perfused for a 30-40 min period, collections of secreted fluid being made very 10 min. EA-(L)cysteine caused a profound reduction in both fluid and Cl secretion to about 12% of the control values; on replacement with drug-free Ringer's the control rate returned almost immediately. Subsequent perfusion with 10^{-4} M EA-(D)cysteine caused a slight decline in secretion rate, however, the control rate following drug removal was lower than the previous control, possibly indicating some deterioration in the gland. A separate experiment demonstrating the absence of an inhibitory effect of 10^{-4} M EA is also shown. It was found that EA only became effective in reducing fluid secretion at 10^{-3} M, and then only to about 50% of control values, confirming the results of Solomon et al (Bull. MDIBL, 18:13, 1978). At this concentration the saturated derivative of EA, DHEA, which has no -SH binding capacity, was about as effective. This observation, coupled with the ready reversibility of EA (Solomon et al., op cit.), suggests that EA is not binding to tissue -SH groups in inhibiting fluid secretion. A slightly different situation obtains in the avian erythrocyte, where EA is more effective than DHEA, but is "irreversible" (i.e., cannot be simply washed out, as can DHEA) implying an interaction with cellular -SH groups related to Na/K/Cl cotransport (Palfrey and Greengard, op cit.).

A preliminary attempt to define the dose-response relationship for inhibition of fluid and Cl secretion by EA-(L)cysteine suggested an IC_{50} value of $\sim 3 \times 10^{-5}$ M for this compound. This would make it about 30-40-fold more potent than the parent drug EA, in agreement with the differentials mentioned above for avian erythrocytes and perfused rabbit tubules. This data supports the hypothesis that the transport systems affected by these drugs in the three different tissues have similar properties. In this regard, it is of interest to note that we recently were able to demonstrate stoichiometric diuretic-sensitive movements of ^{36}Cl with cations following cAMP stimulation of avian erythrocytes (Palfrey and Greengard, unpublished results), directly analogous to results obtained with NaCl transporting epithelia.

CALCIUM AND RECTAL GLAND SECRETION

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Because calcium has beenshown to play a key role in stimulus-secretion coupling in a variety of tissues, we attempted to investigate its importance in stimulated chloride secretion of the isolated perfused rectal gland of Squalus acanthias, using pharmacological agents known to influence the cellular actions of calcium in other cells.

Rectal glands were perfused by the technique described by Solomon et al., (Bull, MDIBL 18:13-16, 1978). Duct fluid and venous effluent were collected every 10 minutes for a total of 60 - 90 min. Glands were usually stimulatedby the addition of 0.05 mM dibutyryl - cyclic-AMP and 0.25 mM theophylline to the perfusate. When vasoactive intestinal peptide was used to stimulate the gland, 10 μg were dissolved in 1-2 ml of shark Ringer's and injected directly into the artery of the gland at the previous rate of perfusion.

CALCIUM IONOPHORES

The divalent cation-selective ionophore A23187 was added to rectal gland perfusions at a concentration of 10^{-4}M (n = 3) or 10^{-5}M (n = 2), after 30 minutes of perfusion in the basal state, without producing a significant increase in chloride secretion. In two additional experiments, the concentration of calcium in the perfusate was increased from 2.5 mM to 10 mM during preliminary basal perfusion, and A23187 in a concentration of 10^{-6}M or 5×10^{-6}M was superimposed after 30 minutes. No change in rectal gland secretion was seen with the lower dose

of the ionophore while at the higher concentration of A23187, duct fluid increased in volume but its chloride concentration fell to that of the perfusate, suggesting a loss of membrane integrity, rather than a stimulation of secretion.

The ionophore lasalocid A (X-537A) was employed in 6 experiments. At 10^{-4}M and 10^{-5}M, duct flow increased, but chloride concentration fell to that of the perfusate. Against a background of 10 mM calcium in the perfusate, 10^{-7}M, 10^{-6}M and 5×10^{-6}M of X-537A had no effect on basal secretory rate, while 10^{-5}M again produced an increase in the volume of secretion at the expense of a complete loss of the ability to concentrate chloride. This behavior should be compared with the action of cAMP or vasoactive intestinal peptide to stimulate the perfused rectal gland, which are characterized by a four-to-ten-fold increase in secretory volume with a chloride concentration 1.5 to 2 times that of the perfusate. Thus, in contrast to the reported action of calcium ionophores to increase chloride secretion by mammalian intestine, they do not appear to stimulate secretion by elasmobranch rectal gland.

VERAPAMIL

In two experiments 10 μg of VIP was injected into an isolated rectal gland that had been perfused for 30 min with 10^{-4}M verapamil, a compound that inhibits calcium influx across the plasma membrane of cells. Verapamil did not prevent stimulation of chloride secretion by VIP, secretory volume increasing from a mean of 0.3 to 1.9 ml/hr/g and chloride secretion from 0.14 to 0.89 mEq/hr/g. In three additional experiments 10^{-5}M verapamil did not alter the rate of chloride secretion in glands that had been stimulated by perfusing with 0.05 mM cAMP and 0.25 mM theophylline.

