J. Membrane Biol. 74, 85–94 (1983)

The Journal of
Membrane Biology
© Springer-Verlag 1983

Na^+, Li^+, and Cl^- Transport by Brush Border Membranes from Rabbit Jejunum

Robert D. Gunther and Ernest M. Wright
Department of Physiology, UCLA School of Medicine, Los Angeles, California 90024

Summary. Na^+, Li^+, K^+, Rb^+, Br^-, Cl^- and SO_4^{2-} transport were studied in brush border membrane vesicles isolated from rabbit jejunum. Li^+ uptakes were measured by flameless atomic absorption spectroscopy, and all others were measured using isotopic flux and liquid scintillation counting. All uptakes were performed with a rapid filtration procedure. A method is presented for separating various components of ion uptake: 1) passive diffusion, 2) mediated transport and 3) binding. It was concluded that a Na^+/H^+ exchange mechanism exists in the jejunal brush border. The exchanger was inhibited with 300 µM amiloride or harmaline. The kinetic parameters for sodium transport by this mechanism depend on the pH of the intravesicular solution. The application of a pH gradient ($pH_{in}=5.5$, $pH_{out}=7.5$) causes an increase in J_{max} (50 to 125 pmol/mg protein·sec) with no change in K_t ($\simeq 4.5$ mM). Competition experiments show that other monovalent cations, e.g. Li^+ and NH_4^+, share the Na^+/H^+ exchanger. This was confirmed with direct measurements of Li^+ uptakes. Saturable uptake mechanisms were also observed for K^+, Rb^+ and SO_4^{2-}, but not for Br^-. The J_{max} for K^+ and Rb^+ are similar to the J_{max} for Na^+, suggesting that they may share a transporter. The SO_4^{2-} system appears to be a Na^+/SO_4^{2-} cotransport system. There does not appear to be either a Cl^-/OH^- transport mechanism of the type observed in ileum or a specific Na^+/Cl^- symporter.

Key Words sodium·lithium·chloride·pH·transport·kinetics·ion permeability

Introduction

It has long been established that the mammalian jejunum is capable of absorbing Na^+, Cl^- and HCO_3^-. Turnberg et al. [24] have shown that, in the absence of sugars or amino acids, the absorption of Na^+ is obligatorily linked to the absorption of HCO_3^- in human jejunum *in vivo*. They went on to show that the apparent HCO_3^- absorption was actually a result of H^+ secretion. The type of Na^+/H^+ exchange system predicted by this model has since been observed in brush border membranes isolated from rat intestine [16] and rabbit ileum [13].

Turnberg et al. [23] postulated that in the ileum, where Na^+ and Cl^- are absorbed and HCO_3^- secreted, that there was a Cl^-/HCO_3^- exchange mechanism operating in parallel to the Na^+/H^+ exchanger. These two systems, operating in parallel would produce NaCl absorption and CO_2 secretion. Other researchers have postulated a directly coupled Na^+/Cl^- cotransport mechanism in ileum [17].

Our results suggest that the jejunal brush borders contain only the Na^+/H^+ exchanger, with no Na^+/Cl^- system. Further experiments were performed to elucidate the mechanism of the Na^+/H^+ transport system in terms of its kinetics and its interactions with other cations, anions and inhibitors. The possible *in vivo* functions of the Na^+/H^+ exchanger are considered.

This work has been presented previously in preliminary form [6].

Materials and Methods

Isolation of Brush Border Membranes

Brush border membrane vesicles were prepared by calcium precipitation and differential centrifugation, and stored in liquid nitrogen as described by Stevens et al. [21]. The membranes were suspended in 300 mM mannitol and either 50 mM Tris/HEPES,[1] pH 7.5, or 50 mM Tris/MES, pH 5.5. The specific activity of alkaline phosphatase was typically enriched by 15 to 23-fold in the brush border membranes as compared to the initial homogenate.

Transport Measurements

Fifteen µl of vesicles (10 mg/ml) were placed at the bottom of a small polystyrene test tube. The uptake reaction was initiated

[1] *Abbreviations:* diS$-C_3-$(5) – 3,3′-dipropylthiadicarbocyanine iodide; HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MES – 2-[N-morpholino]ethanesulfonic acid; Tris – Tris(hydroxymethyl)aminomethane; SITS – 4-acetamide-4′-isothiocyanostilbene-2,2′-disulfonic acid.

with a jet of 85 µl of a reaction mix which contained the isotope plus any experimental variables such as inhibitors and other ions. The uptake was stopped with a jet of 825 µl of ice-cold quench solution. The entire quenched reaction was then quickly filtered through a prerinsed Sartorius or Schleicher and Schuell filter (0.45 µm pore size, cellulose nitrate) and rinsed with 4 ml of ice-cold quench solution. The quench solution consisted of 158 mM KCl, 5 mM Tris/HEPES buffer, pH 7.5, and 30 mM mannitol for the experiments involving the uptake of Na^+ or Li^+. For experiments involving the uptake of Cl^-, the KCl was replaced with $MgSO_4$. The filters were assayed in a liquid scintillation counter. A filter blank (normal uptake procedure, but without vesicles) was subtracted from each uptake.

Li^+ uptake measurements were performed in a similar manner, except that the filters were assayed for Li^+ with a flameless atomic absorption spectrometer [4]. The filters were washed three times in distilled water to lyse the vesicles. The washes were pooled, dried and the residue was resuspended in a small volume of 40 mM NH_4NO_3 to bring the Li^+ concentration into the range of 1 to 30 µM. Control experiments show that 100% of ^{22}Na in vesicles was recovered after three washes in distilled water. The resuspended Li^+ was measured using a Varian flameless atomic absorption spectrometer.

Curve Fitting Procedures

The diffusion component for ion transport into the vesicles was determined by two methods: 1) calculating the best fit of the data to a modified kinetic equation:

$$J_i = \frac{(J_{max} \cdot a_i)}{(K_t + a_i)} + (P_i' \cdot a_i) \qquad (1)$$

where J_i = ion flux rate, a_i = calculated ion activity, J_{max} = maximum rate of saturable flux, K_t = the a_i that yields $^1/_2\, J_{max}$ and P_i' = apparent permeability coefficient, with a computer program (Diffit) using an iterative nonlinear regression technique. 2) Computer generated curves were fitted to the data on a Woolf-Augustinsson-Hofstee plot (J_i *vs.* J_i/a_i), and the best-fitting curve selected by visual inspection. The P_i' determined from Eq. (1) is an oversimplification since it does not include the electrical driving force for the ion. Experiments measuring diffusion potentials have shown that $P_{Cl}/P_{Na} = 1.5$ [7] and the membrane potential caused by addition of NaCl to the outside of the vesicles is negative. This results in an overestimate of the true P_{Na}.

