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 Another study (Raguse-Degener et al., unpublished) has shown that the epithelium is highly impermeable for sodium ($P_{Na} = 4.7 \pm 2.6\ 10^{-6}$ μmol mm^{-2} sec^{-1}, n = 8) and water (Lp $4.9 \pm 4.2\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

Dan Halm, Edward Krasny and Raymond Frizzell, Departments of Physiology and Biophysics, University of Alabama in Birmingham, University Station, Birmingham, Al.

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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

an apical K conductance (Stewart et al., Bull. MDIBL 20:92, 1980). Measurements of ψa during changes in mucosal solution Na or Cl concentration provide no evidence for conductive movements of these ions across the apical membrane (see also Helman and Beyenbach, Bull. MDIBL 18:51, 1978). Complete replacement of mucosal solution Na with choline did not affect ψa (-56 $\pm$ 3 mV vs. -57 $\pm$ 2 mV, n = 3), and complete replacement of mucosal solution Cl with gluconate hyperpolarized ψa (-61 mV vs. -71 mV, n = 2), which is opposite to the result expected for removal of a permeant anion from the mucosal solution.

The complete replacement of K with Rb in both the mucosal and serosal bathing media does not affect NaCl absorption, transepithelial electrical potential difference (ψt) or tissue conductance (Gt) (Stewart et al., Bull. MDIBL 20:92, 1980). For tissues bathed by the normal K Ringer's solution, the dependence of ψa on mucosal solution K concentration yields a slope of 37 $\pm$ 4 mV/decade [K] (n = 3, Figure 1). Tissues bathed in Rb-substituted

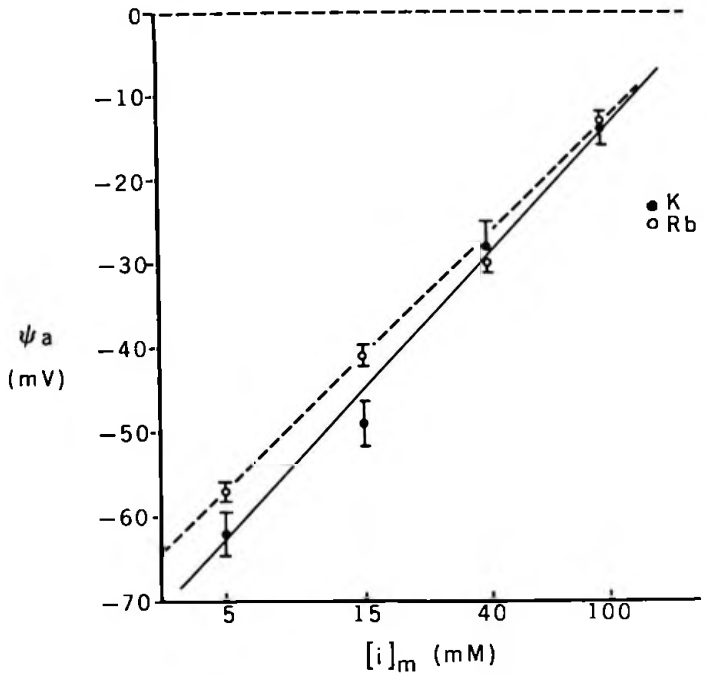

Figure 1.--Dependence of ψa on mucosal K or Rb concentration. Tissues were incubated in Ringer's solution containing either 5 mM K or 5 mM Rb. Tissues were bathed in Rb-containing Ringer's solution for 60-90 minutes before measurement of ψa in order to allow replacement of intracellular K with Rb. The points are the mean $\pm$ SEM of the ψa obtained successively at 5 mM, at a higher concentration and again at 5 mM for each of three concentrations 15, 40, 100 mM.

Ringer's solution showed a similar dependence of ψa on mucosal solution Rb concentration (33 $\pm$ 2 mV/decade [Rb]; n = 3; Figure 1). When tissues were bathed alternately by K Ringer's or Rb Ringer's solution, the depolarization of ψa produced by mucosal addition of Ba (5 mM) was similar (20 mV vs. 26 mV and 14 mV vs. 14 mV). These results suggest that the conductance pathways of the apical membrane do not distinguish between K and Rb, consistent with the finding that the rates of active K or Rb secretion across flounder intestine are identical (Stewart et al., Bull. MDIBL 20:92, 1980). The depolarization of ψa by elevated mucosal solution [K] can be used as a measure of g^a_K; a decrease in g^a_K would reduce the slope of the relation illustrated in Figure 1. In subsequent studies the depolarization of ψa elicited by an increase in mucosal solution [K] from 5 to 40 mM was employed as a measure of g^a_K.

