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# Sodium–proton exchange in human ileal brush-border membrane vesicles

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**This study examines the characteristics of $Na^+$ and $H^+$ transport as well as $Na^+$–$H^+$ exchange in human ileal brush-border membrane vesicles from organ donor intestine. $^{22}Na^+$ uptake into vesicles and the fluorescence quenching of Acridine orange were employed to measure $Na^+$ and $H^+$ transport, respectively. Concentrative uptake of $^{22}Na^+$ (4-fold overshoot above equilibrium) was observed under conditions of an outward proton gradient ($pH_i$ 5.5; $pH_o$ 7.5). Voltage-clamping ($K_i^+ = K_o^+$ + valinomycin) reduced the uptake of $^{22}Na^+$ by 40–50% indicating the presence of $Na^+$ conductance. Dissipation of the Acridine orange fluorescence quench in ileal vesicles with a preformed pH gradient ($pH_i$ 5.5; $pH_o$ 7.5) was accelerated by either external $Na^+$ or voltage-clamping in the absence of $Na^+$. The effects of $Na^+$ and voltage-clamping were additive under the above conditions. In the absence of a pH gradient, Acridine orange quenching was induced by intravesicular $Na^+$ as well as an interior negative $K^+$ diffusion potential. In voltage-clamped BBMV, pH-driven $Na^+$ uptake was inhibited by amiloride ($K_i$ = 140 μM). The initial rate of pH-driven $Na^+$ uptake was saturable and conformed to Michaelis-Menten kinetics with apparent $K_m$ and $V_{max}$ values of 27 ± 1 mM and 47 ± 1 nmol · (mg protein)$^{-1}$ · (3 s)$^{-1}$, respectively. $Li^+$ and $NH_4^+$, but not $Cs^+$, $K^+$, $Rb^+$ or choline$^+$ inhibited pH gradient-driven $^{22}Na^+$ uptake. The results demonstrate in human ileal brush-border membrane vesicles the presence of an $Na^+/H^+$ exchanger and conductive transport pathways for $Na^+$ and $H^+$.**

## Introduction

Perfusion studies of human ileum in vivo demonstrated that $Na^+$ and $Cl^-$ were absorbed and $HCO_3^-$ secreted against electrochemical gradients and that there was a reciprocal movement of $Cl^-$ and $HCO_3^-$ when $Na^+$ flux was zero [1]. These changes in transport appeared to be electrically neutral. From their results, Turnberg et al. [1] postulated that the mechanism of $Na^+$ and $Cl^-$ absorption in human ileum was one involving the simultaneous exchange of $Na^+$ for $H^+$ and $Cl^-$ for $HCO_3^-$. Extensive studies with rabbit ileum in Ussing chambers [2,3] suggested that $Na^+$ absorption is dependent on the presence of $Cl^-$ and vice versa, and that this process is blocked by increased cellular levels of cyclic nucleotides. These results led to the hypothesis that there was a direct coupling of $Na^+$ to $Cl^-$ [2,3,4]. Studies using brush-border membrane vesicles from rat ileum [5,6] and from rabbit ileum [7,8], however, showed no evidence of direct coupling of $Na^+$ to $Cl^-$ but demonstrated convincing proof for the presence of dual exchanges ($Na^+/H^+$, $Cl^-/HCO_3^-$). To date, there are few studies of NaCl absorption by the human ileum using brush-border membrane vesicles.

We have established that intestinal tissue from organ donors provides a viable source for human intestinal brush-border membrane vesicles to study nutrient absorption at the membrane level [9–11]. Our current studies were performed using brush-border membrane vesicles from human ileum to investigate the mechanisms of $Na^+$ and $H^+$ ion transport in human ileum. Our results demonstrate the presence of $Na^+$–$H^+$ exchange and conductive transport pathways for $Na^+$ and $H^+$ in human ileum and further characterize this $Na^+$–$H^+$ exchange process. An abstract of this study has been published [12].