The diffusion-corrected data were fitted to Woolf-Augustinsson-Hofstee and Lineweaver-Burk plots by linear regression analysis and to Michaelis-Menten plots by a computer program (Mmplot) which used a nonlinear least-squares analysis. The activities were calculated using activity coefficients from Robinson and Stokes [19].

Results

Sodium Transport

The uptake of Na^+ (100 mM NaCl) into brush border membrane vesicles as a function of time is shown in Fig. 1. Na^+ equilibrated with the vesicles with a half-time of 3 to 4 min. In this, but not all, experiments there was a transient "accumulation" (~1.2-fold) above the 5-hr equilibrium value. This may simply reflect vesicular volume

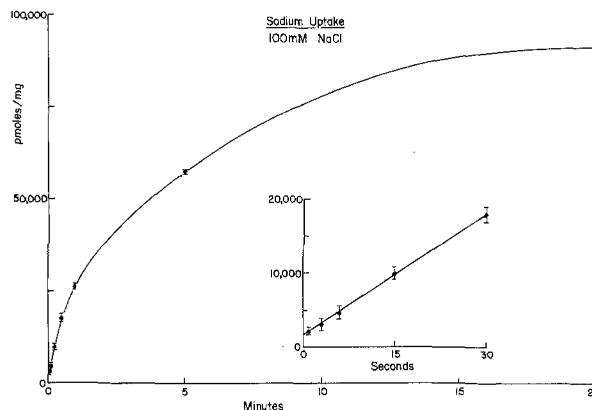

Fig. 1. Time course of sodium uptake into jejunal brush border membrane vesicles. The intravesicular solution contained 50 mM HEPES/Tris (pH 7.5). The extravesicular solution contained 100 mM NaCl in 50 mM HEPES/Tris (pH 7.5). Osmolarity was maintained at 350 mOsm/liter inside and out with D-mannitol. Data points are the mean of five samples ± standard error of the mean. The sodium uptake at 5 hr was 58,700 pmol/mg ± 1050 SE

changes during the course of the experiment. Na^+ uptake was linear from 2 to 30 sec in all experiments (inset of Fig. 1). This linear regression ($r = 0.965$) corresponded to an initial rate (J_{Na}) of uptake of 550 pmol/(mg protein · sec) and an intercept on the ordinate of 1500 pmol/mg protein. The intercept of the initial rate line on the ordinate was always positive. This type of linear regression was used as a measure of the initial rate of transport in all the experiments discussed in this paper.

When Na^+ influx was measured in the presence of various anions (SCN^-, NO_3^-, Cl^- and SO_4^{2-}), there was little effect in either the presence or absence of a pH gradient. Uptake of 850 µM Na^+ in the presence of various 15 mM salts varied less than 14% from the mean rate in the presence or absence of a pH gradient. The small effects that were observed were consistent with an effect on the diffusion potential across the vesicle membrane caused by the permeability differences between the anions. The more permeant anions permitted the greater influx of sodium ($NO_3^- > SCN^- > Cl^- > SO_4^{2-}$). Other evidence that membrane potential affects Na^+ influx in the absence of a pH gradient came from a "voltage clamp" experiment where a K^+ equilibrium potential of 28 mV (inside negative) was generated with a K^+ gradient in the presence of valinomycin. This potential stimulates influx of carrier-free $^{22}Na^+$ about twofold compared to vesicles with the membrane potential clamped at 0 mV.

The initial rate of Na uptake (J_{Na}) was measured as a function of extravesicular Na^+ activity (a_{Na}). Figure 2 shows a Woolf-Augustinsson-Hof-

stee plot of this type of data, where J is plotted against J/a_{Na}. In this plot the intercept on the ordinate is J_{max} and the slope is $-K_t$ (a_{Na} yielding $^1/_2\ J_{max}$). The data points can be fitted to a two-component curve, consisting of a diffusional flux and a saturable component with a J_{max} of 41 pmol/(mg protein·sec) and a K_t of 5 mM. The diffusional flux is a vertical line on this plot, with the intercept on the abscissa giving the apparent permeability coefficient (P'_{Na}). In this experiment P'_{Na} was 1.9 nl/(mg protein·sec). This coefficient, when multiplied by a_{Na} in moles/liter gives the magnitude of the diffusional flux, e.g. at 100 mM Na^+ the diffusional flux equals 190 pmol/(mg protein·sec).

The diffusional component was subtracted from the total rate of uptake at each concentration, and the saturable component fitted by computer to Eq. (1) (with $P'_i = 0$). The kinetic parameters for the carrier-mediated transport shown in Fig. 2 were $K_t \cong 5$ mM and $J_{max} = 49$ pmol/(mg protein·sec) when fitted to the Michaelis-Menten equation.

In one experiment the data could be fitted to a model describing two-carrier systems and a diffusional component as well as to a one-carrier model as described above. It was not clear whether the data was best described by one carrier or two in this experiment. If the two-carrier model was applied, one was characterized by a low K_t and low J_{max} ($K_t < 1$ mM and $J_{max} \cong 3$ pmol/(mg protein·sec)): in view of the low J_{max} of this carrier it was not considered further in the present paper. When a one-carrier model was applied to the same data, the J_{max} was 60 pmol/(mg protein·sec), the K_t was 5.5 mM and P'_{Na} was 2.2 nl/(mg protein·sec) (*see* Table 4).