REMOVAL OF CALCIUM FROM PERFUSATE

In six experiments, isolated rectal glands were perfused with a shark Ringer's solution modified by the omission of calcium and by the addition of 2 millimolar EGTA. Duct flow increased progressively in all and chloride concentration in the secreted fluid rapidly decreased to the level of the perfusate, suggesting that ionic calcium is necessary to the integrity of the epithelial membrane. Stimulation with cAMP and theophylline did not alter this pattern of deterioration.

TRIFLUOPERAZINE (TFP)

The inhibitory effect of trifluoperazine (Stelazine^{R}, Schering) on rectal gland secretion was tested by adding it to the rectal gland perfusate for 30 min and then perfusing with 0.05 mM dibutyryl cAMP and 0.25 mM theophylline in the presence of TFP for an additional 30-40 min. TFP inhibited secretion in a dose-dependent manner (Figure 1).

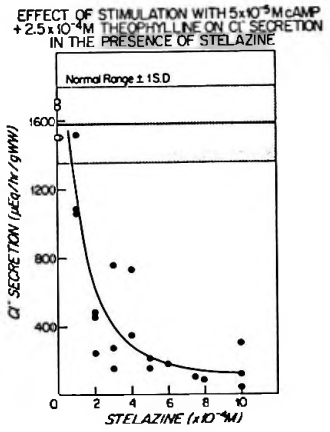

Figure 1

Complete inhibition at this level of stimulation by cAMP and theophylline was obtained at concentrations of TFP above 5×10^{-4} with half-maximal inhibition at approximately $2\text{-}3 \times 10^{-4}$M. The inhibition could be overcome by increasing the stimulus to secretion. When the dose of cAMP-theophylline was increased by 10 times (to 0.5 mM dibutyryl-cAMP and 2.5 mM theophylline) in 3 glands perfused by 5×10^{-4} TFP, a prompt increase in secretion was observed. A similar increase was induced in 2 other experiments by a bolus injection of 10 μg vasoactive intestinal peptide superimposed on 5×10^{-4}TFP.

At 10^{-3}M TFP, however, neither a concentration of 10 μg of VIP nor the higher concentration of cAMP-theophylline stimulated rectal gland secretion (n = 2 for each).

Because calcium ionophores did not stimulate, and verapamil did not inhibit rectal gland secretion, these experiments suggest that an influx of external calcium is not required for cAMP-induced secretion by the dogfish rectal gland. The results with trifluoperazine, on the other hand, raise the possibility of a role for calcium, since this compound binds to and inhibits the calcium-calmodulin complex that is thought to mediate the intracellular regulatory actions of calcium in almost all animal cells (C.B. Klee, T.H. Crouch and P.G. Richman, Ann. Rev. Biochem., 49:489-575, 1980). Calmodulin has been identified in rectal gland cells (H.C. Palfrey et al., this issue) and it is possible that the release of calcium from intracellular stores modulates at least a portion of rectal gland secretion. The results may be compared to those obtained by P.C. Smith and M. Field (Gastroenterology, 78:1545-1553, 1980) who found that calcium ionophores served as a submaximal stimulus to chloride secretion by rabbit small intestine, while trifluoperazine inhibited, though incompletely. The effect of trifluoperazine in the rectal gland may indicate a role for calmodulin in mediating secretion in this organ, but does not prove the case since it may also reflect other cellular actions of this drug.

OXYGEN CONSUMPTION OF CELLS ISOLATED FROM THE RECTAL GLAND OF THE SPINY DOGFISH SQUALUS ACANTHIAS

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The use of tissue slices for studies of intracellular electrolytes or for the binding of hormones or inhibitors of electrolyte transport is fraught with many uncertainties. The unavoidable problems arise from the fact that slices have a dead space that not only requires measurement, but also retards the binding or the effects of inhibitors or hormones. In large measure, these problems can be circumvented by using isolated tubules or cell preparations. Isolation of cells from the rectal gland of the shark is desirable for the measurement of the changes in intracellular concentration of electrolytes that attend activation of secretion by the gland with cyclic AMP, as well as for the measurement of the binding of ouabain to the Na-K-ATPase present in the membrane which is probably activated as a result of that stimulation. We report here the isolation of cells from the rectal gland of the shark and measurements of their oxygen consumption in vitro.

The rectal glands of two sharks were removed and perfused with 100 ml each of shark Ringer's in vitro as described (Am. J. Physiol. 233:F298-F306, 1977), for over 10-20 minutes to remove all red cells. The glands were then perfused with 10 ml of shark Ringer's containing 0.2% collagenase, 0.2% hyaluronidase and 10% fetal calf serum. Perfusion was then stopped, the glands sectioned longitudinally in half, then minced into 0.5 mm squares using a McIlwain tissue slicer. The minced tissue was placed in oxygenated shark Ringer's containing 0.2% collagenase and 0.2% hyaluronidase and 10% fetal calf serum and digested for variable periods of time at room temperature. The digestion process was followed by examining the tissue suspension under light microscopy at timed intervals. It was found that one hour of digestion yielded the largest proportion of viable isolated cells. The tissue digest was centrifuged at 500 rpm in a refrigerated international centrifuge for 1 minute to remove the undigested tubules; and the supernatant (containing the cells) was then centrifuged at 1500 rpm for 3 minutes to harvest the cells. The cells were washed once with shark Ringer's, then suspended in a final volume of 1 ml of shark Ringer's and stored in an ice bath until used for experiments.