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Amiloride Sensitivity of Proton-Conductive Pathways in Gastric and Intestinal Apical Membrane Vesicles

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Summary. Passive proton permeability of gastrointestinal apical membrane vesicles was determined. The nature of the pathways for proton permeation was investigated using amiloride. The rate of proton permeation (k_H^+) was determined by addition of vesicles ($pH_i = 6.5$) to a pH 8.0 solution containing acridine orange. The rate of recovery of acridine orange fluorescence after quenching by the acidic vesicles ranged from 4×10^{-3} (gastric parietal cell stimulation-associated vesicles; SAV) and 5×10^{-3} (duodenal brush-border membrane vesicles; dBBMV) to 11×10^{-3} sec^{-1} (ileal BBMV; iBBMV). Amiloride, 0.03 and 0.1 mM, significantly reduced the rate of proton permeation in dBBMV and iBBMV, but not gastric SAV. The decreases in k_H^+ were proportionately greater in iBBMV as compared with dBBMV. The presence of Na^+/H^+ exchange was demonstrated in both dBBMV and iBBMV by proton-driven ($pH_i < pH_o$) $^{22}Na^+$ uptake. Evidence was also sought for the conductive nature of pathways for proton permeation. Intravesicular acidification, again determined by quenching of acridine orange fluorescence, was observed during imposition of K^+-diffusion potential ($[K^+]_i \gg [K^+]_o$). In dBBMV and iBBMV, intravesicular acidification was enhanced in the presence of the K^+-ionophore valinomycin, indicating that the native K^+ permeability is rate limiting. In the presence of valinomycin, the K^+-diffusion potential drove BBMV intravesicular acidification to levels close to the electrochemical potential. In gastric SAV, acidification was not limited by the K^+ permeability. Valinomycin was without effect, but the K^+/H^+ ionophore nigericin enhanced acidification in gastric SAV, illustrating the low proton permeability of these membranes. Amiloride, 0.03–1 mM, resulted in concentration-dependent reductions of K^+-diffusion potential-driven acidification in dBBMV and iBBMV but not in gastric SAV. These data demonstrate that proton permeation in the three membrane types is rheogenic. The sensitivity of the proton-conductive pathways in intestinal BBMV to high concentrations of amiloride correlated with the presence of the Na^+/H^+ antiport and indicates that this transmembrane protein may represent a pathway for proton permeation.

Key Words amiloride · brush-border membranes · intestine · Na^+/H^+ antiport · parietal cell · proton conductance

Introduction

The epithelial cells lining the gastrointestinal mucosa are able to resist the erosive properties of the normal luminal contents of the gastrointestinal tract. The cells of the upper gastrointestinal tract will be exposed on their apical surface to H^+, in excess of 0.1 M in the case of the stomach, and the properties of the apical plasmalemma and the intercellular tight junctions are integral components of the mechanism resisting damage by H^+ (Powell, 1984; Hirst, 1989, 1990, 1991). We have previously quantified the passive permeability of upper gastrointestinal epithelial apical membranes to protons and related this permeability to the lipid dynamics of these membranes (Wilkes et al., 1989). The passive proton permeability of gastric parietal cell membranes and intestinal brush-border membranes was in the range $4–10 \times 10^{-4}$ cm sec^{-1}, an order of magnitude lower than that for renal cortical brush-border membranes (Wilkes et al., 1989; Hirst, 1991). Gastric membranes had the lowest permeability to protons, correlating with their physiological function, but had the highest membrane fluidity as quantified by diphenyl hexatriene fluorescence anisotropy studies (Ballard, Wilkes & Hirst, 1988; Wilkes et al., 1989). Thus the fluidity of the lipid bilayer is not the major determinant of proton permeability under basal conditions.

A number of pathways and mechanisms have been postulated for the transmembrane transport of protons, including weak acid protonophores, water wires and diffusion of molecular, uncharged acid species (Deamer, 1987, Gutknecht, 1987). In biological membranes, the presence of membrane spanning proteins, such as those involved in transmembrane transport functions, are likely to provide an important pathway for proton permeation (Verkman, 1987). We have used amiloride to provide evidence for such protein pathways for proton permeation in gastric and intestinal apical membranes. We demonstrate that the proton permeation in these gastrointestinal membranes is rheogenic in nature. In the intestinal membranes, proton permeability is re-

duced by amiloride, whereas gastric membranes are insensitive to this compound. The concentration-response relationship for amiloride inhibition of proton conductance in duodenal and ileal brush-border membranes, and the correlation with the presence of Na^+/H^+ exchange, leads us to suggest that, in the absence of Na^+ the Na^+/H^+ antiport in an unusual mode of operation may provide a route for transmembrane rheogenic proton permeation.

