the rabbit ileum is independent of changes in cyclic AMP but dependent on extracellular calcium. The present studies are consistent with the hypothesis that changes in intracellular calcium and cholinergic stimuli may modulate chloride secretion in the dogfish rectal gland.

We emphasize that in contrast to the modest effect of A23187 seen in the present studies, chloride secretion in the rabbit colon following ionophore is quantitatively similar to that observed following cyclic AMP (Frizzel, J. Membrane Biol. 35:175-187, 1977). Indeed the response to ionophore in the dogfish rectal gland is quantitatively much less than that observed in numerous other secretory epithelia (Candia et al., Amer. J. Physiol. 233(2):F94-F101, 1977). The reason for these differences is not apparent from the present studies.

FURTHER STUDIES ON THE MECHANISM OF CHLORIDE TRANSPORT IN THE RECTAL GLAND OF *Squalus acanthias*

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The rectal gland of the spiny dogfish, *Squalus acanthias*, secretes chloride against a steep electrochemical gradient. We have postulated that the secretion of chloride depends on the linked movement of sodium and chloride across the basolateral cell membrane, thus chloride entry into the cell follows the passive movement of sodium down its electrochemical gradient. The gradient for sodium is maintained by the continued activity of NaKATPase. The efflux of chloride from the cell into the duct lumen, completing the transcellular passage, would be effected down an electrical gradient, the lumen being less negative than the cell, and the potential difference overcoming the very steep chemical gradient. Sodium on the other hand would move through intercellular junctions since its extrusion across the luminal membrane against a large electrochemical gradient precludes simple diffusion. This putative model for the active secretion of chloride involves several different steps: (1) The linked entry of chloride and sodium into the cell. (2) The continued activity of NaKATPase. (3) The passive diffusion of chloride across the luminal membrane. (4) The paracellular movement of sodium. Previous reports from our laboratory have given evidence for the involvement of NaKATPase in this process, described the requirement for either sodium or chloride and shown that inhibition of chloride entry into the cells by pharmacological means impairs its transcellular movement. The present report provides additional evidence for the specificity of the carrier system for chloride and presents further support for the notion that the entry of chloride into the cell is necessary for transepithelial transport. In addition, the effect of an attempt at pharmacological blockade of the intercellular pathway for sodium is described.

Dogfish of either sex were taken by hook and line from Frenchman Bay and kept in marine livecars until they were killed, usually within three days of capture. The dogfish were killed by segmental transection of the cord and the rectal gland removed through an abdominal incision. The rectal gland artery, vein and duct were cannulated with PE90 tubing. The glands were then placed in either a plexiglass and aluminum or all glass perfusion chamber and kept at 15°-17°C by running seawater. The rectal glands were perfused by gravity at a pressure of 4 mm Hg and a flow of 1.2 to 7 ml/min. The perfusion medium composition, unless otherwise specified, was (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 and 1% CO_2. In all experiments the glands were stimulated continuously with 0.25 mM theophylline and 0.05 mM dibutyryl cyclic-AMP. Rectal gland fluid was collected over periods of ten minutes. Changes in the composition of the perfusate were made at the end of two or more collection periods. Experimental periods were usually of 30 minutes duration divided into 10-minute intervals. Determination of chloride was done by amperometric titration in a Buchler-Cotlove chloridometer. Sodium and potassium were measured in an IL 143 flame photometer. Results are expressed as mean ± SEM (n).

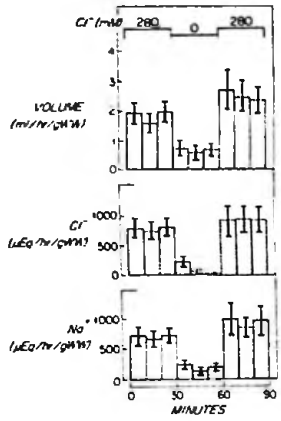

Figure 1. Substitution of sulfate for chloride. Sulfate cannot replace chloride and inhibits reversibly the rate of volume and sodium secretion. Bars represent mean ± SEM (n=6).

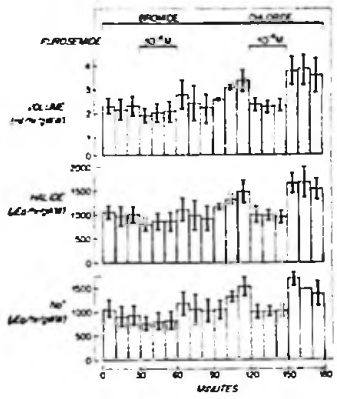

Figure 2. Effect of 10^{-4} M furosemide on the rate of volume, bromide or chloride, and sodium secretion in isolated rectal glands perfused with bromide for the first ninety minutes and with chloride for the rest of the experiment. Furosemide at this concentration does not inhibit significantly bromide secretion while it inhibits chloride secretion. Bars represent mean ± SEM. Number of experiments: bromide = 6, chloride = 4.

