Table 4. Effects of serosal or mucosal K concentration or Ba on electrical properties of alkaline gland.

condition	ψ_t (mV)	ψ_a (mV)	ψ_b (mV)	R_t (ohms cm^2)	f_r
control (4,95)	5.3±0.7	-45.2±3.7	50.4±3.6	102±16	0.39±0.09
50 mM K on M (4,13)	4.4±0.5	-42.1±2.9	46.6±2.6	108±21	0.27±0.08
control (5,107)	5.4±0.4	-44.7±2.9	50.0±2.8	103±12	0.39±0.09
50 mM K on S (5,21)	2.7±0.2	-29.0±1.4	31.7±1.2	90±10	0.63±0.13
control (3,70)	4.6±0.1	-44.3±6.6	48.9±6.7	74±13	0.44±0.10
Ba 1mM on S (3,18)	3.5±0.6	-35.3±5.1	38.9±5.6	79±16	0.41±0.07

Numbers in parentheses represent the number of glands and number of impalements, respectively. See text for composition of solutions.

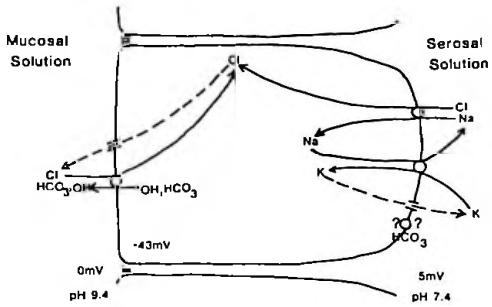

Figure 1.--Model of electrolyte transport by the alkaline gland.

K conductance and may largely determine the value of ψ_b; and (5) luminal alkalinization is accomplished by an increase in luminal solution HCO_3^-/CO_3^{-2} (OH^-) concentration which is Cl dependent. The exact mechanism of this process may involve an anion exchange mechanism at the apical membrane. Cl entering the cell by this process could recycle back to the mucosal solution thru the Cl conductance in this membrane. Whether the HCO_3^-/CO_3^{-2} (OH^-) which accumulates in the lumen arises from a net movement of HCO_3^-/CO_3^{-2} (OH^-), from cellular metabolism or from some combination of these processes is not known.

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SODIUM CHLORIDE COTRANSPORT BY SQUALUS ACANTHIAS RECTAL GLAND: POTENTIAL SITES OF REGULATION BY CYCLIC AMP

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INTRODUCTION

Experiments performed on isolated perfused rectal glands have demonstrated that secretion of sodium chloride is markedly stimulated by cyclic AMP or vasoactive intestinal peptide (Stoff et al., J. Exp. Zoology

199:443-448, 1977). It has been proposed by Silva et al (Am. J. Physiol. 233:F298-306, 1977) that the chain of events comprising active chloride secretion involves transfer of chloride from the blood across the basal-lateral membrane of the cell via a sodium-chloride cotransport system whereas the transfer across the luminal membrane is sodium independent. In order to elucidate the mechanism by which intracellular cyclic AMP affects sodium chloride secretion the transport properties of plasma membrane vesicles isolated from stimulated and nonstimulated rectal glands were investigated. It was found that the transport properties of the membranes as investigated sofar in vitro are not affected by stimulation of the gland in vivo.

MATERIALS AND METHODS

Dogfish caught by hook and line in Frenchman Bay were maintained in live cars and used within two days of capture. They were killed by a blow to the head followed by transection of the spinal cord. The glands were removed and perfused through the rectal gland artery for 30 minutes as described by Solomon et al (Bull. MDIBL 18:13-16, 1978). For experiments requiring stimulation of the gland 0.05 mM dibutyryl-cAMP and 0.25 mM theophylline were added to the dogfish Ringer's. Isolation of plasma membrane vesicles was carried out at 0-4°C as described by Eveloff et al (Pfluegers Arch. 378:87-92, 1978) with minor modifications. Protein was determined by the method of Lowry et al (J. Biol. Chem. 193:265-275, 1951) using bovine serum albumin as standard.

^{22}Na, [^{3}H]-mannitol and [^{3}H]-alanine uptake were followed by the filtration techniques described by Eveloff et al (Pflueger's Arch. 378:87-92, 1978). All reactions were carried out at 15°C. Composition of incubation media are given in the figure legends. ^{36}Cl efflux was studied in plasma membrane vesicles preincubated for two hours at 15°C in medium containing ^{36}Cl. The efflux reaction was initiated by adding 20 µl of preincubated membranes to 300 µl of incubation medium without isotope, followed by removal of 50 µl of the reaction mixture at timed intervals and rapid dilution into 1 ml ice cold stop solution. For evaluation of the amount of isotopes taken up by the vesicles, the amount of radioactivity present on the filter was corrected for radioactivity present on the filter in the absence of protein (usually less than 10% of final uptake).