The Hyperpolarization of ψa by Removal of Mucosal Solution Cl

The hyperpolarization of ψa that accompanies replacement of Cl with gluconate is not consistent with conductive Cl movement across the apical membrane as discussed above. Both Cl replacement and addition of the "loop" diuretic bumetanide inhibit NaCl absorption by blocking NaCl co-transport across the apical membrane; their effects on ψa and g_K^a were evaluated. Figure 2 shows ψa (the total height of the bar) and the change in ψa

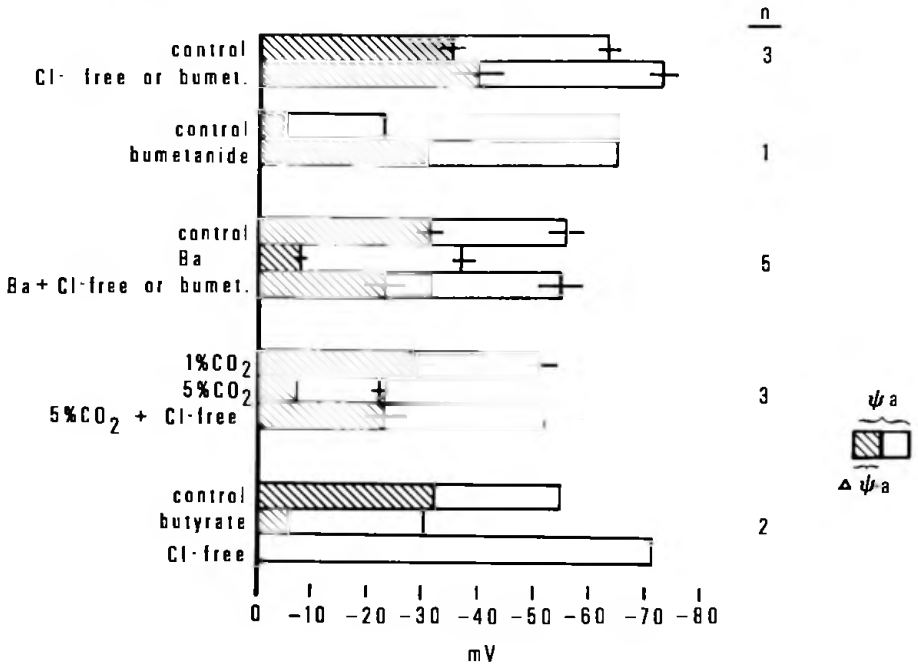

Figure 2.--Hyperpolarization of ψa induced by inhibition of Cl transport. ψa was measured before and after inhibition of Cl transport by Cl removal from the mucosal solution or addition of bumetanide (10^{-5}M) to the mucosal solution. The change in ψa induced by increasing mucosal [K] from 5 to 40 mM (the hatched bar) was taken as a measure of g_K^a. Barium (1 mM) and butyrate (8 mM) were added to the mucosal solution. Medium pCO_2 was increased from 8 to 40 mm Hg while leaving [HCO_3] fixed (20 mM). The error bars are SEM.

produced by increasing mucosal solution K from 5 mM to 40 mM (the height of the cross-hatched bar) under a variety of conditions. A small, but consistent hyperpolarization of ψa and increase in g_K^a was observed in control tissues exposed to either Cl-free mucosal solutions or bumetanide. When ψa was spontaneously low, the increase in ψa and g_K^a induced by bumetanide or Cl replacement was more dramatic. Indeed in one tissue (Figure 2) where the spontaneous value of ψa was -23 mV due to a low g_K^a and where the short-circuit current was spontaneously high (approximately 140 $\mu A/cm^2$), bumetanide increased ψa and g_K^a to normal values.

The responses of ψa and g_K^a to Cl replacement or bumetanide addition were also examined following experimental conditions that previously were shown to decrease ψa and g_K^a: 1) addition of Ba to the mucosal solution and 2) increased ambient pCO_2. As shown in Figure 2, the decrease in ψa and g_K^a produced by mucosal Ba or increased pCO_2 could be reversed either by addition of bumetanide to the mucosal solution or by replacement of mucosal solution Cl with gluconate. A representative experiment is shown in Figure 3 and illustrates the effects of Cl replacement and mucosal Ba on g_K^a. The hyperpolarization of ψa induced by Cl replacement under control conditions or following exposure to either Ba (or elevated pCO_2) rapidly reverses upon return of Cl to the mucosal solution (data not shown).

