ported per K. In these experiments, however, TEA was substituted for Na and the Hill plot for Rb influx when Na is replaced by TEA is biphasic. Although at 60 mM Na (the concentration used for the simultaneous ^{22}Na and ^{42}K influxes) the slope of the Hill plot is close to 2.0, the non-linearity renders any estimate of stoichiometry tenuous. Possibly there are more than one brush border cotransport systems for NaCl with varying K requirements and varying Na:Cl stoichiometries.

The effect of ouabain indicates that there is some type of feedback between the two borders of the cell to control electrolyte transport. The fact that ouabain increases cellular cGMP 5-fold suggests that this cyclic nucleotide may mediate the feedback inhibition and reinforces its central role in regulating intestinal salt absorption in the flounder.

MEASUREMENTS OF MEMBRANE POTENTIAL AND MEMBRANE RESISTANCE IN ISOLATED CELLS FROM DOGFISH RECTAL GLANDS

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The active secretion of Cl^- in the rectal gland of the dogfish (Squalus acanthias) is enhanced by c-AMP or theophylline (a phosphodiesterase inhibitor). Cl^- is thought to be actively transported across the baso-lateral membrane and to be passively extruded across the luminal membrane into the duct (Silva et al., Am. J. Physiol. 233:F298-F306, 1977). According to this scheme, Cl^- secretion would be enhanced by either hyperpolarization or increased Cl^- conductance. The suppression of the Cl^- secretion in the isolated perfused gland by Ba^{2+} is consistent with this theory since Ba^{2+} is expected to depolarize the gland by blocking K^+ channels (Silva et al., MDIBL Bull 21:12-13, 1981). To study the electrophysiology of the secretory process we decided to use the enzymatically separated single cell preparation (Segall et al., MDIBL Bull. 20:38-39, 1980) to measure changes in membrane potential and membrane conductance which might occur following activation by c-AMP. Judged by changes in O_2 consumption, single cells are known to respond much like the intact gland to c-AMP, furosemide, ouabain and changes in the Na^+ and Cl^- concentrations (Spokes et al., MDIBL Bull. 21:13-14, 1981).

METHOD--Single cells were suspended in Shark-Ringer (in millimoles per liter: Na, 280; K, 5: Cl, 280; bicarbonate, 8; Ca, 5.0; Mg 3.0; phosphate, 1; sulphate, 0.5; urea 350; glucose, 20) and were impaled by single conventional microelectrodes (3M KCl, 5-30 Mohm) on the stage of an inverted microscope.

RESULTS--Seen under the microscope the cellular suspension consisted of spherical single cells, clusters of a few (2-10) spherical cells and some larger tubular fragments. Impalement of single cells or cells in small clusters yielded membrane potentials close to -100 mV. Strict screening of the results was performed to verify this surprisingly large membrane potential. Results were considered reliable only if the potential dropped abruptly upon impalement and was stable for several minutes. In some cases the measured membrane potential hyperpolarized slowly by 10 to 20 mV following impalement. In these cases the electrode was often pulled out to check if the tip potential had changed. Taking such precautions, the resting membrane potential was found to be -90 mV $\pm$ 12 mV (S.D., n=45).

In order to measure the membrane conductance of the single cell preparations current pulses of varying amplitude were injected through the intracellular electrode and the resulting potential deflection was measured with the same electrode (Figure 1a). This method depends critically on the proper compensation for stray capacitance and electrode resistance. The compensation was carried out always before impalement. Figure 1a shows that the voltage transients resulting from uncompensated capacitance are substantially faster than the charging of the membrane capacitance. The records suggest a small change in the electrode resistance following impalement. Readjustment of the resistance compensation would improve the appearance of the records without actually improving the accuracy

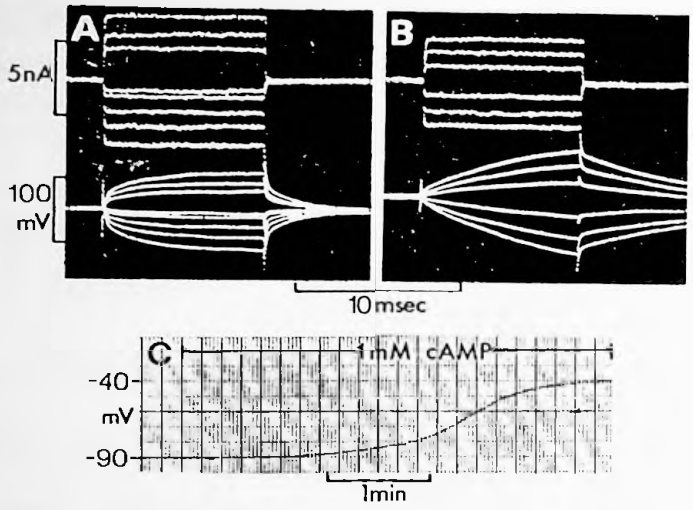

Figure 1.--Measurements of membrane conductance in the absence and presence of c-AMP. The panels A and B show the potential deflections (lower traces) caused by 10 msec current pulses (upper traces) before and after addition of c-AMP, respectively. The observed increase in the time constant of the membrane from 2.1 msec to 11.5 msec corresponds to a decrease in the membrane conductance from 140 nS to 25 nS. The membrane capacitance is unchanged, 390 pF, and the diameter of the impaled cluster of cells was 60 μm. Some uncompensated electrode resistance is noticeable in panel A. Panel C shows the time course of the c-AMP induced depolarization in another preparation.