RESULTS

Two potential sites of regulation were investigated using plasma membrane vesicles: the sodium chloride cotransport system located in the basal-lateral membrane and the membrane permeability to chloride.

Sodium uptake under several experimental conditions is shown in Figure 1. Replacement of chloride by

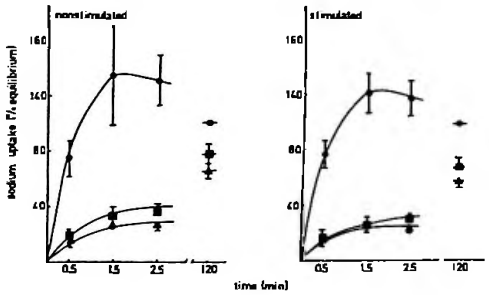

Figure 1.--Sodium uptake into plasma membrane vesicles prepared from dogfish rectal gland. The vesicles were suspended in 200 mM mannitol, 20 mM Tris-Hepes, 1.2 mM Mg $(NO_3)_2$ pH 7.6. The incubation media contained 20 mM Tris-Hepes, 1.2 mM Mg $(NO_3)_2$, 2 mM $NaNO_3$, pH 7.6 and either 200 mM KCl (o(, 200 mM KNO_3 (■) or 200 mM KCl plus 5 x 10^{-4}M bumetanide (Δ).

Vesicles from stimulated glands were prepared in the presence of dibutyryl cyclic AMP and theophylline in the homogenization medium. Vesicles from non-stimulated glands were prepared in the absence of cAMP and theophylline. Prior to the transport vesicles were preincubated in bumetanide for 5 minutes. Mean values and standard error of the mean derived from seven experiments are expressed as percent of equilibrium value obtained in the presence of 200 mM KCl. The equilibrium value amounted to 2.99 nmoles/mg protein for stimulated gland and 2.68 nmoles/mg protein for non-stimulated gland.

nitrate results in a reduction in uptake to the level obtained in the presence of 5×10^{-4} M bumetanide, a specific inhibitor of the sodium-chloride cotransport system. In the presence of 200 mM chloride the time course of Na uptake shows an overshoot wherein the amount of ^{22}Na accumulated in the vesicle transiently exceeds that seen at equilibrium. Maximal uptake occurs at 1.5 minutes and amounts to 135% of the equilibrium value in the nonstimulated gland and 122% of the equilibrium value in the stimulated gland. No statistically significant difference as measured by the Student's t-test can be demonstrated between stimulated and nonstimulated glands. Mannitol uptake was identical under all experimental conditions (data not shown).

The efflux of ^{36}Cl from preloaded plasma membrane vesicles is shown in Figure 2. There is no significant

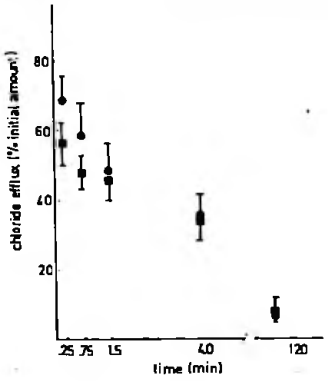

Figure 2.--Chloride efflux from plasma membrane vesicles prepared from dogfish rectal gland. The vesicles were suspended in 200 mm mannitol, 20 mM Tris-HEPES, 1.2 mm $Mg(NO_3)_2$, pH 7.6. The vesicles prepared from stimulated (●) and non-stimulated glands (▣) were preloaded for two hours in a medium which contained 100 mM mannitol, 75 mM $K^{36}Cl$, 50 mM Tris-HEPES, 1.2 mM Mg $(NO_3)_2$, pH 7.6. Mean values and standard error of the mean derived from three experiments (except t=4 minutes where the mean is derived from two experiments) are given as percent of initial amount of chloride present in the vesicle. The initial amount was 73.2 nmole/mg protein for stimulated gland and 72.2 nmole/mg protein for non-stimulated gland.

difference in efflux between vesicles prepared from nonstimulated glands and those prepared from stimulated glands.