Materials and Methods

Preparation of Duodenal and Ileal Brush-Border Membrane Vesicles (BBMV)

Adult New Zealand White rabbits were sacrificed by intravenous barbiturate. The small intestine was removed and the duodenum and ileum identified. In some experiments, the duodenal bulb (first 5 cm after pyloric junction) was also used. Intestinal segments were washed with ice-cold saline, opened longitudinally and blotted to remove adherent mucus. The mucosa was scraped from the underlying muscularis and transferred to 20 vol. of homogenizing buffer (50 mM mannitol, 2 mM Tris-HCl, pH 7.4). Mucosa was homogenized for 2 min at 4°C in a Serval Omnimixer operating at 3000 rpm.

Duodenal (dBBMV) and ileal (iBBMV) brush-border membrane vesicles were purified from the small intestinal mucosal homogenates by a modification of the method of Kessler et al. (1978). The homogenate was stirred on ice, with $MgCl_2$ added to a final concentration of 10 mM, for 20 min. The homogenate was centrifuged for 20 min at 3,000 $\times$ g_{av}, 4°C. The vesicles were harvested from the supernatant by centrifugation at 27,000 g_{av} for 40 min at 4°C. The membrane vesicles were resuspended in an isotonic buffer (300 mM mannitol, 10 mM Tris-HCl, pH 7.4) and washed by sequential low speed (5,000 $\times$ g_{av}, 15 min, 4°C) and high speed (27,000 $\times$ g_{av}, 40 min, 4°C) centrifugation. Membranes were resuspended at a protein concentration of 15–30 $\mu g\ \mu l^{-1}$ in isotonic resuspension buffer. Membranes were frozen in liquid nitrogen and stored at −70°C for less than two mo.

Preparation of Parietal Cell Apical Membrane Vesicles (Gastric SAV)

Gastric SAV were prepared by a modification of the method of Hirst and Forte (1985). New Zealand White rabbits were treated with histamine to prepare stimulated gastric fundic mucosa. The mucosa was homogenized at 4°C in MSEP solution (125 mM mannitol, 40 mM sucrose, 1 mM EDTA, 5 mM PIPES-Tris, pH 6.7) by 16–18 passes of a Potter-Elvehjem homogenizer with a loose-fitting pestle operating at 200 rpm.

Stimulation-associated vesicles (SAV) were isolated by differential, followed by density gradient centrifugation. The 7,000 $\times$ g_{av} fraction was harvested from the 16% interface of a discontinuous Ficoll gradient, as described previously (Wilkes et al., 1989). Following washing by dilution into 20–50 vol. of sucrose (300 mM)-Tris-HCl (10 mM, pH 7.4) and centrifugation at 20,000 $\times$ g_{av} for 15 min at 4°C, membranes were resuspended at 6 mg ml^{-1} protein, diluted with 0.8 vol. of 2 M sucrose, frozen in liquid nitrogen and stored at −70°C for up to 1 mo.

Analysis of Proton Permeation

Proton permeation in all membrane vesicle preparations was determined by a modification of the method of Ives and Verkman (1985). Membrane vesicles were equilibrated overnight at 4°C in a solution containing 250 mM sucrose, 50 mM N-methyl-D-glucamine-(NMG) gluconate, 10 mM HEPES-Tris, pH 6.5. Vesicles were collected by centrifugation at 27,000 $\times$ g_{av} for 30 min and voltage clamped by resuspension in 150 mM K-gluconate, 4 μM valinomycin, 10 mM HEPES-Tris, pH 6.5 for 1–2 hr at 4°C. Proton permeation was monitored by diluting vesicles to a final concentration of 0.1–0.3 mg ml^{-1} in 150 mM K-gluconate, 6 μM acridine orange, 10 mM HEPES-Tris, pH 8.0. The appropriate concentration of amiloride was added to both of the above solutions. The fluorescence signal from the acridine orange (excitation 492 nm, emission 530 nm) was monitored in a Perkin Elmer LS-5 spectrofluorimeter. Proton permeation was determined from the rate of equilibration of pH as described previously (Wilkes et al., 1989), and values are given as the apparent rate constants for proton permeation, k_H^+.

Determination of H^+ Conductance in Membrane Vesicles

Rheogenic proton permeation of membrane vesicles was investigated by observing intravesicular acidification following imposition of a K^+-diffusion potential (Muallem et al., 1985). Membrane vesicles were suspended to a final concentration of 3–6 mg ml^{-1} in a buffer containing 150 mM K-gluconate, 10 mM MOPS-Tris, pH 7.6, and incubated overnight at 0°C. Amiloride was added to vesicles 1 hr prior to the experiment. Experiments were initiated by dilution of the equilibrated vesicles into 9 vol. of a buffer containing 150 mM NMG-gluconate, 6 μM acridine orange, 10 mM MOPS-Tris, pH 7.6, and the appropriate amiloride concentration. Acidification of the intravesicular space was monitored by the quenching of acridine orange fluorescence in a Perkin Elmer LS-5 spectrofluorimeter. Ionophores were added in small volumes (<0.5%) at appropriate points according to the protocols.