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## Materials and Methods

*Preparation of brush-border membrane vesicles*

Human small intestine from the ligament of Treitz to the end of ileum was obtained at the time of organ donation after removal of all transplantable organs. The small intestine was opened along its antimesenteric border, rinsed with ice-cold 0.9% NaCl, and divided into four equal segments. The last segment was used to obtain ileal mucosa which was kept frozen at −70 °C in 5 to 10 g quantities. On the morning of each experiment, brush-border membranes were obtained by $CaCl_2$ or $MgCl_2$ precipitation method from the thawed ileal mucosa by the method of Schmitz et al. [13] and vesiculated as described by Hopfer et al. [14] and as modified in this laboratory [15]. A 20-fold enrichment of sucrase activity measured by the method of Dahlqvist [16] was routinely observed in ileal vesicles. Protein content was assessed by the method of Lowry et al. [17] using bovine serum albumin as the standard.

*Transport studies*

*$Na^+$ transport.* Transport studies were carried out by the rapid filtration method of Hopfer et al. [14] as previously described [15]. Details of our methods for loading and resuspension of the vesicles in the desired media are as already reported by us [10,18]. The vesicles were resuspended with the final resuspension buffer to a final protein concentration of approx. 10 mg membrane protein/ml. Uptake was initiated by diluting the vesicle suspension 10-fold into an incubation medium containing $^{22}Na^+$. Details of transport measurement, washing of the filters, corrections applied to uptake values for binding of $^{22}Na^+$ to filters and counting procedures are as previously described [18]. The composition of the final resuspension solution and incubation media is given in the legends of the figures. In all cases 0.1 mM $MgSO_4$ was present in both intra- and extravesicular media. Results are expressed as nmoles of $Na^+$ uptake per mg of protein. Uptake studies were always performed in triplicate and experiments were repeated in vesicle preparations prepared from at least three different intestines. The variations of the triplicates were always ±5% of the mean value. By use of different membrane preparations, qualitatively similar results were obtained but in view of the significant variations in intravesicular space between preparations, only results of typical experiments are shown in the figures.

*$H^+$ transport.* $H^+$ transport was estimated from the variations of pH gradients between extra and intravesicular environments as determined from fluorescence quenching of Acridine orange [19]. An intravesicular pH that is more acidic than the external medium, either because of preincubation of vesicles with an acid-buffered medium or by $H^+$ entry, causes the vesicles to concentrate acridine orange, the weak base. This results in self-quenching of Acridine orange fluorescence within the vesicles and decreases Acridine orange fluorescence measured in the external medium. Conversely, dissipation of the intravesicular acid pH gradient leads to the release of Acridine orange from the vesicles and an increase in fluorescence measured in the external medium. Although these fluorescence changes have been determined to be rapid enough to reflect actual rates of $H^+$ transport [19], their use in this fashion has been critized recently [20]. Binding to the negatively charged cell membrane, influenced by cations and the polar headgroups of phospholipids, excimer formation, and temperature may affect the rate of recovery of Acridine orange fluorescence quench. Nevertheless, since these effects are likely to be present equally in all samples of a given vesicle preparation, it is likely that comparative rates of $H^+$ transport can be estimated using this technique.

Vesicles were loaded with the desired media as indicated above. 30-μl aliquots of a concentrated vesicle suspension were diluted into 3 ml of external media of defined ionic composition (given in figure legends) containing 6 μM Acridine orange. Extravesicular fluorescence was monitored at 23 or 4 °C, as noted, in a Perkin-Elmer LS-5 Fluorescence Spectrophotometer, with 494 nm excitation wavelength (5 nm slit width) and 530 nm emission wavelength (10 nm slit width). Fluorescence of the media prior to vesicle addition was always adjusted to a relative value of 90%. Protocols were repeated in at least three different vesicle preparations. Tracings shown in the figures depict the results of representative studies.

*Materials*

$^{22}Na^+$ (9.52 Ci/mmol) was obtained from Amersham Corp., Arlington Heights, Illinois, and L-[1(n)-$^3$H]glucose (10.7 Ci/mmol) was obtained from New England Nuclear Corp., Boston, MA. Amiloride was a gift from Merck Sharp and Dohme. All other chemicals were obtained from Sigma Chemical Co., St. Louis, MO. Membrane filters (0.45 μm) were obtained from Sartorius Filters Inc., Hayward, CA. Stock solutions of 1 M *N*-methylglucamine gluconate or 1 M tetramethylammonium gluconate were made by titrating aqueous 1 M *N*-methylglucamine or 1 M tetramethylammonium hydroxide with solid D-gluconic acid lactone to pH 7.5. Valinomycin, Acridine orange, and nigericin were prepared as ethanolic stock solutions and final concentrations of ethanol in incubation media were at or below 0.7%.