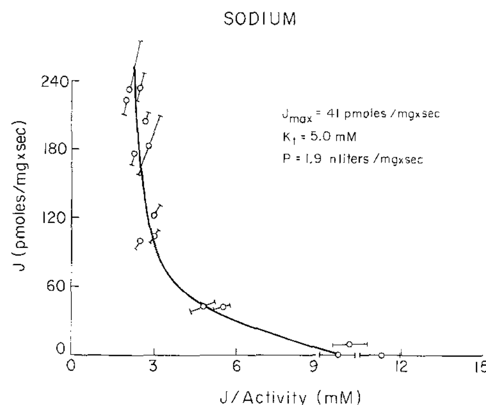

Fig. 2. Woolf-Augustinsson-Hofstee plot of sodium kinetics, where the initial rate of Na^+ flux (J) is plotted against J/sodium activity. The intravesicular solution contained 50 mM HEPES/Tris (pH 7.5). The extravesicular solution contained NaCl at concentrations ranging from 10 µM to 150 mM in 50 mM HEPES/Tris (pH 7.5). Osmolarity was maintained at 350 mOsm/liter inside and out with D-mannitol. Initial flux rates were determined by linear regression through time points in the first 30 sec of uptake. The points shown are data points ± standard deviations, and the line was fitted as described in Materials and Methods. This data is from two different batches of membranes. The points at high concentrations (8.5 to 150 mM) are from one batch and were normalized to those at low concentrations (10 µM to 10 mM) by comparing the fluxes at 10 mM, a concentration that was measured in both batches. *See also* Fig. 4

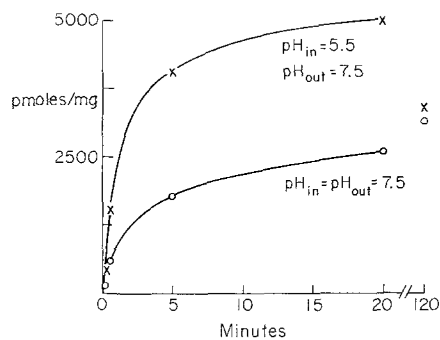

Fig. 3. Influence of a pH gradient on sodium uptake. The intravesicular solution contained either 50 mM HEPES/Tris (pH 7.5) or 50 mM MES/Tris (pH 5.5). The extravesicular solution contained 850 µM NaCl in 50 mM HEPES/Tris (pH 7.5). Osmolarity was maintained at 350 mOsm/liter inside and out with D-mannitol. Each time point is the mean of three samples with the exception of the 3-sec, 5-min and 20-min points of the $pH_{in} = pH_{out}$ experiment which were the mean of two samples. With the exception of these points, all standard errors were less than 5% of the mean

pH-Dependent Sodium Transport

Na^+ influx was measured in the presence and absence of a pH gradient. Figure 3 shows the effect of an outwardly directed H^+ gradient of two pH units. The H^+ gradient doubled J_{Na} and caused a twofold overshoot of Na^+ above the equilibrium concentration.

The effect of the pH gradient on the kinetics of Na^+ uptake was determined, and the results of one experiment are illustrated in Fig. 4. It is apparent that the pH gradient increased J_{max} with little or no effect on either K_t or P'_{Na}. J_{max} rose from 50 to 90 pmol/(mg protein·sec), while the K_t changed very little (from 5.5 to 5.0 mM) and the P'_{Na} changed very little (from 2.2 to 2.5 nl/(mg protein·sec)). Analysis of the saturable component was carried out in four different ways as discussed in Materials and Methods, and all yielded similar kinetic parameters. The Hofstee plot gave an increase in J_{max} (66 to 94 pmol/(mg protein·sec)) and little change in K_t (5.5 to 5.2 mM). The Michaelis-Menten plot yielded an increase in J_{max} (50 to 95 pmol/(mg protein·sec)) and an increase in K_t (4.4 to 5.4 mM), the Lineweaver-Burk plot yielded

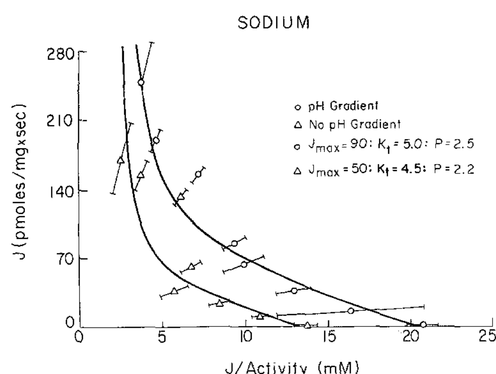

Fig. 4. Influence of a pH gradient on sodium uptake kinetics, shown as a Woolf-Augustinsson-Hofstee plot. The intravesicular solutions were either 50 mM HEPES/Tris (pH 7.5) (Δ) or 50 mM MES/Tris (pH 5.5) (o). The extravesicular solution contained NaCl at concentrations ranging from 100 μM to 85 mM in 50 mM HEPES/Tris (pH 7.5). Osmolarity was maintained at 350 mOsm/liter inside and out with D-mannitol. Points shown are data points ± standard deviation. Curves were fitted to the points as described in Materials and Methods

an increase in J_{max} (48 to 80 pmol/(mg protein·sec)) and little change in K_t (3.9 to 4.1 mM), and the Cornish-Bowden plot yielded an increase in J_{max} (61 to 98 pmol/(mg protein·sec)) and little change in K_t (5.1 to 5.3 mM).

If a two-carrier model was used on the same data, the low-capacity, high-affinity system showed little change in either J_{max} (≅3 pmol/(mg protein·sec)) or K_t (≅0.4 mM) when the pH gradient was applied.

This kinetic analysis was performed on data from several experiments on different batches of vesicles with the same result: application of a pH gradient increased the J_{max} with little effect on K_t. The average P'_{Na} for six experiments on three different batches of vesicles was 4.6 ± 1.0 (SE) nl/(mg protein·sec). In two experiments on different batches the J_{max} increased (from 57 to 93 and from 45 to 161 pmol/(mg protein·sec)) with little change in K_t (from 4.8 to 5.0 and from 4.8 to 3.8 mM).

Inhibitors

To determine the specificity of the Na^+ transport mechanism(s) the ability of various ions and drugs to inhibit Na^+ transport was tested. In all of the inhibition experiments 1 mM Na was used to insure that a large percentage of the observed Na transport was due to the Na^+/H^+ exchanger. Given the kinetic parameters for Na^+ transport described above it can be calculated that in the absence of a pH gradient 66% of the Na^+ flux should be carrier mediated and in the presence of a pH gradient 83% of the Na^+ flux should be carrier mediated.

