Furosemide appeared to block the accelerated changes in electrolyte composition produced by cAMP and theophylline in ouabain-treated glands. When furosemide was added together with ouabain in previously stimulated glands, the resultant changes in intracellular electrolytes over the ensuing 20 minutes resembled those seen in basal glands not exposed to cAMP (Figure 2).

These experiments indicate that when the Na-K-ATPase pump is blocked by ouabain, passive movements of sodium and chloride into the rectal gland cell, and of potassium out of the cell, are accelerated by cAMP. They suggest a possible mode of action of cAMP in enhancing rectal gland secretion.

Finally, these results suggest, in the light of the analogous effects of cAMP on ion movements in avian erythrocytes, that a general action of cAMP may be to enhance the linked transport of ions across cell membranes down an electrochemical gradient. Aided by N.I.H. grant AM 18078 and N.S.F. grant PCM 77-01146.

OUABAIN BINDING IN RECTAL GLAND OF SQUALUS ACANTHIAS

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Chloride secretion by the rectal gland of the dogfish Squalus acanthias depends on the activity of Na-K-ATPase located in the basolateral membrane of the cells lining the secretory tubules. We have previously reported indirect evidence supporting activation of Na-K-ATPase when chloride secretion is stimulated by cyclic AMP and theophylline (Bull. M.D.I.B.L., 18: 16-19, 1978). When these agents are used to stimulate secretion, that portion of the rectal gland oxygen consumption which can be inhibited by ouabain increases six-fold, suggesting a similar increase in the activity of Na-K-ATPase. After stimulation the intracellular concentration of sodium decreases and that of potassium rises, a finding consistent with primary activation of Na-K-ATPase. Recently Shuttleworth and Thompson (J. Exper. Zool., 206:297-302, 1978), reported that ouabain binding by slices of the rectal gland of the dogfish shark Scyliorhinus canicola was increased after incubation with cyclic AMP and theophylline. On the other hand, previous attempts in our laboratory to demonstrate that rectal gland stimulation increases ouabain binding in slices and perfused glands of Squalus acanthias have been unsuccessful. The present experiments were therefore designed to investigate further the nature of ouabain binding by rectal gland slices and its possible modification by cAMP stimulation.

Rectal gland slices were prepared within 20 minutes prior to incubation. Coronal slices were cut using a Stadie-Riggs tissue slices except for one experiment for which slices were cut using a mechanical tissue chopper (McIlwain and Buddle, Biochem. J., 53:412-420, 1953) supplied by Drs. Shuttleworth and Thompson. The freshly cut slices were placed in oxygenated shark Ringers solution at room temperature. A single slice weighing an average of 20 mg was placed in a 25 ml Erlenmeyer flask in 3 ml of shark Ringers containing ouabain at concentrations of 10^{-9}, 10^{-8}, 10^{-6}, 10^{-4} and 10^{-3}M. Tritiated ouabain, 19.3 Ci/mmol, was used as a marker for ouabain. At ouabain concentrations of 10^{-9} and 10^{-8}M no carrier ouabain was added. Inulin (^{14}C-inulin) at a concentration of 0.04 μCi/ml was added as an indicator of the adequacy of marking of the extracellular space. Half of the slices were incubated with 0.5 mM dibutyryl cyclic AMP and 2.5 mM theophylline while the other half served as a control. Evidence that these concentrations of theophylline and cyclic AMP stimulated transport was provided by the fact that in five similar experiments, oxygen consumption of slices measured with a Clark electrode rose an average of five-fold, from an unstimulated rate of 0.019 ± 0.005 μmoles O_2/mg tissue/hr (mean $\pm$ s.e.).