## Results

*Effects of pH gradients on $Na^+$ uptake*

Fig. 1 depicts the effects of pH gradients on $Na^+$ uptake. $Na^+$ uptake was enhanced by an outwardly

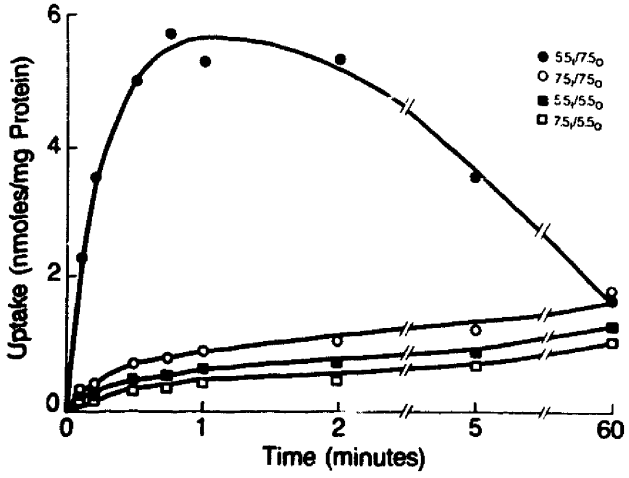


Fig. 1. Effect of pH gradients on $^{22}Na^+$ uptake in human ileal BBMV. Vesicles were preloaded with 100 mM TMA gluconate; 100 mM mannitol and 50 mM Tris-Hepes buffer (pH 7.5) or 100 mM TMA gluconate, 115 mM mannitol and 50 mM Tris-Mes buffer (pH 5.5). Uptake was determined after diluting vesicles 10-times into incubation media of identical composition except for buffer (appropriate to external buffer) and containing 1 mM $^{22}Na^+$ as NaCl. TMA, tetramethylammonium.

directed $H^+$ gradient ($pH_i$ 5.5; $pH_o$ 7.5) which led to an overshoot of about 3–4-fold over the equilibrium value. There was no stimulation of $Na^+$ uptake when either the inside of the vesicles was alkaline ($pH_i$ 7.5; $pH_o$ 5.5) or when a pH gradient was absent ($pH_i = pH_o$ 5.5 or 7.5). These results indicate that $Na^+$ uptake is coupled to $H^+$ efflux from the vesicles.

It is possible that the enhancement of $Na^+$ uptake observed when the inside of the vesicles was acidic is due to an intravesicular negative potential created by a $H^+$-diffusion potential. To investigate this possibility, $^{22}Na^+$ uptake studies were carried out under voltage-clamped conditions of equal internal and external $K^+$ concentrations and the presence of valinomycin to negate interference by any diffusion potential. Fig. 2 shows that voltage-clamping reduced $^{22}Na^+$ uptake compared to its absence. However, even under voltage-clamped conditions, an outward pH gradient stimulated sodium uptake and yielded a 2-fold overshoot. Studies on the effect of voltage-clamping on $^{22}Na^+$ uptake were also carried out employing vesicles prepared by the $Mg^{2+}$-precipitation method and the results obtained were identical to those in Fig. 2. Figs. 1 and 2, therefore, demonstrate evidence for an $Na^+/H^+$ exchange process as well as $Na^+$ conductance in ileal vesicles.

*Effect of $Na^+$ gradients on $H^+$ transport*

To determine the effects of $Na^+$ gradients on $H^+$ transport, we utilized the pH-sensitive fluorescent dye Acridine orange. Dilution of vesicles preincubated in a pH 5.5 medium into a pH 7.5 extravesicular medium was associated with an immediate decrease in Acridine orange fluorescence from about 90% to about 45%, with a slower, spontaneous recovery thereafter (tracing (a) of Fig. 3). Nigericin alone did not affect the spontaneous fluorescence recovery; the initial portion of tracing (b) is superimposed on tracing (a). However, the subsequent addition of potassium gluconate (arrow) led to an immediate recovery of Acridine orange fluorescence. This demonstrates that Acridine orange fluorescence responds to the dissipation of the preformed pH gradient. External $Na^+$, in the form of gluconate, (tracing (c)) also accelerated the dissipation of the $\Delta pH$, as indicated by a rapid decrease in Acridine orange fluorescence quenching. In the absence of a pH gradient (tracing (d)), fluorescence quenching of only about 10% was observed, presumably due to light scattering by the vesicles. Additions of nigericin, $K^+$, and $Na^+$ had no effect on the fluorescence intensity, indicating that no pH gradient or cation modifiable Acridine orange binding had occurred.

