In conclusion, the role of carbon anhydrase in the rectal gland appears to be in the dissimilation of metabolic CO_2. Enzyme inhibition generates high pCO_2 in the rectal gland fluid, and the resulting low intracellular pH reduces the normal rate of saline-induced secretion of NaCl. This work was supported by NIH grant HL-22258.

FURTHER STUDIES ON OUABAIN BINDING IN THE RECTAL GLAND

Patricio Silva, Katherine Spokes, Patricio Silva, Jr., Jonathan Epstein, Arthur Stevens and Franklin H. Epstein, Charles A. Dana Research Institute and the Harvard-Thorndike Laboratory of Beth Israel Hospital Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, Mass.

Stimulation of rectal gland secretion by dibutyryl cAMP and theophylline increases the binding of ouabain by rectal gland cells, but the mechanism of the increase is not clear. We have previously shown that maneuvers that interfere with sodium chloride cotransport do not prevent this phenomenon in rectal gland slices (Bull MDIBL 21:103, 1981). Both bumetanide 10^{-5}M and removal of sodium or chloride or both were used to interfere with sodium and chloride cotransport. Stimulation of ouabain binding in the presence of bumetanide 10^{-5}M could be due to the use of insufficient amount of the inhibitor, therefore, the experiment was repeated using a concentration ten-fold larger. Binding was measured using the technique previously described (Bull MDIBL 21:103, 1981). As shown in Table 1,

Table I

Effect of bumetanide on the stimulation with dibutyryl cyclic AMP (10^{-3}M) and theophylline (10^{-3}M) of ouabain binding by rectal gland slices.

	10^{-9}M ouabain		10^{-8}M ouabain		mean % increase with stimulation
	Basal	Stimulated	Basal	Stimulated	
Control	14.3	26.9	154.4±16.4(3)	226.4±20.5(3)	58±11(4)***
Bumetanide 10^{-5}M	15.2	19.8	186.2±14.5(3)	278.3±32.1(3)	46±16(4)*
Control			113.3±10.9(6)	173.0±20.8(6)	55±12(6)***
Bumetanide 10^{-4}M			139.3±14.2(3)	200.7±35.5(6)	42±16(6)**
Solvent			144.3±19.8(4)	179.8±8.5(4)	30±13.4(4)*

Units are picomoles of ouabain bound per gram wet weight. Values are Mean ± SEM(n)
* $p < 0.05$
** $p < 0.025$
*** $P < 0.01$

theophylline and dibutyryl cyclic AMP increased ouabain binding even in the presence of bumetanide 10^{-4}M. Thus, the increased ouabain binding after stimulation with theophylline and dibutyryl cyclic AMP appears to be independent of the entry of sodium and chloride into the cell via the coupled sodium and chloride carrier system since high concentrations of bumetanide fail to alter the increase in binding at a time when coupled entry of NaCl should be slowed or stopped.

If the effect of dibutyryl cyclic AMP to increase ouabain binding were mediated through an increase in Na-K-ATPase activity secondary to the admission of sodium into the cell, other maneuvers that increase sodium influx should have the same effect. Monensin, an ionophore with a cation selectivity $Ag^+ > Na^+ > K^+ > Rb^+ > Cs^+ > Li^+ > NH_4^+$, at a concentration of 1.5×10^{-5}M, did not (control 129.4±7.7, monensin 144.5±16.5, solvent 137.4±6.0, $p < 0.1$). In two of these slice preparations monensin increased ouabain inhibitable oxygen consumption from an average of 29.4 to 36.7 micromoles of O_2/hr/g wet weight or 26±2%, confirming the action of monensin in these concentrations to activate Na-K-ATPase by enhancing sodium entry. Since monensin, which increases the entry of sodium into the cell as judged by the increase in ouabain inhibitable oxygen consumption, does

not increase the binding of ouabain in slices of rectal gland, this mechanism cannot be the explanation for the increased binding of ouabain induced by dibutyryl cyclic AMP.

The increase in ouabain binding evoked by dibutyryl cyclic AMP and theophylline could be the expression of an increase in the number of membrane binding sites as a result of transfer to the cell membrane of already formed binding sites available in the cytoplasm. If such is the case, a role for membrane bound or cytoplasmic microtubules could be envisioned as the mechanism for the transfer of the preformed sites to the cell membrane. Table 2

Table 2

Effect of colchicine on the stimulation with dibutyryl cyclic AMP (10^{-3}M) and theophylline (10^{-3}M) of ouabain binding by rectal gland slices.

	10^{-8}M ^{3}H-ouabain		mean % increase with stimulation
	Basal	Stimulated	
Control	127.3±6.6(6)	187.6±19.9(6)	46±12(6)***
Colchicine 10^{-4}M	138.0±7.9(6)	200.7±35.5(6)	44±12(6)***

Units are picomoles of ouabain bound per gram wet weight. Values are Mean ± SEM(n)
* $p < 0.05$
** $p < 0.025$
*** $P < 0.01$

shows that colchicine at a concentration of 10^{-4}M has no effect on the increased ouabain binding brought about by dibutyryl cyclic AMP and theophylline. This experiment suggests that tubulin, as far as can be determined by the lack of effect of colchicine, does not appear to be involved in the process of stimulation of ouabain binding by dibutyryl cyclic AMP and theophylline.