In addition to experiments involving the potential site of regulation by cyclic AMP, preliminary experiments on alanine uptake were performed. Figure 3 demonstrates L-alanine uptake in rectal gland plasma membrane

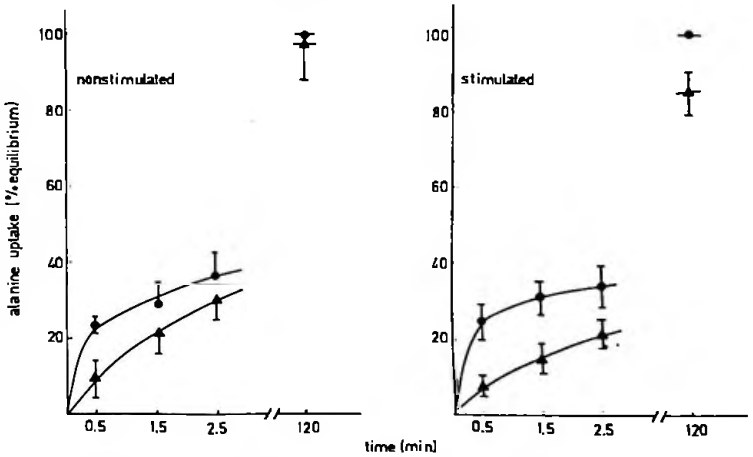

Figure 3.--Alanine uptake into plasma membrane vesicles prepared from dogfish rectal gland. The vesicles were suspended in 200 mM mannitol, 20 mM Tris-HEPES, 1.2 mM $Mg(NO_3)_2$, pH 7.6. The incubation media contained 20 mM Tris-HEPES, 1.2 mM $Mg(NO_3)_2$, 0.1 mM alanine, pH 7.6 and either 200 mM NaCl (o) or 200 mM KCl (Δ). Mean values and standard error of the mean derived from five experiments are expressed as percent of equilibrium value obtained in the presence of 200 mM KCl. The equilibrium value amounted to .129 nmoles/mg protein for stimulated gland and .110 nmoles/mg protein for non-stimulated gland.

vesicles. Replacement of 200 mM Na with 200 mM K results in a statistically significant decrease in alanine uptake. Cyclic AMP and theophylline have no demonstrable effect.

DISCUSSION

Plasma membrane vesicles isolated from the rectal gland of Squalus acanthias appear to contain a NaCl cotransport system as indicated by chloride dependence of sodium transport in the vesicles (Eveloff et al., Pflueger's Archiv. 378:87-92, 1978). This cotransport system is inhibited by furosemide, bumetanide and other "loop diuretics" which have been shown to inhibit chloride secretion in the intact gland (Kinne et al., Bull. MDIBL 19: 92-95, 1979). Demonstration herein of an overshoot in sodium uptake which is abolished by anion replacement or the presence of bumetanide provides further support for the existence of a sodium chloride cotransport system in these vesicles. It was not possible in the present studies to demonstrate a cyclic AMP effect on sodium uptake or chloride efflux in isolated vesicles. Considering that this fraction contains predominantly basal-lateral membrane vesicles and that ten-fold stimulation of secretion by cAMP occurs in the intact gland it is highly unlikely that regulation by cAMP involves the sodium chloride uptake step. Since only approximately 10% of the surface area of the cell is comprised of luminal membrane, the membrane fraction used is not well suited to study luminal membrane properties, thus a change in luminal membrane permeability to chloride may be masked. The experiments should be repeated with membrane fractions highly enriched in luminal membranes.

Preliminary data were also presented demonstrating a significant effect of a sodium grandient on uptake of L-alanine into rectal gland plasma membrane vesicles, suggesting the existence of a sodium dependent system for L-alanine uptake.

Finally it should be noted, that there are some limitations in the approach used in this study to investigate regulation of transport processes by cAMP. The possibility cannot be overlooked that the failure to demonstrate an alteration of the transport properties might be due to the rapid reversibility of the transport change. In this instance the cAMP mediated alteration might not be detectable in the isolated vesicles. This work was supported by American Heart Association-Maine Affiliate Grant to J. Eveloff, NIH Grant AM 27441 to R. Kinne, NIH Training Grant T32GM 7288 to the Medical Scientist Training Program, Albert Einstein College of Medicine.

BIOCHEMICAL BASIS FOR TRANSPORT PROCESSES IN THE ARCHINEPHRIC DUCT OF THE ATLANTIC HAGFISH (MYXINE GLUTINOSA)

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The urine forming system of the early vertebrate Myxine glutinosa consists of large glomeruli and short neck segments which drain the glomerular filtrate into the archinephric duct (Eisenbach, G.M., et al., Bull. MDIBL 11:11-15, 1971; Stolte, H., and Eisenbach, G.M., Bull. MDIBL 13:120-121, 1973). Earlier experiments revealed the importance of glomerular filtration for volume regulation in this species. In microperfusion experiments, first evidence was obtained that no net reabsorption of sodium and fluid takes place in the archinephric duct, but that potassium secretion and D-glucose reabsorption occurs (Alt, J.M. et al., J. Exp. Biol. 91:323-330, 1981).

To get further insight into the mechanisms underlying sodium and sugar transport, micro stopped flow perfusion experiments, histochemical investigations and transport studies with isolated brush border membranes were performed.

METHODS AND MATERIALS

The handling of the fish, anesthesia and further technical procedures have been described earlier (Alt et al.,