Fluorescence quenching of Acridine orange was induced by diluting vesicles loaded with a pH 7.5 medium containing $Na^+$ into external medium at the same pH but lacking $Na^+$ (Fig. 4, tracing (a)), indicating the development of an intravesicular acid pH gradient. Vesicles lacking $Na^+$ (tracing (b)) induced only a small degree of quenching unaffected by nigericin and $K^+$ similar to that described above.

Although these results are consistent with electroneutral $Na^+$–$H^+$ exchange, it is also possible that the

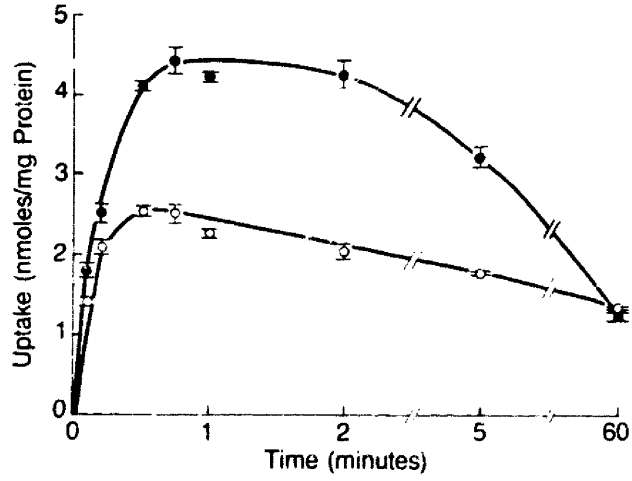


Fig. 2. Effect of voltage clamping on $^{22}Na^+$ uptake into human ileal BBMV. Vesicles were preloaded with 20 mM potassium gluconate, 80 mM TMA gluconate, 115 mM mannitol and 50 mM Tris-Mes buffer (pH 5.5) and incubated in a medium containing 20 mM potassium gluconate, 80 mM TMA gluconate, 100 mM mannitol, 50 mM Tris-Hepes (pH 7.5), 21 $\mu$M valinomycin and 1 mM $^{22}NaCl$ (○——○). Vesicles were preloaded with 100 mM TMA gluconate, 115 mM mannitol and 50 mM Tris-Mes buffer (pH 5.5) and incubated in a medium containing 100 mM TMA gluconate, 100 mM mannitol, 50 mM Tris-Hepes buffer (pH 7.5) and 1 mM $^{22}NaCl$ (●——●). TMA, tetramethylammonium.

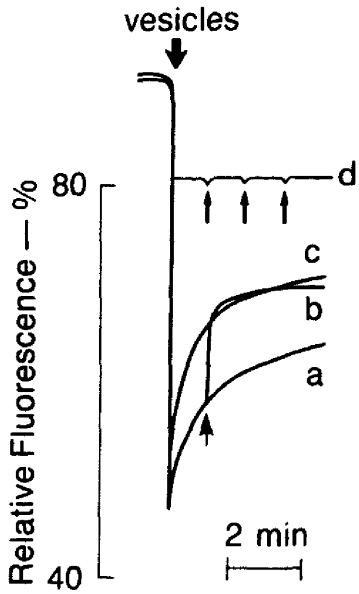


Fig. 3. Effect of $Na^+$ on Acridine orange fluorescence quench induced by a preformed pH gradient. Vesicles were preloaded with 100 mM *N*-methylglucamine (NMG) gluconate, 115 mM mannitol and 50 mM Tris-Mes buffer (pH 5.5). External solutions contained 6 $\mu$M Acridine orange, 100 mM NMG gluconate and 100 mM mannitol. The external buffer was either 50 mM Tris-Hepes (pH 7.5), (a–c), or 50 mM Tris-Mes (pH 5.5) (d). Curve a, vesicles diluted into external pH 7.5 without $Na^+$ or $K^+$. Curve b, external pH 7.5 and 6 $\mu$M nigericin. 25 mM potassium gluconate added at arrow. Curve c, external pH 7.5 containing 25 mM Na gluconate. Curve d, external pH 5.5, additions of nigericin, $K^+$, $Na^+$ at arrows.