Table 1. Inhibition of Na/H exchange by cations[a]

Ion	No Gradient	pH Gradient	Specific
Lithium	50 ± 11 (n=3)	62 ± 4 (n=3)	73 ± 3 (n=3)
Sodium	60 ± 2 (n=3)	61 ± 5 (n=3)	61 ± 9 (n=3)
Potassium	33 ± 5	29 ± 5	26 ± 10
	34 ± 3	27 ± 5	21 ± 9
Rubidium	28 ± 7	34 ± 4	39 ± 9
	33 ± 5	23 ± 5	13 ± 9
Cesium	30 ± 10	23 ± 4	18 ± 10
	32 ± 3	29 ± 6	27 ± 10
Ammonium	48 ± 4	67 ± 4	82 ± 8
	40 ± 3	54 ± 5	68 ± 10

[a] Percent inhibition of J_{Na} (1 mM Na^+) by 15 mM chloride salts. Specific uptake was the rate of uptake in the absence of a pH gradient subtracted from the rate of uptake in the presence of a pH gradient. The data for two series of experiments is given as the percent inhibition of the initial rate as compared to control (1 mM Na only) ± standard deviation. The mean ± standard deviation of three experiments are given for sodium and lithium competition.

Table 2. Inhibitors of Na uptake[a]

Inhibitor	No Gradient	pH Gradient	Specific
300 μM Amiloride	41 ± 15	44 ± 6	47 ± 17
300 μM Harmaline	39 ± 1	53 ± 6	64 ± 11
300 μM Amil + Harm	37 ± 4	58 ± 6	73 ± 11
100 μM SITS	−18 ± 6	−3 ± 6	12 ± 14
1 mM cAMP	34 ± 14	12 ± 11	−1 ± 20
10 mM Theophylline	−4 ± 17	−3 ± 9	−3 ± 18

[a] Percentage inhibition of J_{Na} (1 mM Na^+). Specific uptake is the uptake in the absence of a pH gradient subtracted from the rate of uptake in the presence of a pH gradient. The data is given as percent inhibition of the initial rate of uptake as compared to a control uptake (no inhibitor) ± standard deviation.

Ions

Table 1 shows the effects of 15 mM chloride salts on the initial rate of uptake of 1 mM Na^+. In the absence of a pH gradient the ions fell into two groups. Na^+, Li^+ and NH_4^+ inhibited 41 to 59% while K^+, Rb^+ and Cs^+ all inhibited about 30%. When a pH gradient was applied the pattern of specific inhibition was similar: Na^+, Li^+ and NH_4^+ inhibited by 51 to 82% while K^+, Rb^+ and Cs^+ inhibited by 13 to 39%.

Drugs

The effects of drugs on J_{Na} are illustrated in Table 2. Harmaline and amiloride (300 μM) each inhibited transport of 1 mM Na^+ about 40% in the absence of a pH gradient. When a pH gradient was applied, the specific inhibition of pH gradient stimulated Na^+ transport was 47% for 300 μM

Table 3. Relationship between rate of Na transport and external binding[a]

Experimental Conditions	Slope	Intercept	r
Experiment I:			
Control	17±0.2	63±3	0.9999
300 μM Amiloride	10±3	103±56	0.9386
300 μM Harmaline	10±0.1	113±2	0.9999
300 μM Amil+300 μM Harm	11±0.5	72±13	0.9969
Experiment II:			
Control	11±1	82±30	0.9655
pH Gradient	28±3	64±55	0.9837
10 mM Proline	29±2	55±39	0.9952
10 mM Glucose	69±2	132±34	0.9989

[a] Relationship between rate of Na transport (slope) and external binding (intercept). Experiments I and II were performed on different batches of membranes. Uptake of 1 mM Na^+ was measured using radioactive tracer flux and a linear regression of uptakes between 2 and 30 sec was done for each experimental condition. The slopes are expressed as pmol/(mg protein × sec) ± SD and the intercepts are expressed as pmol/mg protein ± SD. All experiments were performed with pH 7.5 inside and out except for the pH gradient group in experiment II where the pH was 5.5 inside and 7.5 outside as described in the text. All substrates and inhibitors were present on the outside only.

amiloride and 64% for 300 μM harmaline. Together they inhibited specific transport 73%. Ten mM theophylline, 100 μM SITS and 1 mM cAMP had no effect on specific pH gradient-stimulated Na^+ transport.

Binding

Linear regressions through uptakes between 2 and 30 sec always had a positive intercept on the ordinate. There was no correlation between the magnitude of the intercept and the rate of transport described by the slope of the regression line (Table 3). Although the Na^+ transport rates varied sevenfold, there was no significant change in the intercept. This suggests that the intercept represents binding of Na^+ to the external surface of the vesicles. Plotting binding as a function of Na concentration on a Hofstee plot yielded a maximum binding capacity of 11 nmol/mg (±1 SD), and an affinity of 90 mM (±12 SD).

Lithium Uptake

In light of the fact that Li^+ appears to be a good inhibitor for Na^+/H^+ exchange, experiments were undertaken to determine if Li^+ was actually transported by the exchanger. The uptake of Li^+ into brush border membrane vesicles was measured in the presence or absence of a pH gradient (Fig. 5).

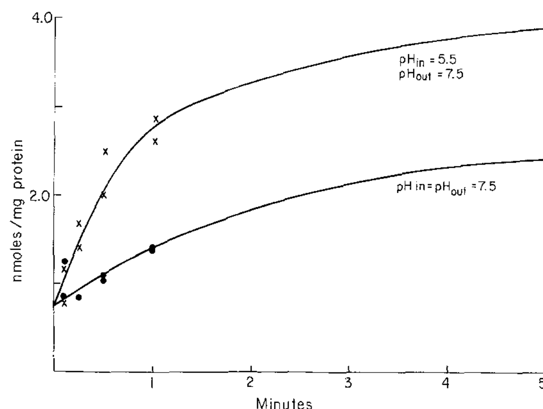

Fig. 5. Influence of a pH gradient on lithium uptake. The intravesicular solution was either 50 mM HEPES/Tris (pH 7.5) (●) or 50 mM MES/Tris (pH 5.5) (×). The extravesicular solution contained 1 mM LiCl in 50 mM HEPES/Tris (pH 7.5). Osmolarity was maintained at 350 mOsm/liter inside and out with D-mannitol. Points shown are individual data points