Determination of Na^+/H^+ Exchange by $^{22}Na^+$ Uptake in Brush-Border Membranes

BBMV were resuspended at 10 mg ml^{-1} in 300 mM mannitol, 20 mM K-gluconate, 50 mM MES-Tris, pH 5.5, 9 μM valinomycin. Five μl of BBMV preparation were diluted into 95 μl of 300 mM mannitol, 20 mM K-gluconate, 0.1 mM $^{22}NaCl$ (1.6 kBq ^{22}Na) and either 50 mM MES-Tris, pH 5.5, or 50 mM HEPES-Tris, pH 7.5. ^{22}Na uptake was followed with time at room temperature before stopping by addition of 5 ml ice-cold stop solution containing 150 mM mannitol, 120 mM K-gluconate, 5 mM Tris-gluconic acid lactone, pH 5.5, and immediate filtration using a 0.45-μm nitrocel-

lulose filter. Filters were washed, dried and added to scintillation cocktail, and radioactivity was determined by scintillation counting.

Protein was determined by the method of Bradford (1976), using γ-globulin (Cohn fraction V) as standard.

Results

Passive Permeation of Protons through Apical Membrane Vesicles

The determination of the apparent rate constant for proton permeation (k_H^+) in dBBMV is illustrated in Fig. 1. Addition of dBBMV (intravesicular pH 6.5) to the acridine orange solution (pH 8.0) resulted in an immediate quenching of acridine orange fluorescence. The fluorescence then recovers with time. A semi-log plot of this recovery *versus* time is linear (Fig. 1, *inset*) and gives k_H^+ (Wilkes et al., 1989). Addition of increasing concentrations of amiloride resulted in concentration-related decreases in the rate of recovery of fluorescence (Fig. 1). Values of k_H^+ were significantly decreased by amiloride, 3×10^{-5} and 10^{-4} M (Fig. 2). Values for k_H^+ were much greater in iBBMV, and amiloride resulted in proportionately greater decreases in k_H^+ (Fig. 2). In gastric SAV, basal k_H^+ was of the same order as in dBBMV, but amiloride did not significantly alter k_H^+ in these vesicles (Fig. 2).