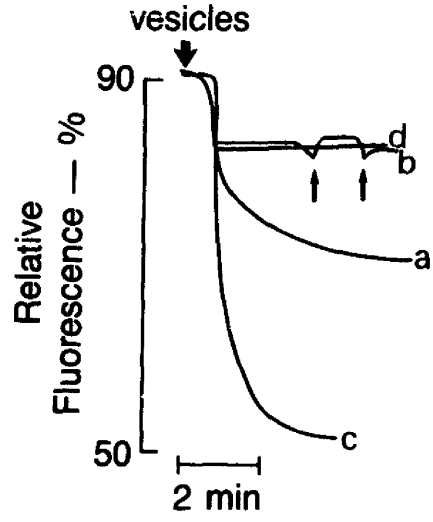


Fig. 4. Effects of a $Na^+$ gradient (*a*, b) and of a $K^+$ diffusion potential (c, d) to induce Acridine orange fluorescence quench. BBMV were preloaded with 100 mM mannitol, 10 mM Tris-Hepes buffer (pH 7.5) and either 100 mM sodium gluconate (a) or 100 mM NMG gluconate (b) and diluted into incubation media containing 6 $\mu$M dye, 100 mM mannitol, 10 mM Tris-Hepes buffer (pH 7.5) and 100 mM NMG gluconate. Additions of nigericin and $K^+$ at arrows. Vesicles were preloaded with 100 mM potassium gluconate, 100 mM mannitol, 10 mM Tris-Hepes buffer (pH 7.5) and were diluted into a medium containing 100 mM NMG gluconate, 100 mM mannitol, 10 mM Tris-Hepes buffer (pH 7.5), 6 $\mu$M Acridine orange and 15 $\mu$M valinomycin (c). Vesicles were also diluted (d) into the same external media except for the presence of 100 mM potassium gluconate instead of 100 mM NMG gluconate.

vesicles contain an $H^+$ conductive pathway that could be shunted by $Na^+$, providing, in effect, electrical coupling between $Na^+$ and $H^+$ transport. To determine whether a $H^+$ conductance pathway was present, vesicles were preincubated in pH 7.5 medium containing 100 mM potassium gluconate. Vesicles were subsequently diluted into an external pH 7.5 medium lacking $K^+$ but containing valinomycin. As shown also in Fig. 4, fluorescence quenching of Acridine orange was observed (tracing (c)), indicating the development of an acid intravesicular pH gradient. Dilution into medium with an equivalent $K^+$ concentration (tracing (d)), even in the presence of valinomycin, did not induce fluorescence quenching anymore than that which could be attributable to light scattering. In subsequent experiments performed at 4°C (vide infra), vesicles were loaded with a pH 5.5 medium containing 50 mM potassium gluconate and diluted into a pH 7.5 external medium with an equivalent potassium gluconate concentration, which resulted in the Acridine orange fluorescence quenching with spontaneous dissipation (tracing (a) of Fig. 5). Inclusion of valinomycin in the external medium resulted in more rapid reappearance of Acridine orange fluorescence (tracing (b) of Fig. 5), indicating enhancement of the rate of pH gradient dissipation. Taken together, these two experiments are consistent with the presence of electrogenic $H^+$ transport mechanism in human ileal BBMV.

In order to distinguish between an electrical coupling between $Na^+$ and $H^+$ transport and electroneutral $Na^+$–$H^+$ exchange, the effect of $Na^+$ on a preformed

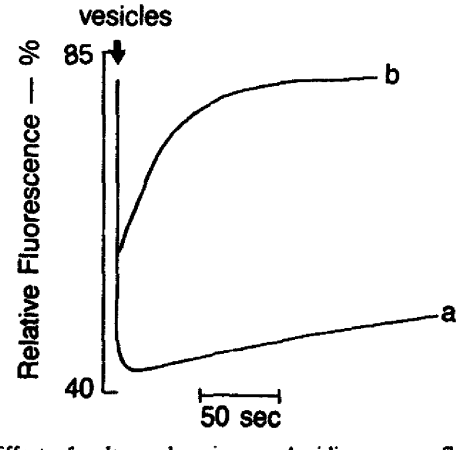


Fig. 5. Effect of voltage clamping on Acridine orange fluorescence quench. BBMV were preloaded with 50 mM potassium gluconate, 50 mM NMG gluconate, 115 mM mannitol, 50 mM Tris-Mes buffer (pH 5.5) and were diluted into a medium containing 6 $\mu$M Acridine orange, 50 mM potassium gluconate, 50 mM NMG gluconate, 100 mM mannitol, and 50 mM Tris-Hepes buffer (pH 7.5) (curve a) or to an identical medium containing 15 $\mu$M valinomycin (curve b).