of the measurements. Instead it was preferred to estimate the change in the membrane potential from the exponentially decaying potential following the turn off of the current pulse. This method depends on a clear separation of capacitive artifacts from the membrane capacitance but is independent of the slight non-linearity of the electrode resistance which was occassionally observed. Figure 2 shows that the steady state voltage deflection is a linear function of the injected current.

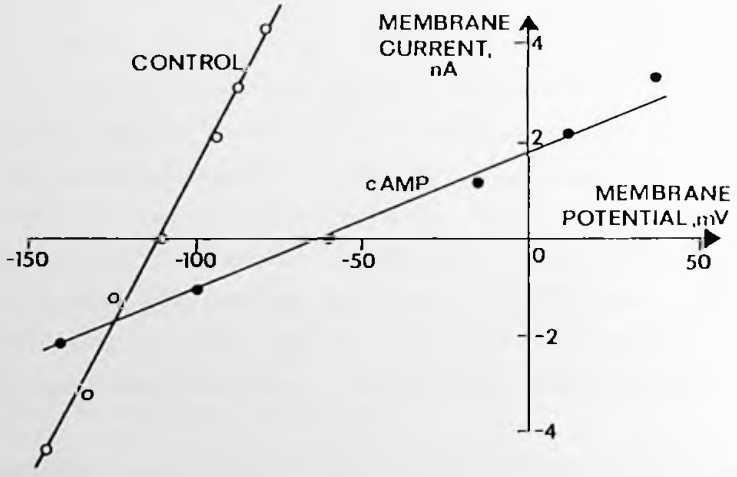

Figure 2.--Current-voltage relations before and after addition of cAMP. The results are from the experiment also illustrated in Figure 1a and b, but the results with c-AMP (filled circles) are measured with longer current pulses after longer drug exposure. The current-voltage relations are approximately linear.

Application of c-AMP reduced the membrane potential over a period of a few minutes as shown in Figure 1c. Typically the membrane depolarized to potential between -60 to -20 mV. Depolarization of the membrane induced by c-AMP was accompanied by a decrease in membrane conductance. Figure 1b also shows that the time constant of

charging the membrane capacitance is increased. In Figure 2 the steady state current-voltage relations, measured with longer current pulses (20 msec), are compared in the presence and absence of c-AMP. The curves are linear and a decrease in membrane conductance is indicated upon addition of c-AMP.

Measurements of the membrane capacitance give approximately the same value before and after addition of c-AMP. The membrane area calculated from Coles constant (1 $\mu F/cm^2$) was found to be three times larger than that of a sphere with the same diameter as the impaled cluster of cells, suggesting possible invagination or infolding of the surface membrane by a factor of 3 - 4.

In some instances a spontaneous depolarization was observed. Figure 3 shows that such spontaneous depolarizations were also accompanied by an increase in membrane resistance.

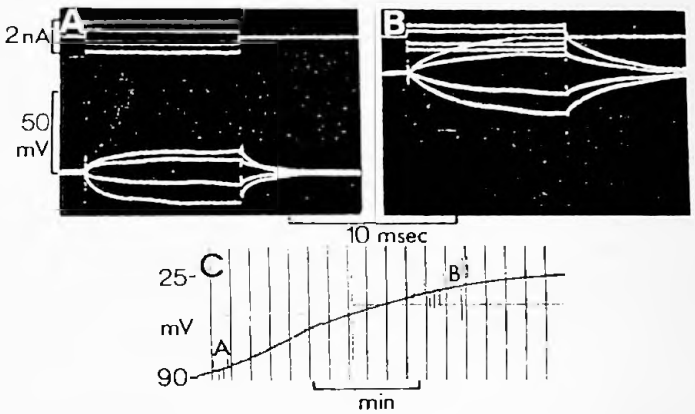

Figure 3.--Spontaneous depolarization and decrease in membrane conductance. The panels A and B show the potential response to current pulses measured before and after spontaneous depolarization. Panel C shows the time course of the depolarization with labels indicating the measurements in panels A and B. The depolarization occurred shortly after impalement and before the membrane potential had stabilized. The diameter of the impaled c cell was 25 μm and the membrane capacitance was about 100 pF.