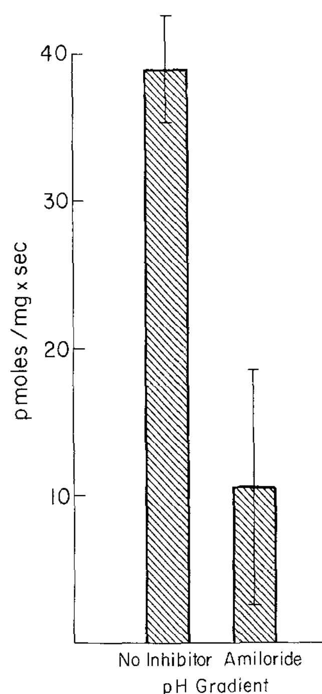

Fig. 6. Effect of amiloride (0.1 mM) on pH gradient-stimulated lithium uptake. Experimental conditions were as given for Fig. 5. Initial rates were determined by linear regression through four points during the first 30 sec of uptake

The initial rate of uptake of 1 mM Li^+ was stimulated approximately fivefold by the two-unit pH gradient in this experiment (an average of 4.1±0.4 SE in three experiments). This pH gradient stimulation was inhibited 97% by 0.1 mM amiloride (Fig. 6). A second experiment involving the effect of amiloride on the pH gradient-stimulated Li^+ transport yielded an 80% inhibition.

Table 4. Kinetic parameters of ion transport[a]

Ion	K_t	J_{max}
Na^+	4.7±0.8 (n=3)	47±4 (n=3)
K^+	3.8±1.4 (n=3)	58±25 (n=3)
Rb^+	3.0, 5.9	75, 52
SO_4^{2-}	0.12, 0.10	1.8, 0.4
Br^-	None	None

[a] Kinetic parameters for ion transport determined by radioactive tracer flux experiments. K_t's are given in mM and J_{max}'s in pmol/(mg protein × sec). Errors where noted are standard errors of the mean. If less than three measurements were available, data from individual experiments is given with no errors noted. Cl^- was the counterion for Na^+, K^+ and Rb^+ experiments, Na^+ or K^+ for Br^- experiments and Rb^+ for SO_4^{2-} experiments. The Na^+ data is from experiments in which no pH gradient was present.

Potassium and Rubidium Uptake

K^+ and Rb^+ uptakes were linear for 30 sec. Kinetic analysis of the uptakes showed saturable mechanisms for both. K^+ had a K_t of 3.8±1.4 mM and J_{max} of 58±25 pmol/(mg·sec) (n=3) and Rb^+ had K_t's of 3.0 and 5.9 mM and J_{max}'s of 75 and 52 pmol/(mg × sec) in two experiments (*see* Table 4).

Chloride and Bromide Uptake

A time course of Cl^- uptake (127 mM NaCl) is illustrated in Fig. 7. The half-time to equilibration was about 4 min, and uptake was linear from 2 to 30 sec (inset). A linear regression (r=0.958) of the 2 to 30-sec uptakes yielded an initial rate (J_{Cl}) of 286 pmol/(mg protein·sec) and an intercept of 2767 pmol/mg.

In preliminary experiments to test for the presence of a Cl^-/OH^- exchanger, it was found that a pH gradient (pH 7.5 in, 5.5 out) caused a 49% stimulation of Cl^- uptake at 128 mM Cl^- but did not cause an overshoot. Cl^- uptake was not inhibitable by 5 mM SITS or 0.5 mM furosemide. As a test for NaCl symport, Cl^- uptake (10 mM KCl) was measured in the presence of either Rb_2SO_4 or Na_2SO_4 (50 mM) in the extravesicular solution. J_{Cl} was 31 pmol/(mg protein·sec) in the presence of Rb^+ and 27 pmol/(mg protein·sec) in the presence of Na^+. Experiments using the fluorescent dye diS$-C_3-$(5) to measure diffusion potentials show that Rb^+ is more permeable than Na^+ [7]. Therefore the slight stimulation of Cl^- transport by Rb^+ can be explained by the more positive membrane potential in the presence of Rb^+.

Kinetic experiments on Br^- uptake showed a purely diffusive mechanism. No saturable component was observed.

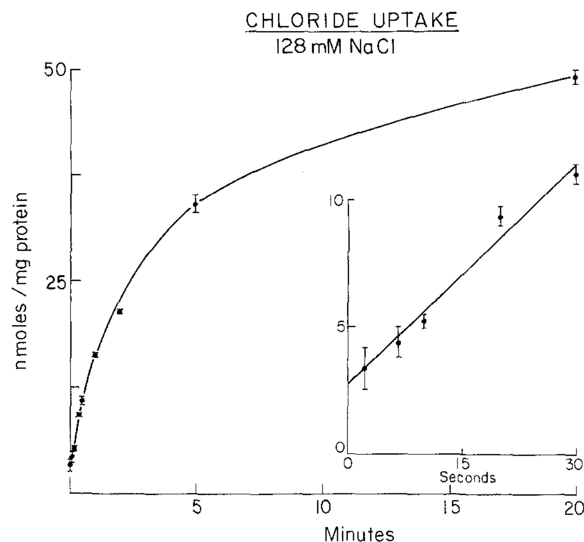

Fig. 7. Time course of chloride uptake into jejunal brush border membrane vesicles. The intravesicular solution contained 50 mM HEPES/Tris (pH 7.5). The extravesicular solution contained 128 mM NaCl in 50 mM HEPES/Tris (pH 7.5). Osmolarity was maintained at 350 mOsm/liter inside and out with D-mannitol. Data points are the means of three samples ± standard error. The chloride uptake at 3.5 hr was 65.7 nmol/mg ± 0.9 SE

Sulfate Uptake

SO_4^{2-} uptakes were also linear for at least 30 sec. Kinetic analysis of SO_4^{2-} flux revealed a saturable component which was Na^+ dependent. The initial rate of uptake of carrier-free (0.76 nM) $^{35}SO_4^{2-}$ was stimulated 10-fold in the presence of 15 mM NaCl as compared to 15 mM RbCl [5]. This suggests that a Na^+/SO_4^{2-} cotransporter, similar to the one observed in rat intestine [15] exists in rabbit jejunum as well. In two kinetics experiments the K_t's were 0.10 and 0.12 mM and the J_{max}'s were 1.8 and 0.4 pmol/(mg × sec) (*see* Table 4).