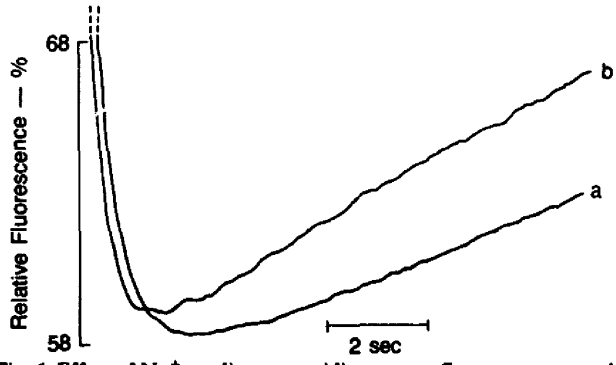


Fig. 6. Effect of $Na^+$ gradient on acridine orange fluorescence quench under voltage-clamped conditions. BBMV were preloaded with 50 mM potassium gluconate, 50 mM NMG gluconate, 115 mM mannitol, 50 mM Tris-Mes buffer (pH 5.5) and diluted into a medium containing 50 mM potassium gluconate, 50 mM NMG gluconate, 100 mM mannitol, 50 mM Tris-Hepes buffer (pH 7.5), 6 μM Acridine orange and 15 μM valinomycin (tracing a) or 25 mM sodium gluconate replaced 25 mM NMG gluconate in the above medium (tracing b). This experiment was performed at 4°C. Note that only the nadir of the Acridine orange quenching and the first 8 s of recovery are shown.

pH gradient was examined in vesicles that were chemically voltage-clamped with equivalent internal and external $K^+$ concentrations and valinomycin. These studies were performed at 4°C in order to slow down the processes sufficiently so that the effect of $Na^+$ could be determined before dissipation of the preformed gradient. Dilution of vesicles loaded with a pH 5.5 medium containing 50 mM potassium gluconate into a pH 7.5 external medium with an equivalent potassium gluconate concentration and containing valinomycin was associated with Acridine orange fluorescence quenching with spontaneous dissipation (tracing (a) of Fig. 6, note the greatly expanded time scale). When 25 mM sodium

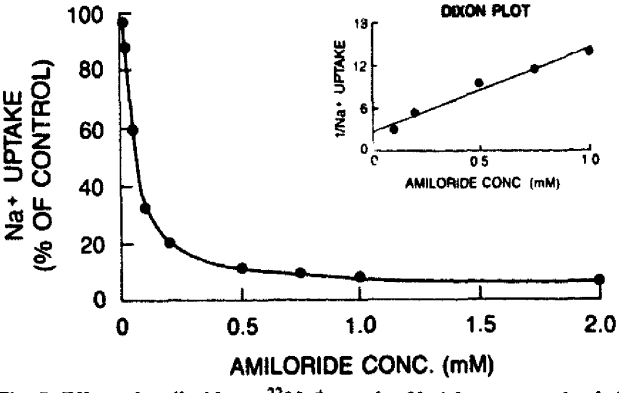


Fig. 7. Effect of amiloride on $^{22}Na^+$ uptake. Vesicles were preloaded with 20 mM potassium gluconate, 80 mM NMG gluconate, 115 mM mannitol and 50 mM Tris-Mes buffer (pH 5.5) and incubated in media containing 20 mM K gluconate, 80 mM NMG gluconate, 100 mM mannitol, and 50 mM Tris-Hepes buffer (pH 7.5), 21 μM valinomycin, 1 mM $^{22}NaCl$ and various concentrations of amiloride. $^{22}Na^+$ (3 s) uptake is expressed as a percent of that for vesicles without amiloride. The inset shows a Dixon plot of the absolute values of $Na^+$ uptake with various amiloride concentrations.