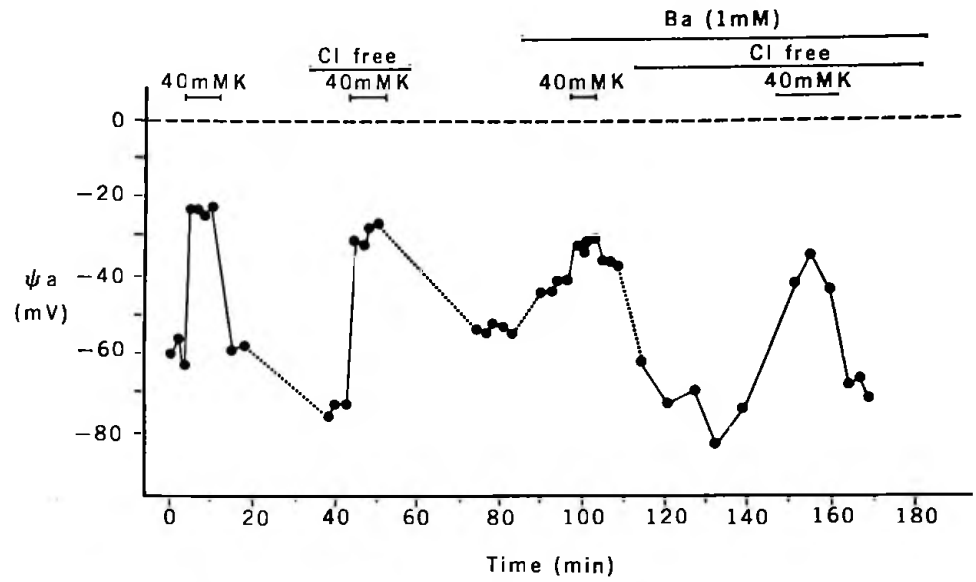

Figure 3.--Hyperpolarization of ψa and increase of g_K^a during removal of mucosal Cl. Successive values of ψa obtained during a representative experiment where mucosal solution Cl was replaced with gluconate and after the addition of Ba to the mucosal solution. The effect on ψa of increasing the mucosal solution [K] from 5 to 40 mM was used as an estimate of g_K^a.

The increase of g_K^a with Cl replacement or bumetanide addition is consistent with the opening of latent K channels. The decrease in ψa and g_K^a produced by Ba presumably involves a direct blockage of the K channel, as proposed for Ba inhibition of K conductance pathways in other epithelia and in excitable membranes. Inhibition by Ba is thought to be competitive, so that an increase in the total number of K channels with Cl replacement or bumetanide would not be expected to alter the percentage of channels blocked by Ba. Under these conditions, the number of open channels would increase such that ψa hyperpolarizes and a large K-induced depolarization of ψa reappears.

A decrease in serosal pH, produced by an elevation of pCO_2, has been shown to reduce ψa and g_K^a and inhibit Cl absorption (Smith et al., Bull MDIBL 19:24, 1979). A decrease in serosal solution pH from 8.0 to 7.0, produced by an adjustment in the TRIS-MES buffer ratio, did not reduce ψa or g_K^a, but did inhibit I_{sc} (74 ± 12 $\mu A/cm^2$ vs. 35 ± 5 $\mu A/cm^2$, $n = 4$) reflecting a decreased Cl absorption rate. The high lipid solubility of CO_2 would lead to rapid alterations in cell pH when pCO_2 is varied, whereas TRIS and MES would not be expected to markedly affect cell pH during the time course of these studies (ca. 30 min). This suggests that the reduction in g_K^a observed with elevated pCO_2 results from acidification of the cell interior while inhibition of Cl absorption is due to acidification of the serosal solution alone, as previously suggested (Smith et al., Bull. MDIBL 20: 96, 1980).