DISCUSSION--The most intriguing finding of these experiments is that the c-AMP-induced depolarization of the membrane is accompanied by an increase in the membrane resistance. This finding is contrary to the predictions of the model based on experiments with the intact isolated perfused gland. According to this model, the increase in the Cl^- secretion caused by c-AMP corresponds to an increase in the passive Cl^- flux across the luminal membrane. This means that the increased Cl^- secretion must involve an increase in the luminal Cl^- conductance or an increase in the electrochemical gradient for Cl^-. If only the Cl^- conductance were to increase, then the total conductance should also increase. Our measurements show that the total membrane conductance is reduced several fold upon addition of c-AMP. This result suggests, therefore, that an increase in the Cl^- conductance cannot occur unless accompanied by an even larger decrease in another ionic component of the total membrane conductance. Similarly, the increase in the electrochemical gradient for Cl^- suggested by the model implies a hyperpolarization which is contrary to the measured depolarization (Figure 1). It must be remembered, however, that the electrophysiological measurements in this study were carried out on isolated cells which may have ionic gradients and extracellular environments quite different from those of the intact tissue. There is as yet no evidence that in the intact tissue c-AMP depolarizes the membrane or decreases the membrane conductance.

If Cl^- secretion really does occur in the isolated cells in response to exposure to c-AMP, then the measured depolarization and the decrease in membrane conductance might represent a shift from a K^+ selective state with high conductance to a Cl^- selective state with significantly lower conductance. This scheme may conserve the

cellular K^+ gradient, especially during long term depolarization caused by the cellular secretory process. Clearly this hypothesis requires rigorous measurements of ionic selectivity to Cl^- and K^+ at the two states. We have no evidence at present that such a change of selecting in fact occurs. We should like to thank Drs. Silva and Epstein for providing us with the isolated cells and for many helpful discussions.

MECHANISMS OF TAUROCHOLATE UPTAKE IN THE SKATE LIVER EFFECTS OF SODIUM ION AND ALBUMIN

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INTRODUCTION--Studies in mammals indicate that Na^+ is required for Taurocholate (Tc) transport into the hepatocyte, a process which may represent coupled transport of Tc^- and Na^+ via a membrane associated carrier (Boyer, Physiol. Rev. 60:303-326, 1980). More recently, serum albumin has been shown to facilitate Tc uptake in mammalian liver (Forker, J. Clin. Invest. 67:1517-1522, 1981), possibly through recognition of an albumin-anion complex at a specific receptor. The latter might show Na^+ dependence either by Na^+ -dependent recognition of the complex of by an effect on the complex association constant under conditions where the complex concentration is limiting.

Our previous studies utilizing single pass clearances in isolated perfused skate livers indicate that hepatic Tc uptake from albumin solutions is temperature dependent, saturable and competitive with bromsulphathalein, supporting a carrier mediated process (Zacks, et al Bull. MDIBL 21:110, 1981). In the present study, we use this system to determine if Tc uptake is Na^+ dependent, and whether this effect can be attributed to a Na^+ dependent membrane carrier for free Tc anion, to changes in Tc affinity for albumin, or to Na^+ dependent membrane recognition of the albumin-Tc complex.

METHODS--Equilibrium Dialysis: To determine whether Na^+ affects Tc binding to albumin, association constants (Ka) were obtained in triplicate by equilibrium dialysis in elasmobranch ringers solution at 37°C prepared with either NaCl (high Nat, 270 mEq) or choline Cl (low Na^+, 20 mEq) media. Dialysis bags, containing 37.5 μM fatty acid free Bovine Serum Albumin (BSA and 37.5 μM ^{14}C-NaTc were immersed in either high or low Na^+ ringers solution and allquots were removed at 12 h intervals until equilibrium was reached at 48 h. Ka was calculated as (CPM in/CPM out) (1/BSA) at equilibrium. Ka's were also measured in NaCl ringers at 15°C to determine effects of low temperatures. Ka values were compared to the published value of 1.22×10^7 measured in a mammalian medium at 37°C. (Green, et al BBA 231:250-252, 1971). Using measured Ka's, the concentration of bound and free Tc were calculated at equilibrium for each of the combinations of Tc and BSA used in skate liver perfusion experiments, to determine the influence of albumin on hepatic uptake rates of Tc.

Tc-Clearance Studies: The hepatic clearances of Tc were determined using a multiple steady state, single pass method adapted for the perfused skate liver as previously described (Weisiger, et al Bull MDIBL 2:108, 1981) using three concentrations of ^{3}H-NaTc (3.73, 26.1 and 74.5 μM). Either Na^+ or choline chloride elasmobranch Ringers was used to assess the effect of Na^+. To assess the effect of albumin, livers were perfused with either 0.25% BSA (7 studies) or 2.0% BSA (5 studies).

^{14}C-Tc-BSA solutions in NaCl or choline or chloride were alternately infused for 3 min. each, to establish a steady extraction rate for Tc. A 15 min wash infusion of the same medium without Tc preceeded each study. Immediately prior to perfusion with the unlabelled wash solution, four 1.5 ml samples of hepatic venous effluent were obtained every 15 seconds and aliquots analyzed by liquid scintillation counting for unlabelled wash solution, four 1.5 ml samples of hepatic venous effluent were obtained every 15" and aliquots analyzed by liquid scintillation counting for determination of Tc extraction.

Additional hepatic venous samples were taken at 1,3,5,10 and 15 min after the unlabelled wash. Infusion