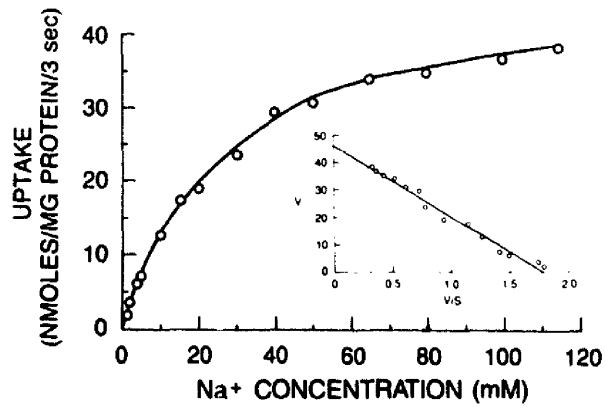


Fig. 8. Effect of external $Na^+$ concentration on initial rates of $^{22}Na^+$ uptake. Vesicles were preloaded and incubated as in Fig. 7 but with increasing concentration of sodium gluconate, uptake values at 3 s were determined. Inset: An Eadie-Hofstee plot of data shown.

gluconate was also present in the external medium, the rate of pH gradient dissipation was enhanced (tracing (b) of Fig. 6).

*Effect of amiloride on $Na^+$ transport*

Since amiloride has been established to be a specific inhibitor of $Na^+$–$H^+$ exchange process, its effect under voltage-clamped conditions was investigated to further confirm the presence of an $Na^+/H^+$ exchanger in human ileal vesicles. Fig. 7 illustrates the inhibiting effects of amiloride in a dose-dependent fashion. Analysis of amiloride inhibition yielded an inhibition constant ($K_i$) of 140 μM at 1 mM $Na^+$.

*Kinetic characteristics of $Na^+$–$H^+$ exchange*

The effect of varying sodium concentration on the initial rates of pH-stimulated sodium uptake under voltage-clamped conditions was determined in ileal vesicles (Fig. 8). It is clear that sodium uptake occurred by a

TABLE I

*Effect of cations on $Na^+$ uptake in human ileal brush-border membrane vesicles*

Values are means ± S.E. ($n = 4$). Vesicles were preincubated in 20 mM potassium gluconate, 80 mM *N*-methylglucamine gluconate, 115 mM mannitol, and 50 mM Tris-Mes buffer (pH 5.5) and diluted into an external medium containing 20 mM potassium gluconate, 80 mM *N*-methylglucamine gluconate, 100 mM mannitol, 50 mM Tris-Hepes buffer (pH 7.5), 21 μM valinomycin, 1 mM $^{22}Na$, and 20 mM of the $Cl^-$ salts of indicated cations. Uptake was measured at 3 s.

| Cation | Inhibition (%) |
|---|---|
| $Li^+$ | 80 ± 2 |
| $NH_4^+$ | 49 ± 3 |
| $Na^+$ | 40 ± 5 |
| $Rb^+$ | 15 ± 2 |
| $Choline^+$ | 5 ± 2 |
| $Cs^+$ | 0 |
| $K^+$ | 0 |

saturable process conforming to Michaelis-Menten kinetics. Kinetic analysis of sodium uptake using an Eadie-Hofstee plot (Fig. 8, inset) yielded a $K_m$ of $27 \pm 1$ mM and a $V_{max}$ of $47 \pm 1$ nmol · (mg protein)$^{-1}$ · (3 s)$^{-1}$.

*Effect of monovalent cations on $Na^+$ uptake*

To determine the specificity of the exchange process, competition experiments with other cations were performed. Table I shows the percentage inhibition of 1 mM $^{22}Na^+$ uptake by 20 mM of various cations under voltage-clamped conditions. $^{22}Na^+$ uptake was inhibited by unlabeled $Na^+$ and by $Li^+$ and $NH_4^+$. The inhibition by other cations was not appreciable. These results suggest the selectivity of the $H^+$ exchange process for $Li^+$, $NH_4^+$ and $Na^+$.

## Discussion

Our results demonstrate that outwardly directed proton gradients stimulate $Na^+$ uptake in the presence and absence of chemical voltage clamping. Our studies with Acridine orange fluorescence quench show that $Na^+$ gradients lead to intravesicular acidification or dissipation of performed pH gradients in directions appropriate for $Na^+$–$H^+$ exchange. Amiloride inhibited pH gradient-driven $Na^+$ uptake under voltage-clamped conditions. $Na^+$ uptake under voltage-clamped conditions was saturable and the process conformed to Michaelis-Menten kinetics. $Li^+$ and $NH_4^+$ inhibited appreciable $Na^+$ uptake under voltage-clamped conditions. These results strongly suggest the presence of an $Na^+$–$H^+$ exchange mechanism in human ileal brush-border membrane vesicles.