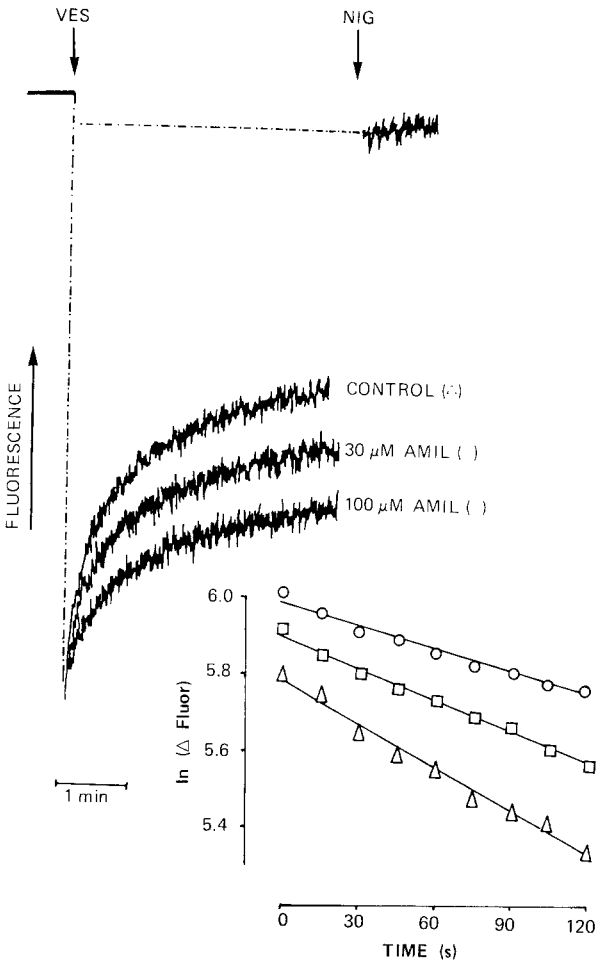

Fig. 1. Effect of amiloride on proton permeation in duodenal BBMV. Addition of dBBMV equilibrated at pH 6.5 (*VES*), into a solution at pH 8.0 containing acridine orange produced a quenching of acridine orange fluorescence due to accumulation of dye into the lower pH compartment. Subsequent dissipation of the pH gradient with time produces a reduction in quenching (△). Complete dissipation of the pH gradient was achieved by addition of nigericin (*NIG*), a K^+/H^+ ionophore. Rate of dissipation is decreased by the presence of amiloride, 30 μM (□) and 100 μM (○). *Inset:* Analysis of fluorescence quench curves. Fluorescence recovery followed first-order kinetics and was linearized by plotting ln ($\Delta Fluor$) *versus* t, where $\Delta Fluor$ is the difference beween fluorescence observed at time t and the fluorescence observed following dissipation by nigericin, 10 μM. This manipulation gave lines of gradient $-k_H^+$, where k_H^+ is the apparent first-order rate constant for the process.

Vesicular Acidification Driven by Diffusion Potentials

The selection of the impermeant cation and anion, NMG and gluconate, leaves only K^+ and H^+ as ionic species of significant permeability across the vesicular membranes. Thus when $[K^+]_i \gg [K^+]_o$, a K^+-diffusion potential is established which drives the entry of protons, resulting in quenching of the acridine orange fluorescence. If K^+ permeability is high, the process is limited by the rate of entry of H^+, i.e., the H^+ conductance. This is demonstrated in Fig. 3, where dBBMV were acidified in response to diffusion potentials. The native K^+ permeability of these vesicles is not large enough to make H^+ permeation the limiting factor. Addition of the K^+ ionophore, valinomycin, produced further acidification. Hence, measurement of H^+ permeation required inclusion of valinomycin in the reaction. The presence of valinomycin drove acidification to levels close to the electrochemical potential (as achieved by the K^+/H^+ ionophore nigericin). The proton gradients may be dissipated by the use of tri-*n*-butyltin. By contrast, the behavior of gastric SAV were consistent with the known properties of these membranes (Fig. 4). The presence of an endogenous K^+ conductance (Wolosin & Forte, 1984) made H^+ entry the limiting factor; intravesicular acidification was influenced only slightly by valinomycin. However, addition of nigericin to gastric SAV resulted in a marked increase in acidification (Fig. 4), consistent with the low proton permeability determined in these membranes in previous studies (Wilkes et al., 1989).

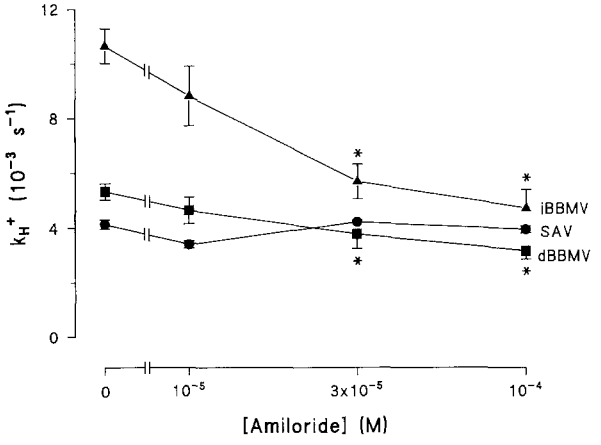

Fig. 2. Proton permeation in gastrointestinal apical membrane vesicles in the presence of increasing concentrations of amiloride. Proton permeation was determined as illustrated in Fig. 1, in the presence of increasing concentrations of amiloride. The calculated values of k_H^+ for gastric SAV and duodenal and ileal BBMV are illustrated as mean with errors bars of 1 SEM for 4–12 determinations. $^*P < 0.05$ compared with k_H^+ in the absence of amiloride.

