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.,

ibid.). The percentage of filtered load remaining was calculated from the concentrations of sodium and glucose in fluid of the archinephric duct and plasma. These calculations are based on the fact that the net fluid reabsorption from the inulin measurements is zero (TF/PF 1.0 ± 0.08, n = 33). Alkaline phosphatase and K-sensitive, ouabain-inhibitable p-nitrophenylphosphatase were localized in aldehyde fixed sections by the method of Ernst (J. Cell Biol. 66:586-608, 1975) and determined directly in tissue homogenate by standard procedures. Brush border membrane vesicles were prepared by a modified calcium precipitation method. D-glucose transport was measured by a rapid filtration technique (Hannafin et al., this Bulletin).

RESULTS

In stopped flow perfusion experiments of the archinephric duct in situ the remaining sodium with 105 ± 8% (n = 67) equalled the filtered load, but 57 ± 10% (n = 15) of filtered glucose was reabsorbed. 0.1 mmol/l phlorizin inhibited the glucose reabsorption completely (TF/PF 1.00 ± 0.07, n = 7). Both alkaline phosphatase and Na-K ATPase activity could be demonstrated in tissue homogenates. According to the histochemical experiments, alkaline phosphatase is localized at the brush border membrane and the ouabain-inhibitable, potassium-sensitive neutral p-nitrophenylphosphatase as indicator for Na-K-ATPase is present only in the basal-lateral plasma membranes (See Figure 1). The isolated brush border membranes were about 8 fold enriched in alkaline

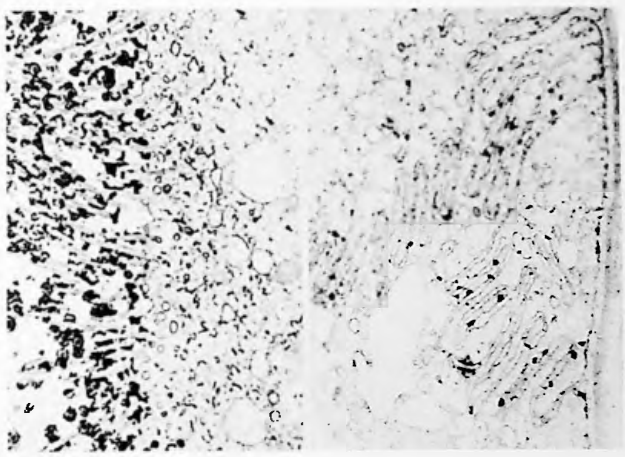

Figure 1.--Histochemical demonstration of alkaline phosphatase activity (left panel) and ouabain-inhibitable, potassium-sensitive neutral p-nitrophenylphosphatase activity (right panel) in the archinephrenic duct of Myxine glutinosa. Magnifications: left panel x 8000, right panel x 16000.

phosphatase. D-glucose uptake by the membrane vesicles was stimulated 5-fold by a 100 mmol/l sodium-thiocyanate gradient compared to a potassium thiocyanate gradient. Phlorizin (0.1 mmol/l) inhibited sodium-dependent D-glucose uptake almost completely (see Figure 2).

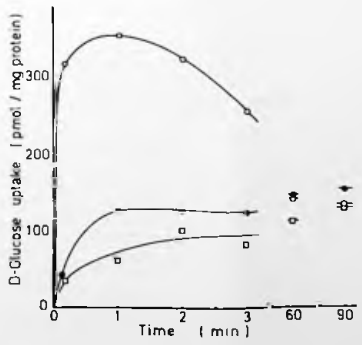

Figure 2.--D-glucose uptake into brush border membrane vesicles isolated from the archinephric duct of Myxine glutinosa. Membranes were isolated from about 0.3 grams of pooled archinephric duct by a modified calcium precipitation method. Uptake of D-glucose was determined at 25 degrees C in a medium containing 0.1 mM D-glucose, 20 mM Tris HEPES buffer, pH 7.4 and 100 mM sodium thiocyanate (O-O) or 100 mM potassium thiocyanate (□-□) or 100 mM sodium thiocyanate and 0.2 mM Phloridzin (●-●). One representative experiment is shown.

