solution of sodium chloride by a process involving the active transport of chloride. This gland is composed of a homogenous population of branching secretory tubules. These tubules are formed by a secretory type of epithelium with rich basal-lateral infoldings and a specialized apical surface demonstrating numerous microvilli (Doyle, Bulletin 15:28-30, 1975). The gland has a single artery, vein and duct and can therefore, easily be removed from the shark and perfused at seawater temperature in the laboratory with artificial shark-Ringers solution as previously described (Stoff et al., J. Exptl. Zool. 199:443-448, 1977). Studies from our laboratory have revealed a number of key characters of rectal gland secretion. (1) The duct fluid contains sodium chloride at 1.5 - 2.0 times the concentration of the shark plasma or artificial perfusate. This is approximately the concentration of sodium chloride in seawater. (2) The duct secretion is isosmotic with plasma by virtue of the fact that it contains little or no urea. (2) Measurement of the electrochemical driving forces across the contraluminal membranes indicate that chloride is the actively transported ion since the electrical potential of the duct is negative to the perfusate. (4) The entry step for chloride is tightly coupled to sodium and probably involves a Na-Cl carrier localized to the contraluminal membrane. (5) This requirement of sodium for chloride entry is further exemplified by the dependence of chloride transport on Na-K-ATPase activity. In these respects, active chloride transport in the rectal gland is similar to that in other electrolyte transporting epithelia like amphibian skin and urinary bladder; mammalian cornea and intestine; and the thick ascending limb of the loop of Henle. Recently we have demonstrated that secretion by the rectal gland was markedly stimulated by theophylline and cyclic AMP (Stoff et al., ibid. 199:443-448, 1977). The responses to theophylline and dibutyryl cyclic AMP are quite prompt and secretion increases by 10- to