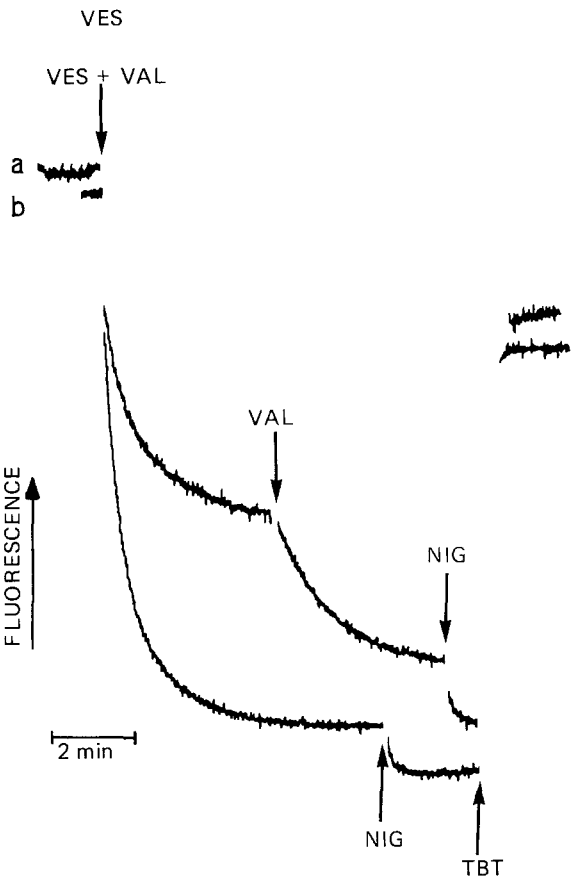

Fig. 3. Intravesicular acidification driven by diffusion potentials in duodenal BBMV. Duodenal BBMV, equilibrated in 150 mM K-gluconate, pH 7.6 were diluted (*VES*) into 150 mM NMG-gluconate, 6 μM acridine orange, pH 7.6 (trace *a*), which resulted in quenching of the fluorescence signal indicative of intravesicular acidification. Subsequent addition of valinomycin, 4 μM (*VAL*) produced further acidification (trace *a*), indicating K^+ permeability was limiting, and maximal acidification was achieved by addition of nigericin, 10 μM (*NIG*). Addition of vesicles preincubated with valinomycin (*VES + VAL;* trace *b*) produced a large intravesicular acidification, only slightly increased by nigericin, indicative of a high passive H^+permeability. In each example, fluorescence quenching was released by addition of 15 μl tri-*n*-butyltin chloride (*TBT*).

In the presence of the K^+ ionophore valinomycin, H^+ permeation is rate limiting; hence the diffusion-limited acidification will be dependent only upon the permeability of H^+ through the vesicle membrane. The apparent inhibition of passive proton permeability by amiloride observed in dBBMV and iBBMV (Figs. 1 and 2) was paralleled by a concentration-dependent inhibition of diffusion-limited acidification by amiloride, as illustrated for iBBMV in Fig. 5. Amiloride had no effect on the ability of nigericin to drive acidification at its electrochemical potential, nor the ability of tri-n-butyltin to dissipate the proton gradients (Fig. 5). By contrast, gastric SAV had no sensitivity of passive permeation to amiloride (Fig. 2), and in these membranes diffusion-limited acidification was unaffected by amiloride, even at the very highest concentrations (Fig. 6).

Na^+/H^+ Exchange in Brush-Border Membranes

The effects of an outward H^+ gradient ($pH_i < pH_o$) on vesicular accumulation of ^{22}Na is illustrated in Fig. 7. ^{22}Na uptake into iBBMV was stimulated when intravesicular pH was lower than extravesicular pH, and this effect was abolished when intravesicular and extracellular pH were similarly acidic. These results are identical to those reported by numerous other groups (*see below*) and are consistent with Na^+/H^+ exchange. In duodenal BBMV, a similar if slightly attenuated stimulation of ^{22}Na uptake, was observed in the presence of an outwardly directed H^+ gradient. In contrast, no evidence for H^+-stimulated ^{22}Na accumulation was observed in BBMV prepared from the duodenal bulb.

In additional experiments, evidence for Na^+/H^+ exchange was provided by measuring the effects of Na^+ on the formation and dissipation of proton gradients using acridine orange fluorescence, as described by Reenstra et al. (1981) using the same conditions as Knickelbein et al. (1983). In iBBMV, Na^+ stimulated both formation and dissipation of intravesicular acidity (*data not shown*). In contrast,