The archinephric duct epithelium in Myxine glutinosa differs from vertebrate epithelium by the lack of net sodium and fluid transport which is compatible with Myxine glutinosa being an osmoconformer. Another study (Raguse-Degener et al., unpublished) has shown that the epithelium is highly impermeable for sodium ($P_{Na} = 4.7 \pm 2.6\ 10^{-6}$ μmol mm^{-2} sec^{-1}, n = 8) and water (Lp $4.9 \pm 4.2\ 10^{-10}$ cm^3 cm^{-2} sec^{-1} cm H_2O^{-1}, n = 24). The zero net fluid reabsorption thus can be explained by the low sodium and water permeability combined probably with a low activity of the sodium pump. However, this activity seems to be sufficient to drive sodium cotransport of organic solutes such as D-glucose. The basic mechanisms involved in epithelial transport in higher vertebrates such as Na-K-ATPase for sodium transport and sodium D-glucose cotransport for glucose reabsorption thus seems to be already present in the archniphric duct of this early vertebrate. The generous supply of hagfish by Dr. Huntsman, Maine Laboratory, St. Andrews, New Brunswick, Canada, is gratefully acknowledged as well as the experienced help of Dr. William Driedzic, Mt. Allison University, Sackville, New Brunswick, Canada, in transferring the animals. Supported by DFG, SFB 146 and NIH grant 27441.

APICAL MEMBRANE POTASSIUM CONDUCTANCE IN FLOUNDER INTESTINE: RELATION TO CHLORIDE ABSORPTION

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INTRODUCTION

Studies of electrolyte transport across isolated flounder intestine have provided evidence for active NaCl absorption and K secretion (Field et al., J. Memb. Biol. 41:265, 1978; Stewart et al., Bull. MDIBL 20:92, 1980). Microelectrode studies demonstrated that the apical membrane is conductive to K (Stewart et al., Bull. MDIBL 20:92, 1980) allowing secretion by passive exit of K across the apical membrane. Similar to the K conductances in other epithelia, the apicalK conductance of flounder intestinal cells is decreased by mucosal Ba, which results in a depolarization of the electrical potential difference across the apical membrane (ψa). In addition, ψa is depolarized by a decrease in bathing solution pH, which also inhibits Cl absorption (Smith et al., Bull MDIBL 20:96, 1980). This suggests that apical membrane K conductance (g_K^a) may be altered by changes in cellular acid-base status and/or Cl transport rates.

In the present study we examined the effects of a variety of agents or conditions on g_K^a. The pH of the bathing media was changed with permeant or impermeant buffers to determine whether mucosal, serosal or intracellular pH is primarily responsible for the decrease in g_K^a and Cl absorption observed when extracellular pH is reduced. A possible role of cell Ca in modulating g_K^a was examined, since increased cellular calcium levels have been shown to activate a K conductance in red blood cell and neuronal membranes (Gardos, Biochim. Biophys. Acta 30:653, 1958; Gorman and Hermann, J. Physiol. 296:393, 1979).

METHODS

Conventional microelectrodes were employed to measure the electrical potential difference across the apical membrane (ψa). The criteria for successful cellular impalement have been described by Duffey et al (J. Memb. Biol. 50:331, 1979); 3 to 6 values of ψa were obtained under each experimental condition. The results are presented as the mean $\pm$ SEM with n equalling the number of tissues examined. The bathing media were buffered with either EPPS, TRIS/MES or CO_2/HCO_3; the standard pH was 8.0.

RESULTS

Ion Selectivity of the Apical Membrane

ψa has been shown to be sensitive to changes in mucosal K concentration, consistent with the presence of