Discussion

These experiments were performed to characterize the ion transport properties of isolated brush border membrane vesicles from rabbit jejunum. The results suggest that there is a Na^+/H^+ exchange and Na^+ diffusion in these membranes. Na^+/H^+ exchange has been observed in a wide variety of tissues, including rat intestine and kidney [12], rabbit kidney [1, 2, 11, 21], rabbit ileum [13] and *E. coli* [22]. Its presence in jejunal brush borders may explain, in part, the $NaHCO_3$ absorption observed in this tissue *in vivo*.

Justification of the Methods

Throughout this paper, the initial rate of transport (J) of a substrate was defined as the slope of the

linear regression line through 2- to 30-sec uptakes. There are several lines of evidence to indicate that the slope of a regression through time points in the first 30 sec corresponds to the initial rate of transport, while the intercept of this line on the ordinate corresponds to binding on the external surface of the vesicle. The linearity of the uptake during this time period suggests that only one process is occurring, and the slope of the line varies as expected for transport; e.g., addition of inhibitors decreases the slope. The intercept on the ordinate, however, behaves as predicted for external binding; e.g., while saturable, it is not affected by specific transport inhibitors. Since other explanations are possible for the "rapid binding" uptake, e.g. transport followed by internal binding, we have chosen to characterize the linear uptake.

The kinetics of the Na^+ transport was studied after correction for the passive diffusion component. The ability to correct for diffusion was important since the magnitude of diffusion was comparable to the magnitude of the saturable component at higher concentrations. The diffusional flux was extracted from Woolf-Augustinsson-Hofstee plots (Figs. 2 & 4). It can also be estimated by a computer program as described in Materials and Methods. Either method yields similar results. Once the permeability coefficient for a given batch of membranes is known, the diffusional flux can be subtracted from the total flux at each concentration, leaving only the saturable component(s), which can be subjected to standard kinetic analysis. The validity of this technique for determining permeability is shown by the observation that the diffusion-corrected fluxes can be described by simple kinetic equations and yield similar kinetic parameters regardless of which transformation of the standard equation the data is fitted to. The diffusion-corrected data was fitted to various transformations by linear regression (Hofstee, Lineweaver-Burk), nonlinear least-squares analysis (Michaelis-Menten) and by the direct linear plot method (Cornish-Bowden). The good agreement obtained with all of these methods, each of which weights the data from high and low concentrations differently, supports the validity of our analysis (*see* pages 87 & 88).

Na/H Exchange

The data indicate that there is an exchange mechanism for Na^+ and H^+ in the brush border membranes from rabbit jejunum. The Na^+/H^+ exchanger was observed as a stimulation of J_{Na} by the application of a pH gradient across the vesicular membrane. Both the increase in J_{Na} and the overshoot were probably caused by the coupling of the energy in the H^+ gradient to the influx of Na^+. The H^+ gradient was undoubtedly dissipating during the course of the experiment, but the observation that the overshoot was still apparent at 20 min suggests that the gradient was not significantly changed during the first 30 sec when the initial rate determination was made. The high concentration of impermeant buffers (50 mM) should have maintained the intra- and extravesicular pH relatively constant during this time.

The ΔJ_{Na} was inhibited by 300 µM amiloride or harmaline, but not by 10 µM amiloride or harmaline, 100 µM SITS, 10 mM theophylline or 1 mM cAMP (Table 2). This result is consistent with the observations of Kinsella and Aronson [12] in renal microvillus membrane vesicles. The effects of harmaline and amiloride on jejunal brush borders were not additive, suggesting that they are both inhibiting the same process for Na^+ translocation and are near saturating concentrations for inhibition at 300 µM. This is the same concentration range which Kinsella and Aronson [12] demonstrated was necessary to inhibit the Na/H exchange system in renal brush border membranes, but 100-fold higher than the concentration necessary to inhibit the amiloride-sensitive Na conductance pathway of other epithelia.

The specificity of the exchanger was examined by measuring the inhibition of pH gradient-stimulated Na^+ transport caused by monovalent cations. The results (Table 1) indicate that the Na/H exchanger is specifically inhibited by Li^+ and NH_4^+, and to a lesser extent by K^+, Rb^+ and Cs^+. Li^+ uptake experiments show that this cation can be transported in exchange for H^+ (Fig. 5), and kinetic experiments with K^+ and Rb^+ reveal saturable transport systems for these ions which resemble the Na^+ system. These results suggest that the inhibition caused by these ions is due to competition for the Na^+ transport site. NH_4^+ also inhibits Na^+/H^+ exchange (although it is possible that NH_4^+ may raise the intravesicular pH due to the high permeability of NH_3, this is considered unlikely in the presence of 50 mM buffer), so the selectivity of the exchanger seems to be for ions with a smaller nonhydrated radius. This selectivity sequence indicates that the Na^+ binding site on the exchanger has a relatively high electrostatic field strength [3]. The same selectivity was observed in renal microvillus membranes [12].

The kinetic analysis of J_{Na} indicates that the K_t is approximately 5 mM and the J_{max} is approximately 50 pmol/(mg protein·sec) in the absence of a pH gradient. When an outwardly directed pH gradient of two pH units is applied across the

membrane, the J_{max} increases to 125 pmol/(mg protein·sec) with little change in the K_t. The experiments with competing cations, amiloride and harmiline suggest that the same Na^+/H^+ exchanger is working in the absence or presence of a pH gradient. It is therefore likely that the effect of a pH gradient is to increase the maximum rate of the exchanger which operates in the absence of a pH gradient.