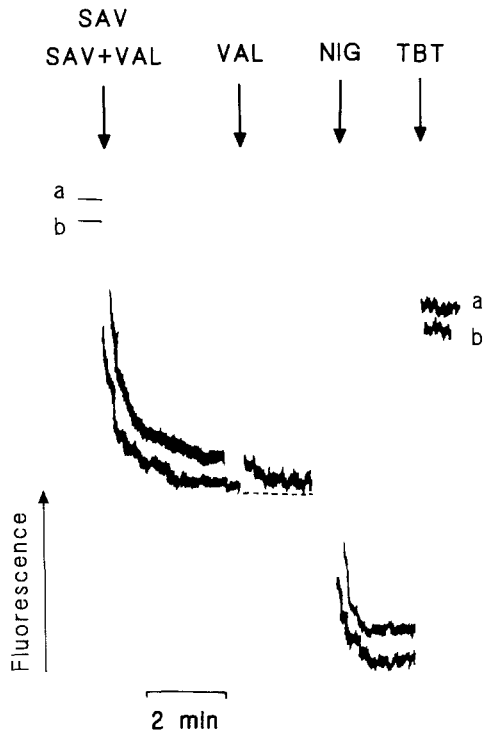

Fig. 4. Intravesicular acidification driven by diffusion potentials in gastric SAV. Gastric SAV, equilibrated in 150 mM K-gluconate, pH 7.6 were diluted (*SAV*) into 150 mM NMG-gluconate, 6 μM acridine orange, pH 7.6 (trace *a*), which resulted in quenching of the fluorescence signal indicative of intravesicular acidification. Subsequent addition of valinomycin (*VAL*) produced little further acidification (trace *a*), indicating K^+ permeability was not limiting. Maximal acidification was achieved by addition of nigericin (*NIG*). Addition of vesicles preincubated with valinomycin (*SAV + VAL;* trace *b*) produced a trace that was superposable on the trace from *SAV* without preincubation with valinomycin, and maximal acidification was again observed upon addition of nigericin, indicating a low passive H^- permeability of the vesicles. In each example, fluorescence quenching was released by addition of 15 μl tri-*n*-butyltin chloride (*TBT*). The two traces are offset as indicated.

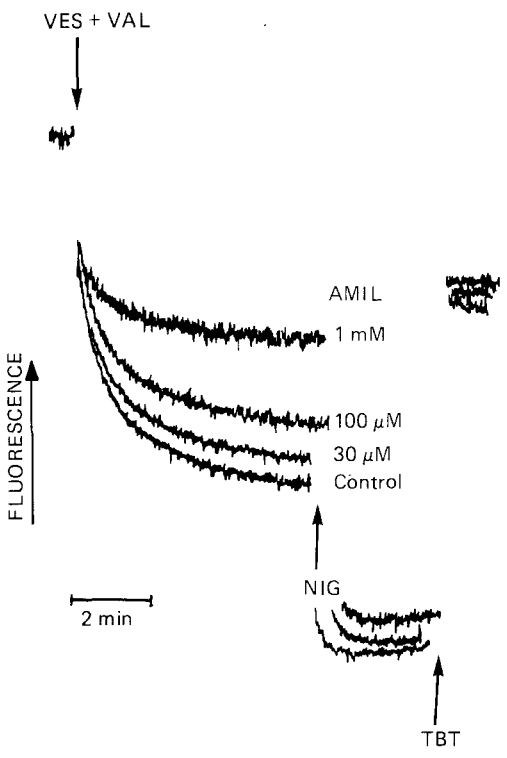

Fig. 5. Effect of amiloride on intravesicular acidification driven by diffusion potentials in ileal BBMV. Ileal BBMV, equilibrated in 150 mM K-gluconate, pH 7.6, and 4 μM valinomycin were diluted (*VES + VAL*) into 150 mM NMG-gluconate, 6 μM acridine orange, pH 7.6 (*Control*), which resulted in quenching of the fluorescence signal indicative of intravesicular acidification. Subsequent addition of nigericin (*NIG*) resulted in maximal acidification. Vesicles preincubated with various concentrations of amiloride (*AMIL*) demonstrated attenuated acidification responses, which was almost maximally inhibited at 1 mM, but addition of nigericin resulted in further acidification and this was similar in the presence and absence of amiloride. In each example, fluorescence quenching was released by addition of 15 μl tri-*n*-butyltin chloride (*TBT*).

in BBMV prepared from duodenal bulb and duodenum, no such stimulation was observed.

Discussion

The present study has demonstrated that proton permeation of three gastrointestinal luminal membranes, gastric parietal cell apical, and duodenal and ileal brush-border membrane, is rheogenic. In the intestinal BBMV, the proton permeability is sensitive to high concentrations ($>10^{-5}$ M) of amiloride. Gastric SAV show no sensitivity to amiloride. We have previously shown that the proton permeability of gastrointestinal membranes is dependent upon the dynamics of the lipid phase (Wilkes et al., 1987, 1989). This process may result from the passage of protons through discontinuities in the lipid bilayer. These same apical membranes contain a variety of integral membrane proteins involved in a range of transport activities. These proteins may facilitate transmembrane passage of protons.

The choice of amiloride was indicated as it is a recognized inhibitor of both Na^+ channels (at 10^{-7}–10^{-5} M) and Na^+/H^+ exchange (at 10^{-4}–10^{-3} M). Its high pK_a (~11) indicates it to be an active, protonated form under all the experimental conditions employed here (Benos, 1982). Na^+/H^+ exchange is involved in NaCl absorption in the intestine and has been clearly demonstrated in rat, rabbit and human jejunal and ileal BBMV (Murer, Hopfer