Potassium concentration in the incubation medium was either 5 or 40 mM. The slices were incubated at 15°C for 2 hours except where otherwise stated and then washed 3 times for 30 minutes at 15°C in a medium of the original composition, free of ouabain and inulin. Preliminary experiments indicated that the inulin marker was completely

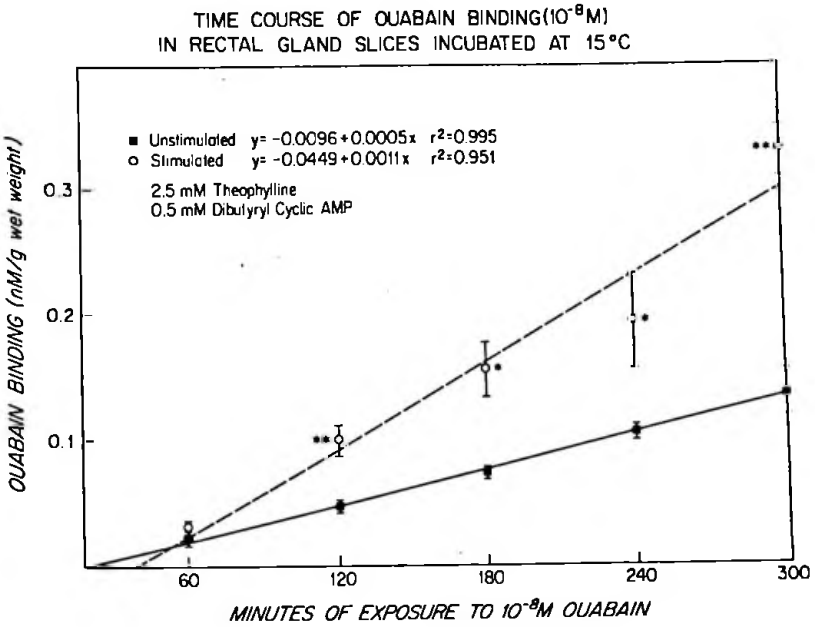

Figure 1. Stimulation of the rate of ouabain binding at 10^{-8}M by cAMP and theophylline. The open circles represent slices incubated with dibutyryl cyclic AMP 5×10^{-4}M. Closed circles are paired controls without stimulation. Binding is expressed in nanomoles of ouabain/g WW. Incubation medium was shark Ringers with 5 mM K^+. The slopes are significantly different ($p < 0.01$). The points represent mean ± SE; n = 3-6 for each point
* = $p < 0.05$; ** = $p < 0.001$.

removed by the end of the third wash, suggesting that this treatment was also adequate to remove unbound ouabain in the extracellular space. The slices were removed from the flasks, lightly blotted in filter paper and weighed in tared scintillation vials where they were dissolved in 1 ml of NCS. The dissolved slices were then counted in Spectrafluor in a liquid scintillation counter set for simultaneous measurement of ^3H and ^{14}C. The counts in each channel were corrected for spillover and converted to dpm using quench curves previously determined with the same batch of scintillation cocktail. Each reported value is the average of the stated number of glands. The values for each gland are the average of 3 or more slices incubated simultaneously.

Previous reports by us (J. Cell. Biol., 1979, in press) and by others (Shuttleworth and Thompson, J. Exper. Zool., 206:297-302, 1978) have shown that ouabain binding at concentrations of 10^{-6} and 5×10^{-6} reaches a steady state at two hours without any significant increase thereafter. At concentrations of 10^{-8}M, on the other hand, binding is much slower and by 5 hours the steady state has not been reached (Figure 1). At this concentration, the rate of binding is clearly increased by the presence of theophylline 2.5 mM and dibutyryl cyclic AMP 0.5 mM. In slices thus stimulated, the rate of ouabain binding was approximately doubled.