The weak acid, butyrate, was also employed in our attempts to experimentally alter cellular pH. To maximize the entry of the protonated form, mucosal pH was lowered to 5.0, a value approaching the pK_a of butyric acid (4.8). Decreasing the pH of the mucosal solution to this value by adjusting the TRIS-MES buffer ratio did not affect ψa, but subsequent addition of 8 mM butyric acid depolarized ψa and decreased g_K^a. In the presence of butyric acid, the success of impalement was lower than under control conditions. Therefore, values of ψa were obtained immediately after removing butyric acid and returning the mucosal pH to 8. During this time, ψa remained depolarized and g_K^a was reduced, as shown in Figure 2. The finding that g_K^a remained low despite removal of butyrate and return of the mucosal solution pH to its normal value suggests that cell pH re-

mained reduced, probably because of the continued presence of the weak acid within the cell. The subsequent replacement of mucosal solution Cl with gluconate hyperpolarized ψa, suggesting that inhibition of g_K^a by butyrate also could be reversed with Cl replacement (Figure 2).

Dependence of ψa on Calcium

A K conductance of neurons and red blood cells has been shown to be activated by increased intracellular Ca. Addition of the Ca ionophore, A23187, to the mucosal solution (1 μg/ml) did not alter ψa as might be expected for an ionophore-induced increase in cell Ca activity and g_K^a. Quinidine (10^{-4}M), which is thought to elevate intracellular Ca activities and/or to block Ca-mediated regulatory events, had no effect on ψa. Furthermore, if Na/Ca exchange across the basolateral membrane plays a role in maintenance of low intracellular Ca levels, as has been proposed for some epithelia, removal of serosal Na should increase intracellular Ca. However, replacement of serosal Na with choline did not alter ψa. Finally, addition of trifluoperazine (0.1 mM) to the serosal solution also had no effect on ψa, so that our attempts to experimentally alter g_K^a through changes in cell Ca activity or Ca-mediated regulatory processes were uniformly unsuccessful.

DISCUSSION

The results of our studies confirm that the apical membranes of flounder intestinal cells are characterized by a high conductance to K, whereas conductances to Na and Cl are not detectable. The conductance pathways traversing this barrier are Ba-sensitive and do not markedly discriminate between K and Rb.

One factor involved in the regulation of g_K^a appears to be cell pH. Elevated pCO_2 or addition of butyric acid to the mucosal solution decreases g_K^a and depolarizes ψa. Reduced serosal solution pH inhibits Cl absorption, as shown previously, but does not reduce g_K^a unless a readily permeant acid is present; reduced mucosal solution pH did not alter g_K^a. Attempts to manipulate cell Ca activity or Ca-mediated events provided no evidence for a Ca-dependent K conductance in this tissue. These findings suggest that cell acidification reduces g_K^a which may provide a mechanistic explanation for the decrease in distal nephron K secretion observed during acidosis.

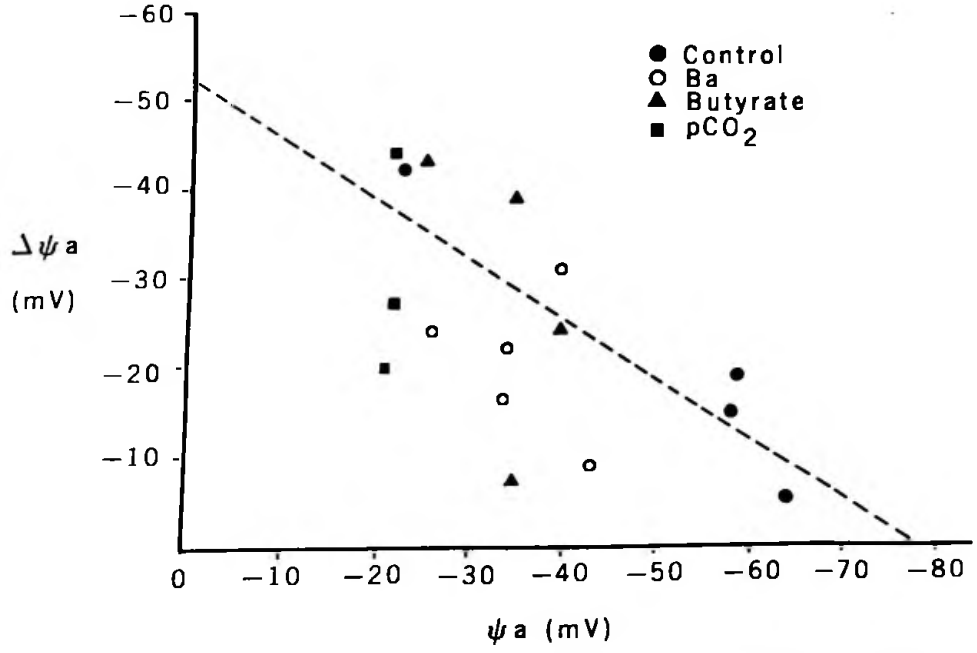

Figure 4.--Relation of initial ψa to the subsequent hyperpolarization induced by inhibition of Cl transport. The data in Figure 2 is plotted to illustrate the dependence of the hyperpolarization of ($\Delta\psi a$), induced by either replacement of mucosal solution Cl with gluconate or addition of bumetanide to the mucosal solution, on the initial value of ψa. The line was obtained by a least-square fit of the data; the intercept on the ψa axis is -79 mV which is close to the E_K across the apical membrane (see text).