In summary, we can conclude that there is a Na^+/H^+ exchange mechanism in jejunal BBMV's by the following criteria: 1) We have observed a pH gradient stimulation of Na^+ uptake into intestinal brush border vesicles, while Murer et al. [16] have observed a Na^+-dependent pH change in similar vesicles. 2) This pH-dependent Na uptake shows the same type of sensitivity to inhibitors and competing cations as the Na^+/H^+ exchanger observed in renal brush borders [12]. 3) The pH gradient stimulation of Na^+ transport observed in the present study cannot be attributed to membrane potential effects because: a) a saturable mechanism is changed by the imposition of a pH gradient across the vesicles. This is not consistent with increased Na^+ flux through a conductance pathway due to a proton diffusion potential, and b) experiments with anion substitution show small effects consistent with a membrane potential effect on diffusive flux. These effects are much smaller (less than 14% change in J_{Na}), than those seen in the presence of a pH gradient.

The jejunal Na^+/H^+ exchanger appears similar to the renal exchanger as observed by Kinsella and Aronson [11, 12], Ives et al. [9] and Murer et al. [16]. It is inhibited by amiloride at a much higher concentration than is necessary for inhibition of conductive Na^+ channels in epithelia, as well as by harmaline. This is in agreement with the work of Kinsella and Aronson [11, 12]. Also in agreement with this work is the cation specificity sequence, with the highest affinity among alkali cations being for Li^+. The Li^+ transport data presented here show that at 1 mM Li^+ concentrations, Li^+ is stimulated far more by a pH gradient than 1 mM Na^+. This is consistent with the data of Ives et al. [9] which show that Li^+ has a lower K_m than Na^+ for causing collapse of a pH gradient across renal brush border vesicles as measured with acridine orange.

In vivo the pH gradient across the brush border of jejunum is not likely to be two pH units. Therefore, the mode of operation of the carrier which is important is probably the low J_{max} mode. A maximum rate of transport of 50 pmol/(mg protein·sec) makes it unlikely that the Na^+/H^+ exchanger is important in the absorption of Na^+ from the jejunum. Table 5 shows some calculated values of Na^+ flux through several known absorptive pathways. The transport rates in this Table were calculated assuming a luminal Na^+ activity of 150 mM and enough of each cotransported solute to drive their respective transport systems at J_{max}. Two different figures are given for Na^+/H^+ exchange, one assuming no pH gradient and one assuming a two-unit pH gradient. It can be concluded that the most important pathways for Na^+ flux across the brush border are diffusion and organic substrate/Na^+ cotransport systems. Diffusion accounts for approximately 700 pmol/(mg protein·sec). Na^+/amino acid cotransport and Na^+/glucose cotransport account for about 900 and 200 pmol/(mg protein·sec), respectively. Na^+/H^+ exchange accounts for less than 3% of the total Na^+ flux under these conditions. In the absence of sugars and amino acids the Na^+/H^+ exchanger would account for less than 7% of the total flux of Na^+.

Table 5. Summary of sodium transport systems in rabbit jejunal brush borders[a]

System	Sodium Flux
Diffusion	700
Na/H exchange (pH gradient)	125
Na/H exchange (no pH gradient)	50
Na/glucose cotransport	170
Na/amino acid cotransport (total)	890
system 1) proline	170
system 2) alanine	100
system 3) phenylalanine	620

[a] Calculated values of sodium flux across brush border of rabbit jejunum. Values were calculated assuming 150 mM luminal sodium activity, and enough of each cotransported solute to saturate their respective systems. Fluxes are given as pmol/mg protein/sec. Two-to-one coupling was assumed for sodium/glucose cotransport [8] and one-to-one coupling for sodium/amino acid cotransport. The sodium/proton exchange flux was calculated assuming either a two-unit pH gradient or no pH gradient, so the actual *in vivo* flux probably falls within these limits. Na/glucose transport data from Kaunitz et al. [10], Na/amino acid transport data from Stevens et al. [20].

Another possible function of the Na^+/H^+ exchanger *in vivo* may be the absorption of HCO_3^-. The jejunum actively absorbs mostly Na^+ and HCO_3^- and some Cl^- *in vivo* [24]. Pancreatic secretion results in a large HCO_3^- load in the jejunum. The net effect of H^+ secretion is the same as HCO_3^- absorption. When intracellular CO_2 combines with H_2O to form H_2CO_3, a H^+ becomes

available for transport. When this H^+ is secreted a HCO_3^- ion is left behind in the cell. The secreted H^+ can then combine with a HCO_3^- ion in the lumen to form H_2CO_3 which dissociates into CO_2 and H_2O. The net effect of a Na^+/H^+ exchange process is the absorption of $NaHCO_3$ and the secretion of CO_2.

The fraction of the pH gradient-stimulated flux that was not inhibitable by amiloride or harmaline (about 25% at 1 mM Na^+) probably indicates that the brush border contains conductive pathways for H^+ as well as for Na^+. The Na^+ conductance was demonstrated by the membrane potential stimulation of Na^+ uptake and by the diffusional flux that is obvious from the kinetic analyses. The Na^+ conductance pathway has also been demonstrated in experiments with the carbocyanine dye diS$-C_3-(5)$ [5]. The existence of a noninhibitable fraction of H^+ gradient-stimulated Na^+ transport suggests that the H^+ gradient induced a membrane potential which stimulated Na^+ transport via the Na^+-conductive pathway. An H^+ gradient-induced membrane potential indicates that a conductive H^+ pathway exists in the vesicles. This is in agreement with the data of Reenstra et al. [18] and Burnham et al. [2] in renal brush border membrane vesicles. These authors determined that a proton conductance exists in renal brush borders and the present result extends this observation to the jejunal brush border.

Chloride Transport

The existence of neutral, coupled Na^+/Cl^+ cotransport has been postulated for the rabbit ileum [17]. Such a transport system does not appear to exist in rabbit jejunal brush borders. If such coupled transport was present in these membranes, Cl^- would be expected to stimulate the transport of Na^+, and Na^+ the transport of Cl^-. These effects were not observed. The fact that no saturable transport mechanism was observed for Br^-, which might be expected to share a Cl^- system to some extent, also supports this conclusion.

Liedtke and Hopfer [14] and Knickelbein et al. [13] have observed a Cl^-/OH^- exchange mechanism in rat and rabbit ileum which was inhibited by either SITS or furosemide. In jejunal brush borders, a pH gradient stimulation of Cl^- transport was observed, but it was not inhibitable by either SITS or furosemide. These results suggest that the type of Cl^-/OH^- exchange mechanism observed in rat and rabbit ileum is not present in rabbit jejunum. This preliminary data is not sufficient to rule out the possibility that a Cl^-/OH^- exchange of a different nature exists in jejunum.