Our results further demonstrate the presence of conductive pathways for both $Na^+$ and $H^+$ transport. The evidence for conductive pathways can be summarized as follows. (1) $Na^+$ uptake energized by an outward $H^+$ gradient was reduced in the presence of equal intra- and extravesicular $K^+$ and valinomycin (i.e., chemical voltage-clamping) compared to the absence of $K^+$ and valinomycin. (2) Dissipation of pH gradient by $Na^+$ was faster in unclamped than clamped vesicles. (3) In the absence of $Na^+$, voltage-clamping led to an enhancement of pH gradient dissipation. (4) Studies done at 4° C also show spontaneous dissipation of performed pH gradient when voltage-clamped with $K^+$ and valinomycin. Our results indicating the presence of $Na^+$ and $H^+$ conductances in human ileal vesicles are different from those in rabbit ileal brush-border membrane vesicles [7]. It should be pointed out, however, that not many rigorous tests for these conductances such as voltage clamping were attempted in the latter studies [7].

Comparison of the methods of preparation of renal brush-border membrane vesicles showed that $Ca^{2+}$-precipitation methods led to higher $Na^+$ and $H^+$ conductances than $Mg^{2+}$-precipitation methods, presumably due to $Ca^{2+}$-stimulated phospholipid breakdown [21]. For our studies, the two methods yielded similar results on $Na^+$ uptake as well as on the effects of voltage-clamping on $Na^+$ uptake. Our results on the presence of $Na^+$ conductance are in contrast to the recent report of Kikuchi et al. [22] of the absence of any conductive $Na^+$ pathway in human ileal brush-border membrane vesicles. The reasons for the differences in results are not known at present. However, the conductive pathways for $Na^+$ and $H^+$ have been demonstrated in our studies using two independent methods, namely $^{22}Na^+$ flux and the quenching of Acridine orange fluorescence. It is not certain, however, that these conductances are true characteristics of intact ileal membranes or that they function under physiological conditions.

Studies on inhibition of $Na^+$ uptake by amiloride under voltage-clamped conditions yielded a typical dose-response curve for an $Na^+$–$H^+$ exchange process. The $K_i$ value of 140 $\mu$M for amiloride inhibition is slightly higher than the value reported by us for human jejunal brush-border membrane vesicles [18] but is in the same range as in rabbit ileal vesicles. A comparison of kinetic characteristics of $Na^+$ uptake reveals that the $K_m$ values for human jejunum and ileum are similar (24 mM and 27 mM) but are much higher than the values (5–16 mM) reported for rabbit ileal brush-border membrane vesicles [7]. $V_{max}$ values for human ileal vesicles are 5-fold higher than the values reported for human jejunum and may be physiologically significant in view of the higher absorption against concentration gradients of NaCl by human ileum compared to jejunum in vivo [1].

Specificity of the human ileal brush-border $Na^+$–$H^+$ exchanger for cations is similar to that reported for $Na^+$–$H^+$ exchangers of renal cortex from various animal species [23–25]. Our studies did not demonstrate any effects of $K^+$ on $^{22}Na^+$ uptake while Knickelbein et al. [7] reported mild $K^+$ inhibition in rabbit ileal vesicles. It is possible that in the latter studies inhibition by $K^+$ might relate to its effect on $Na^+$ diffusion rather than $Na^+$–H exchange activity, since the experiments were not carried out under voltage-clamped conditions.

In conclusion, our studies demonstrate and characterize the processes for $Na^+$ and $H^+$ transport in human ileal brush-border membrane vesicles. Our recent studies have shown evidence for $Cl^-/HCO_3^-$ ($OH^-$) exchange process in human ileal brush-border membrane vesicles [26]. The operation of these two exchangers would result in electroneutral absorption of NaCl reported earlier from in vivo perfusion experiments [1]. Further studies are needed to directly determine the contribution of these two exchangers and of direct coupling of $Na^+$ and $Cl^-$ in the absorption of NaCl by human ileum.

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