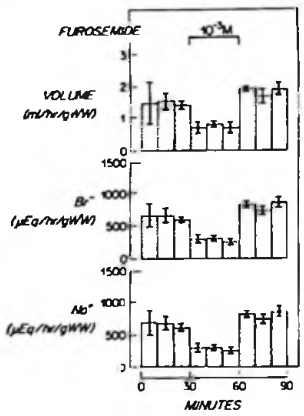

Figure 3. Effect of 10^{-3} M furosemide on the rate of volume, bromide and sodium secretion in three isolated perfused rectal glands. Furosemide reversibly inhibits bromide secretion at this concentration. Bars represent mean ± SEM.

We have previously shown that replacing chloride by either nitrate or acetate in the solution perfusing the rectal glands reduces the rate of secretion to a fraction of its normal value. Replacing chloride in the perfusate by yet another anion, sulfate, also reduces rectal gland secretion as shown graphically in Figure 1. The secretion of sodium fell from 732 ± 114 to 164 ± 69 (n=6) or 80 ± 8%. This effect was rapidly reversible and replacing the sulfate with chloride rapidly returns sodium secretion to control levels (1025 ± 272). The rate of volume secretion follows the same pattern. Chloride secretion drops to 36 ± 16.2 from 805 ± 151 or 95 ± 2% during perfusion with sulfate. The

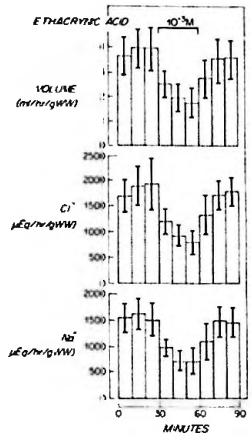

Figure 4. Effect of 10^{-3} M ethacrynic acid on the rate of volume, chloride and sodium secretion in six isolated perfused rectal glands. Ethacrynic acid reversibly inhibits chloride secretion. Bars represent mean ± SEM.

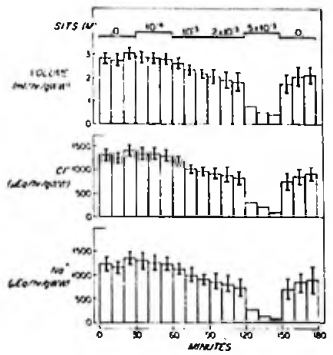

Figure 5. Effect of 4-acetamido-4'-isothiocyano-2,2'-disulfonic stilbene (SITS) on the rate of volume, chloride and sodium in isolated perfused rectal glands. In the preparation of this graph, several experiments in which different concentrations of SITS were used were pooled together. Four glands were perfused with 10^{-4}, 10^{-3} and 2×10^{-3} M SITS, one with 10^{-5}, 10^{-4} and 10^{-3} M SITS, one with 10^{-4}, 10^{-3} and 5×10^{-3} M SITS and one with 10^{-3} and 5×10^{-3} M SITS. SITS inhibits reversibly chloride secretion at concentrations of 10^{-3} M and higher. Bars represent mean ± SEM.

fact that sodium excretion does not drop as much as chloride secretion suggests that sulfate is secreted at a low rate, a phenomenon also noted with nitrate and acetate.

Bromide can partially replace chloride in the rectal gland. It was of interest to determine therefore whether agents such as furosemide, that block chloride entry into cells, would also inhibit bromide secretion. Figure 2 shows the effect of furosemide at 10^{-4} M on the rate of volume, bromide and sodium secretion in six rectal glands perfused with bromide. The same figure shows the effect of furosemide on chloride secretion in four of the six rectal glands that were perfused with chloride immediately after perfusion with bromide. It can be clearly appreciated that 10^{-4} M furosemide has little effect on bromide excretion decreasing it by 24 ± 10% (NS) while it significantly decreases chloride secretion in some of the same rectal glands previously perfused with bromide by 36 ± 4%. Furosemide, 10^{-3} M, decreases bromide excretion by 62 ± 6% while it decreases that of chloride by 82 ± 4%, Figure 3 (chloride data not shown).

Ethacrynic acid inhibits chloride entry into the cell in much the same way as furosemide does. In the rectal gland, ethacrynic acid at a concentration of 10^{-4} M decreases chloride secretion by 31 ± 14% (n=4) a reduction similar in magnitude to that observed with furosemide. At 10^{-3} M ethacrynic acid, reduction in the rate of chloride secretion, shown in Figure 4, is 68 ± 5% (n=6), smaller than that observed with furosemide.

Triaminopirimidine (TAP) is thought to block intestinal sodium secretion by inhibiting sodium movement along the paracellular shunt pathway. Since this is the postulated pathway for sodium

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*

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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.