A simple model for the ion transport characteristics of jejunum and ileum can be postulated from the current knowledge of ion transport mechanisms in brush borders. This model is very similar to the one proposed by Turnberg et al. [23, 24] from their work on human intestine. In the jejunum, which absorbs mostly $NaHCO_3$ in the absence of sugars or amino acids, a Na^+/H^+ exchanger results in net uptake of $NaHCO_3$, whereas in the ileum, which absorbs NaCl, it has a Cl^-/OH^- exchanger and a Na^+/H^+ exchanger in parallel in the brush border membrane. These two systems, operating together, produce the "coupled NaCl" absorption. Coupled NaCl absorption in rat ileum was inhibited by furosemide [8], a drug shown to inhibit Cl^-/OH^- exchange in rabbit ileum [14].

We wish to thank Vincent Fry for the atomic absorption measurements and Drs. Verna Harms, Jon Kaunitz, Bruce Stevens, Steven Wright and Ed Nord for valuable discussions. The computer program Diffit written by Terry Reedy and Mmplot was written by Bruce Stevens. This work was supported by USPHS grants AM 19567 and AM 07270.

References

1. Bichara, M., Paillard, M., Leviel, F., Gardin, J.P. 1980. Hydrogen transport in rabbit kidney proximal tubules – Na:H exchange. *Am. J. Physiol.* **238**:F445–F451
2. Burnham, C., Munzesheimer, C., Rabon, E., Sachs, G. 1982. Ion pathways in renal brush border membranes. *Biochim. Biophys. Acta* **685**:260–272
3. Diamond, J.M., Wright, E.M. 1969. Biological membranes: The physical basis of ion and non-electrolyte selectivity. *Annu. Rev. Physiol.* **31**:581–646
4. Ehrlich, B.E., Diamond, J.M. 1978. An ultramicro method for analysis of lithium and other biologically important cations. *Biochim. Biophys. Acta* **543**:264–268
5. Gunther, R.D. 1981. Sodium transport by intestinal brush border membrane vesicles. Ph.D. Thesis. University of California, Los Angeles
6. Gunther, R.D., Fry, V., Wright, E.M. 1982. Sodium/proton and lithium/proton exchange by rabbit jejunal brush border membranes. *Fed. Proc.* **41**:1494
7. Gunther, R.D., Schell, R., Krasne, S., Wright, E.M. 1982. Ion permeability of jejunal brush border membranes measured with a potential sensitive dye and with radioactive tracers. *Abstr. Am. Physiol. Soc.*
8. Humphreys, M.H. 1976. Inhibition of NaCl absorption from perfused rat ileum by furosemide. *Am. J. Physiol.* **230**:1517–1523
9. Ives, H.E., Yee, V.J., Warnock, D.G. 1982. Effects of Na^+ and Li^+ on the activity of the Na^+/H^+ antiporter of renal brush border membrane vesicles. *Fed. Proc.* **41**:1262
10. Kaunitz, J.D., Gunther, R.D., Wright, E.M. 1982. Involvement of multiple sodium ions in intestinal D-glucose transport. *Proc. Natl. Acad. Sci. USA* **79**:2315–2318

11. Kinsella, J.L., Aronson, P.S. 1980. Properties of the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Am. J. Physiol.* **238**:F461–F469
12. Kinsella, J.L., Aronson, P.S. 1982. Amiloride inhibition of the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Am. J. Physiol.* **241**:F374–F379
13. Knickelbein, R.G., Aronson, P.S., Dobbins, J.W. 1982. Na−H and $Cl-HCO_3$ exchange in rabbit ileal brush border membrane vesicles. *Fed. Proc.* **41**:1494
14. Liedtke, C.M., Hopfer, U. 1982. Mechanism of Cl^- translocation across the small intestinal brush border membrane. II. Demonstration of Cl^-/OH^- exchange and Cl^- conductance. *Am. J. Physiol.* **242**:G272–G280
15. Lucke, H., Stange, G., Murer, H. 1981. Sulfate-sodium cotransport by brush border membrane vesicles isolated from rat ileum. *Gastroenterology* **80**:22–30
16. Murer, H., Hopfer, U., Kinne, R. 1976. Sodium/proton antiport in brush-border-membrane vesicles isolated from rat small intestine and kidney. *Biochemistry* **154**:597–604
17. Nellans, H.N., Frizzell, R.A., Schultz, S.G. 1973. Coupled sodium-chloride influx across the brush border of rabbit ileum. *Am. J. Physiol.* **225**:467–475
18. Reenstra, W.W., Warnock, D.G., Yee, V.J., Forte, J.G. 1981. Proton gradients in renal cortex brush border membrane vesicles. Demonstration of a rheogenic proton flux with acridine orange. *J. Biol. Chem.* **256**:11663–11666
19. Robinson, R.A., Stokes, R.H. 1959. Electrolyte Solutions. Butterworths, London
20. Stevens, B.R., Ross, H.J., Wright, E.M. 1982. Multiple transport pathways for neutral amino acids in rabbit jejunal brush border vesicles. *J. Membrane Biol.* **66**:213–225
21. Stevens, B.R., Wright, S.H., Hirayama, B., Ross, H.J., Gunther, R.D., Nord, E., Kippen, I., Harms, V.K., Wright, E.M. 1982. Liquid nitrogen preservation of organic and inorganic solute transport in renal and intestinal membrane vesicles. *Membr. Biochem.* **4**:*(in press)*
22. Tsuchiya, T., Takeda, K. 1979. Calcium/proton and sodium/proton antiport systems in *Escherichia coli*. *J. Biochem.* **85**:943–951
23. Turnberg, L.A., Bieberdorf, F.A., Morawski, S.G., Fordtran, J.S. 1970. Interrelationships of chloride, bicarbonate, sodium and hydrogen transport in the human ileum. *J. Clin. Invest.* **49**:557–567
24. Turnberg, L.A., Fordtran, J.S., Carter, N.W., Rector, F.C. 1970. Mechanism of bicarbonate absorption and its relationship to sodium transport in the human jejunum. *J. Clin. Invest.* **49**:548–556

Received 17 August 1982; revised 6 January 1983