The stimulation of ouabain binding induced by dibutyryl cyclic AMP and theophylline has been observed both in rectal gland slices and in isolated cells. To test whether intact cells are necessary for the production of the stimulatory effect of cyclic AMP, we examined the effect of dibutyryl cyclic AMP and theophylline on ouabain binding by rectal gland homogenates. Homogenates were prepared from perfused rectal glands. Prior to the preparation of the homogenates, rectal glands were perfused for thirty minutes with shark-Ringers with and without theophylline 2.5×10^{-4}M and dibutyryl cyclic AMP 5×10^{-5}M. The glands were homogenized in a buffer of the following composition (in mM): Na 280; K 5; Cl 295; Mg 3: Ca 2.5; SO_4 0.5; phosphate 1; Urea 350; Hepes 40, pH 7.6, containing in addition an ATP regenerating system (phosphoenol pyruvate 2×10^{-2}M, and pyruvate kinase 16 units/ml). Binding of ouabain was measured using a filtration technique. ^{14}C-Inulin, 0.1 microCi/ml, was used as a label for the incubation solution. The homogenate was incubated in a solution of the same composition as the homogenizing solution in the presence and absence of 10^{-3}M theophylline and 10^{-3}M dibutyryl cyclic AMP. Incubations were stopped by dilution of a measured amount of homogenate, still in the incubation medium, in ten times the volume of ouabain free incubation solution followed by rapid filtration through HA 0.45 micron Millipore filters. The filters were dissolved in 1 ml of ethylacetate and radioactivity counted in a scintillation counter using Hydrofluor^R. Non-specific binding of ^{3}H-ouabain was determined by displacement of the labelled ouabain with 10^{-4}M unlabelled ouabain. Non-specific binding to the filters was measured by filtering incubation solution containing labelled and unlabelled ouabain and labelled inulin but without homogenate. Specific binding to the homogenate was calculated from the total binding minus the non-specific binding after incubation with 10^{-4}M ouabain and minus the non-specific binding to the filter blanks.

Table 3 shows the time course of ouabain binding to homogenates of basal and stimulated rectal glands. Ouabain

Table 3

Ouabain binding by whole homogenates of basal and stimulated rectal glands.

	10^{-8}[^{3}H]-ouabain	
	Basal	Stimulated
0	0.06±0.02(4)	0.04±0.02(5)
15	0.88±0.13(4)	0.63±0.32(5)
30	1.82±0.15(5)	1.15±0.30(5)*
60	3.36±0.26(5)	2.04±0.25(5)*
120	5.72±0.56(5)	3.87±0.32(5)*
180	7.74±1.04(5)	5.23±0.54(5)*
240	9.33±1.11(5)	6.50±0.76(5)*
300	10.41±1.30(5)	7.23±0.86(5)*

Units are picomoles of ouabain bound per milligram of protein. Values are Mean ± SE(n)
* $p < 0.05$

binding was not increased in stimulated glands but rather decreased, indicating that intact cells are necessary for the cyclic AMP induced increase of ouabain binding to become manifest. Cell homogenization may expose all enzymatic sites within the cell to binding by ouabain, whereas only those on the surface of the cell are available in intact cells.

From these experiments we conclude that the stimulation of ouabain binding induced by theophylline and cyclic AMP is independent of the entry of sodium into the cell. The process does not appear to involve the formation of microtubules inasmuch as it cannot be blocked by colchicine. The stimulation of ouabain binding is apparent in intact cells but cannot be demonstrated in whole homogenates of rectal gland in which the cellular architecture has been destroyed.

INHIBITION OF K-INDEPENDENT Na/Cl UPTAKE INCREASES APICAL MEMBRANE K CONDUCTANCE IN FLOUNDER INTESTINE.

Dan R. Halm, Edward J. Krasny, Jr., and Raymond A. Frizzell, Department of Physiology and Biophysics, University of Alabama Medical Center, Birmingham, Al.

Studies of electrolyte transport across isolated flounder intestine have provided evidence for a coupled Na/K/Cl uptake process and for a barium-sensitive K conductance at the apical membrane (Musch et al., Bull MDIBL 21:95, 1981; Field et al., Bull. MDIBL 21:93, 1981; Stewart et al., Bull. MDIBL 20:92, 1980). Inhibition of Cl absorption was shown to hyperpolarize the apical membrane electrical PD (ψ_a) suggesting that the apical membrane K conductance increases, even in the presence of barium (Halm et al., Bull. MDIBL, 21:88, 1982). The results presented here further extend the description of membrane conductive properties and their relationship with salt absorption.

METHODS--Conventional microelectrodes were employed to measure ψ_a. The criteria for successful impalement have been described by Duffey et al (J. Memb. Biol. 50:331, 1979); 3 to 6 values of ψ_a were obtained under each experimental condition.

RESULTS--Figure 1a shows the dependence of ψ_a on mucosal solution K concentration, which is consistent with a high apical membrane K conductance. Addition of barium (2 mM) to the mucosal solution depolarized ψ_a and reduced the dependence of ψ_a on mucosal solution [K], consistent with barium blockade of apical K conductance. As reported previously (Halm et al., Bull MDIBL 21:88, 1981), addition of bumetanide (0.1 mM) to the mucosal solution hyperpolarized ψ_a to near the control value, even though barium was present. Return of the steep dependence of ψ_a on mucosal solution [K] indicates that apical membrane K conductance increased relative to basolateral membrane conductance. Figure 1b shows that the basolateral membrane is conductive to Cl, since