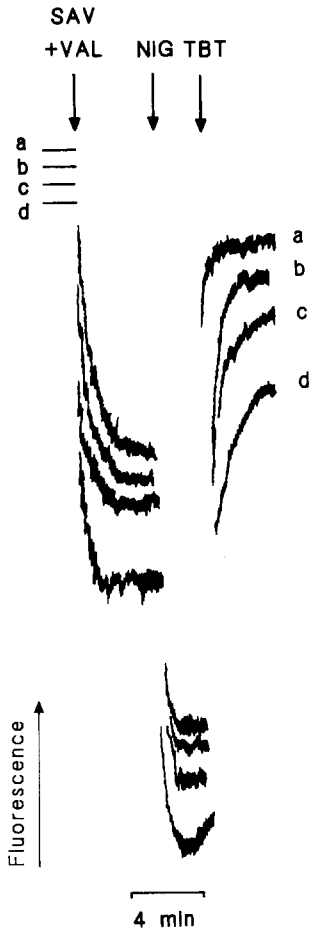

Fig. 6. Effect of amiloride on intravesicular acidification driven by diffusion potentials in gastric SAV. Gastric SAV, equilibrated in 150 mM K-gluconate, pH 7.6, and valinomycin were diluted (*SAV + VAL*) into 150 mM NMG-gluconate, 6 μM acridine orange, pH 7.6 (trace *a*), which resulted in quenching of the fluorescence signal indicative of intravesicular acidification. Subsequent addition of nigericin (*NIG*) resulted in maximal acidification. Vesicles preincubated with various concentrations of amiloride (*b*, 30 μM; c, 100 μM; *d*, 1 mM) demonstrated similar acidification responses to that in the absence of amiloride. Addition of nigericin resulted in maximal acidification which was similar in the presence and absence of amiloride. In each example, fluorescence quenching was released by addition of 15 μl tri-*n*-butyltin chloride (*TBT*). The four traces are offset as indicated.

& Kinne, 1976; Gunther & Wright, 1983; Knickelbein et al., 1983; Cassano, Steiger & Murer, 1984; Kleinman et al., 1988; Ramaswamy et al., 1989). We confirmed the presence of Na^+/H^+ exchange in the rabbit ileum and were also able to demonstrate activity, if to a lesser extent, in the rabbit duodenum by pH-driven $^{22}Na^+$ accumulation. No evidence for Na^+/H^+ exchange activity was detected in the duodenal bulb, thus indicating a gradient of increasing Na^+/H^+ exchange activity down the small intestine. This gradient may correlate with the necessity to control diffusion of high concentrations of acid emptying from the stomach in the duodenal bulb, as compared with the marginally alkaline intraluminal environment in the ileum. Indeed, the duodenum is a net HCO_3^--secreting tissue (Flemström & Garner, 1989), and the presence of apical Na^+/H^+ exchange would compromise such function. Moreover, reversal of Na^+/H^+ exchange is suicidal to cells exposed to extracellular acid (Pouysségur et al., 1984). In the parietal cell, Na^+/H^+ exchange is present in the basolateral membrane (Muallem et al., 1985). The presence of such a mechanism in the apical membrane would short circuit acid production, and no such activity is observed (Wolosin & Forte, 1984).

Passive permeation of protons was observed in the three membrane vesicles studied. Quantitation of passive proton permeability as the apparent first-order rate constant, k_H^+, allows comparisons within one vesicle type but does not allow direct comparisons of the magnitude of permeability between membranes. The absence of Na^+, or a cation able to substitute for it, rules out the possibility that we were observing a Na^+/H^+ exchange mechanism. Amiloride produced a concentration-dependent reduction in k_H^+ in dBBMV and iBBMV but not gastric SAV and required concentrations $\geqslant 10^{-5}$ M. The lack of effect of amiloride on rheogenic-driven acidification in gastric SAV allows us to discount nonspecific interactions between amiloride and valinomycin in BBMV. The nature of the amiloride-sensitive component responsible for the proton conductance in BBMV is of interest. Although rheogenic in nature, the high concentration of amiloride (3×10^{-5} to 10^{-3} M) necessary for inhibition, is inconsistent with inhibition of Na^+ channels (Benos, 1982). In addition, in toad urinary bladder, significant proton conductance through Na^+ channels was reported, but this was maximally inhibited by 2×10^{-5} M amiloride (Palmer, 1984). The low efficacy of amiloride, coupled with the apparent correlation with the distribution of Na^+/H^+ exchange, suggest the Na^+/H^+ antiport may fulfill this role.