Ouabain binding increases linearly with the ouabain concentration in the bath up to concentrations of 10^{-6}M, and reaches saturation at concentrations of 10^{-4}M (Table 1). The binding of ouabain to Na-K-ATPase is displaced by high concentrations of potassium, and this provides a way of distinguishing specific binding to the transport enzyme from non-specific association with other proteins. As shown in Table 1, ouabain is displaced by increasing the concentration of potassium from 5 to 40 mM in the bath when the ouabain concentration is 10^{-6}M or lower, but not when it is 10^{-4}M or 10^{-3}M. Virtually the entire amount of ouabain binding at these high concentrations can therefore

TABLE I

Ouabain Binding in Slices of Dogfish Rectal Gland

Ouabain Concentration	Potassium mM	Control	Theophylline 2.5 mM + Dibutyryl cyclic AMP 0.5 mM	(theo + cAMP)-control
10^{-3}M	5	23.54 ± 6.09 (3)	26.04 ± 10.31 (3)	2.26 ± 1.27 (3) p < 0.05
	40	23.63 ± 6.11 (2)	22.10 ± 10.03 (2)	1.53 ± 3.92 (2)
	5-40	0.19 ± 2.48 (2)	-0.36 ± 3.18 (2)	-0.55 ± 5.66 (2)
10^{-4}M	5	30.51 ± 7.48 (3)	23.79 ± 8.27 (3)	-6.72 ± 2.49 (3) p <0.025
10^{-6}M	5	3.37 ± 1.87 (6)	4.00 ± 1.91 (6)	0.63 ± 0.62 (6) p <0.05
	40	0.54 ± 0.33 (6)	1.21 ± 0.36 (6)	0.67 ± 0.62 (6) p <0.025
	5-40	2.83 ± 1.87 (6)	2.80 ± 1.91 (6)	-0.04 ± 0.62 (6)
10^{-8}M	5	0.047 ± 0.026 (6)	0.076 ± 0.045 (6)	0.027 ± 0.016 (6) p <0.005
	40	0.013 ± 0.008 (3)	0.034 ± 0.022 (3)	0.021 ± 0.017 (3)
	5-40	0.036 ± 0.027 (6)	0.041 ± 0.041 (6)	0.006 ± 0.016 (6)
10^{-9}M	5	0.0057 ± 0.w019 (5)	0.0095 ± 0.0045 (5)	0.0038 ± 0.0025 (5) p <0.01
	40	0.0019 ± 0.0004 (2)	0.0053 ± 0.0017 (2)	0.0034 ± 0.0018 (2)
	5-40	0.0039 ± 0.0019 (5)	0.0042 ± 0.0039 (5)	0.0004 ± 0.0024 (5)

Values are in units of nanomoles per gWW, mean ± SD. Only paired t test were calculated and only for the (theo + cAMP)-control column. Only significant values for p are recorded. Incubation was for 120 min at 15°C in shark Ringer's solution.

be considered non-specific, with the reservation that concentrations of K^+ higher than 40 mM were not used in these experiments.

At lower ouabain concentrations of 10^{-6}, 10^{-8} and 10^{-9}M, ouabain binding was consistently increased by cAMP stimulation. The increase was apparent, however, in slices incubated with 40 mM as well as 5 mM of K^+ (Table 1), suggesting that non-specific as well as specific binding of ouabain was enhanced. No significant changes were noted in the difference between binding in the presence of 40 mM and 5 mM K^+. It is not possible, therefore, to interpret these data as establishing an activation of specific Na-K-ATPase ouabain-binding sites in the stimulated slice. Further experiments will be necessary in the perfused gland and with slices to delineate the nature of the increase in ouabain binding produced when ion transport is stimulated by cAMP in rectal gland cells.

Aided by NIH grant AM 18078 and NSF grant PCM 77-01146.

TELEOST ERYTHROCYTE CARBONIC ANHYDRASE: ISOLATION AND PARTIAL CHARACTERIZATION

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Red cell carbonic anhydrase has been studied and well characterized in a number of species. In addition to physical methods the enzyme can be categorized on the basis of kinetic properties and inhibition by sulfonamides and certain anions. This information has made possible a description of the phylogeny of carbonic anhydrase (Maren and Rittmaster, Bull. MDIBL. 17:35, 1977) and further suggests that the archetypal vertebrate enzyme (as in elasmobranchs) was of low activity, with the first high activity form appearing in teleosts. The kinetics and in-