When g_K^a is low as a result of spontaneous variation or experimentally-induced reduction of g_K^a, inhibition of Cl absorption either by exposure to Cl-free mucosal solutions or by addition of 10^{-5}M bumetanide to the mucosal solution increased g_K^a and hyperpolarized ψa. This effect was more pronounced in tissues showing spontaneously high Cl absorption rates. These findings suggest that the K conductance of the apical membrane varies inversely with the rate of Cl absorption, but the mechanistic details of this relationship remain to be defined.

The hyperpolarization of ψa induced by Cl replacement or bumetanide is given as a function of the initial ψa in Figure 4. The effect of Cl-free media or bumetanide is small when ψa approaches -70 to -80 mV since g_K^a would be near its maximal value. The E_K across the apical membrane of flounder intestinal cells, calculated from cellular and mucosal solution K activities using the Nernst equation, is -77 $\pm$ 3 mV (Smith et al., Bull. MDIBL 20:96, 1980) suggesting that ψa approaches E_K as g_K^a increases. Thus, inhibition of Cl absorption by exposure to Cl-free media or bumetanide increases g_K^a so that ψa approaches E_K. Supported by grants from the NIH (AM 27524) and Merck and Co.

POTASSIUM DEPENDENCE OF CHLORIDE TRANSPORT IN THE INTESTINE OF THE FLOUNDER, PSEUDOPLEURONECTES AMERICANUS

44 Michael Field, Leigh S. Kimberg, Stephanie A. Orellana and Raymond A. Frizzell, Departments of Medicine and Pharmacological and Physiological Sciences, University of Chicago, Chicago, Il., and Department of Physiology, University of Alabama, Birmingham, Al.

Barium-stimulated net Rb absorption[x] and unidirectional Rb influx across the brush border (initial rate of Rb intake from lumen-to-epithelium) are inhibited by furosemide and by replacement of medium Cl with gluconate, suggesting possible co-transport of K and Cl across the brush border (Musch et al, MDIBL, 1981). To further test this possibility we have determined the effects of removing K from the mucosal bathing medium on transepithelial Cl fluxes and on Cl influx.

In addition to possible coupling at the brush border, K and Cl fluxes may also be coupled at the basolateral membrane. Electrophysiological data suggest that the conductance of the basolateral membrane is insufficient to account for a large passive Cl flux, suggesting that Cl movement across that membrane is electroneutral. The most obvious possibility would be KCl cotransport, which would link the large difference in K concentration to Cl efflux across the serosal border. To examine this possibility, we determined the effect of increasing medium K concentration on transepithelial Cl fluxes. [x] ^{86}Rb and ^{42}K fluxes have been shown to be identical in flounder intestine (Stewart et al., Bull. MDIBL 20:92, 1980).

METHODS

Cl fluxes across and into flounder intestine stripped of muscle were determined as previously described (Figle et al., J. Membr. Biol. 41:265, 1978; Frizzell et al., J. Membr. Biol. 46:27, 1979). Bathing solutions used were the same as those described elsewhere in this bulletin (see Musch et al) except that 5 mM KCl replaced 5 mM $RbNO_3$. When K concentration was increased to 50 mM, Na concentration was decreased by 45 mM.

RESULTS

When K was removed from the mucosal medium, net Cl absorption was reduced by 60% (Table 1). This reduction of J_{net}^{Cl} was due entirely to a decrease in J_{ms}^{Cl}. To examine this phenomenon further, we determined the dependence of Cl influx on mucosal K (Table 2). The furosemideinhibitable portion of Cl influx was reduced by 5.4 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ or 70% when K was removed from the mucosal medium. Total Cl influx was also reduced. The relative magnitudes of the K-dependent Cl influx and the Cl-dependent K influx (musch et al., Bull. MDIBL 1981) suggest 2:1 stoichiometry.