Imposition of K^+-diffusion potentials allowed intravesicular acidification via rheogenic proton permeation. Electroneutral K^+/H^+ exchange has been identified in rat iBBMV (Binder & Murer, 1986). In rabbit dBBMV and iBBMV, entry of H^+ was stimulated by addition of the K^+ ionophore valinomycin, inconsistent with an electroneutral mechanism for H^+entry. Valinomycin does not constitute a pathway for proton permeation (Reenstra et al., 1981). The large stimulation of acidification by valinomycin in BBMV indicates a relatively low nonspecific K^+ conductance in these membranes (Gunther, Schell & Wright, 1984). When maximal acidification is observed in dBBMV in the presence of valinomycin, nigericin induced only a moderate further acidi-

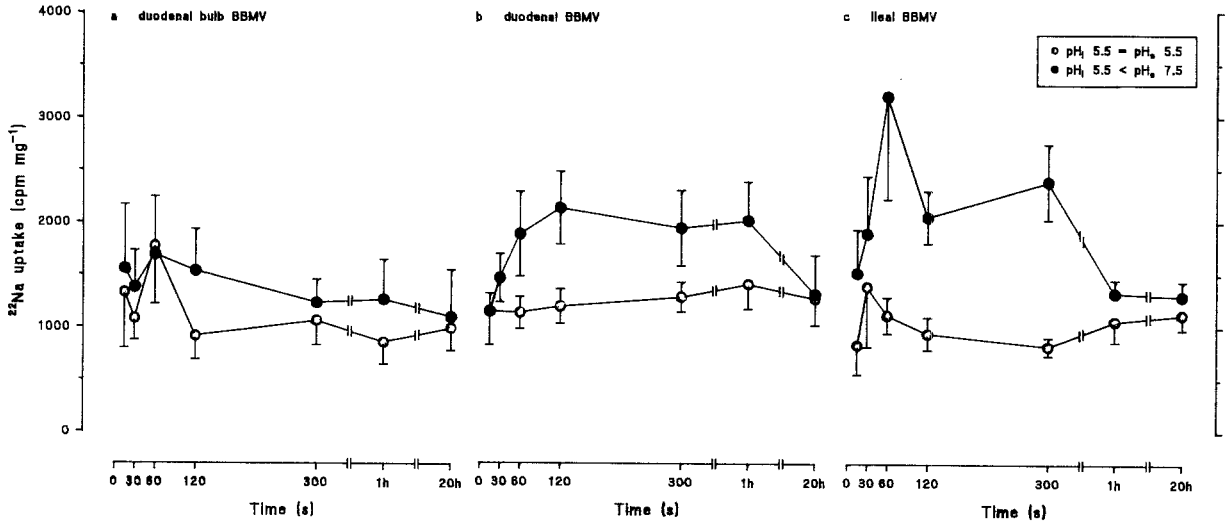

Fig. 7. Time course of ^{22}Na uptake into BBMV prepared from the duodenal bulb, duodenum and ileum. Vesicles were equilibrated at pH 5.5 and diluted into 0.1 mM NaCl containing tracer quantities of ^{22}Na at either pH 5.5 (○) or 7.5 (●). Significantly greater ^{22}Na uptake was observed in duodenal and ileal BBMV in the presence of an outward H^+ gradient. Values are illustrated as mean uptake with error bars of 1 SEM for 6–12 observations.

fication, consistent with the relatively high proton permeability of this membrane (Wilkes et al., 1989). In contrast, in gastric SAV valinomycin induced relatively moderate increases in acidification, while nigericin produced a much greater response. These latter observations are consistent with the high endogenous K^+ conductance (Wolosin & Forte, 1984) and low proton permeability (Wilkes et al., 1989) of gastric SAV.

Proton permeability of planar phospholipid bilayers is surprisingly high, $\sim 10^{-6}$–10^{-7} cm sec^{-1} at around pH 7, as compared with that for other cations such as sodium and potassium (Deamer, 1987; Gutknecht, 1987). Proton conductance in these lipid bilayers may occur, among other mechanisms, via intramembrane water ("water wires"), or weak acid contaminants (Deamer, 1987; Gutknecht, 1987). Proton permeability of biological membranes is generally higher than these model systems, e.g., $\sim 10^{-2}$, $\sim 10^{-3}$ and $\sim 10^{-4}$ cm sec^{-1}, for renal BBMV, small intestinal BBMV and gastric SAV, respectively (Ives & Verkman, 1985; Wilkes et al., 1989; Hirst, 1991). The presence of transmembrane proteins may explain the greater permeability to protons of the biological membranes (Verkman, 1987). Such proteins may increase proton permeability through nonspecific perturbations of the lipid bilayer or may provide specific channels for proton conductance. In small intestinal BBMV, proton conductance was reduced by high concentrations of amiloride, suggesting that the Na^+/H^+ antiport, at least in the absence of Na^+, may represent such a protein pathway for proton permeation in this membrane. In contrast, gastric SAV have a lower proton permeability and are insensitive to amiloride, consistent with the function of this membrane.

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