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Ontogeny of the Na^+-H^+ Exchanger in Rat Ileal Brush-Border Membrane Vesicles

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Summary. The developmental maturation of Na^+-H^+ antiporter was determined using a well-validated brush-border membrane vesicles (BBMV's) technique. Na^+ uptake represented transport into an osmotically sensitive intravesicular space as evidenced by an osmolality study at equilibrium. An outwardly directed pH gradient (pH inside/pH outside = 5.2/7.5) significantly stimulated Na^+ uptake compared with no pH gradient conditions at all age groups; however, the magnitude of stimulation was significantly different between the age groups. Moreover, the imposition of greater pH gradient across the vesicles resulted in marked stimulation of Na^+ uptake which increased with advancing age. Na^+ uptake represented an electroneutral process.

The amiloride sensitivity of the pH-stimulated Na^+ uptake was investigated using [amiloride] 10^{-2}-10^{-5} M. At 10^{-3} M amiloride concentration, Na^+ uptake under pH gradient conditions was inhibited 80, 45, and 20% in BBMV's of adolescent, weanling and suckling rats, respectively. Kinetic studies revealed a K_m for amiloride-sensitive Na^+ uptake of 21.8 ± 6.4, 24.9 ± 10.9 and 11.8 ± 4.17 mM and V_{max} of 8.76 ± 1.21, 5.38 ± 1.16 and 1.99 ± 0.28 nmol/mg protein/5 sec in adolescent, weanling and suckling rats, respectively. The rate of pH dissipation, as determined by the fluorescence quenching of acridine orange, was similar across membrane preparation of all age groups studied. These findings suggest for the first time the presence of an ileal brush-border membrane Na^+-H^+ antiporter system in all ages studied. This system exhibits changes in regard to amiloride sensitivity and kinetic parameters.

Key Words: intestine · maturation · transport · sodium · hydrogen exchange

Introduction

Diarrheal disorders in infants results in fluid and electrolyte imbalances which could be related to differences in electrolyte transport during early life. Understanding of the functional changes in the transport characteristics of the small intestine is essential for management of intestinal diseases in neonates and infants. Perfusion studies in man (Turnberg et al., 1970) and in vitro studies in adult animals (Gunther & Wright, 1983; Knickelbein et al., 1984) suggested the presence of Na^+-H^+ exchanger in the small intestine.

The rate of cellular proliferation and migration in the intestinal epithelium of postnatal rat is slower than of mature adult (Koldovsky, Sunshine & Kretchmer, 1966). While the turnover of intestinal epithelia in the adult jejunum is about 48 to 72 hr, the rate in the suckling animal is at least twice that long (Herbst & Sunshine, 1969). At the time of weaning, rates of cellular migration and proliferation increase rapidly. Recent studies revealed that there was evidence to link Na^+/H^+ exchanger to cell growth (L'Allemain, Paris & Pouyssegur, 1984). Therefore, it was hypothesized that the activity of intestinal Na^+/H^+ exchanger in the postnatal rat could be low as compared to mature adult rat. No study is available, however, describing the developmental aspects of Na^+-H^+ exchanger in the rat small intestine during maturation.

To be able to understand the pathophysiological alterations in electrolyte imbalances and intestinal damage repair resulting from infant diarrheal states, it is important to define the characteristics of Na^+-H^+ exchanger in the infancy period as it relates to growth. The present study was designed to examine the postnatal development of Na^+-H^+ exchange system in the brush-border membrane vesicles of the rat ileum.

Materials and Methods

Materials

^{22}Na and ^{14}C-glucose was obtained from New England Nuclear, Boston, MA. Amiloride was kindly provided by Merck Research Laboratories, West Point, PA. Valinomycin, enzymes and substrates for leucine aminopeptidase were obtained from Sigma

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Chemical, St. Louis, MO. TMA-gluconate was made by titrating solutions of TMA-hydroxide with gluconic acid. Cellulose nitrate filters, 0.45-μm pore size, were obtained from Sartorius Filters, Hayward, CA. All other chemicals were of the highest purity available.

Animals

Two days after birth, Sprague-Dawley rat pups (Harlan Sprague Dawley, Madison, WI) were distributed among mothers to maintain a litter size of 9–10 pups until the time of the study. Adolescent Sprague-Dawley rats (6 weeks old) were purchased directly from the same supplier. Mothers, weanling, and adolescent rats were fed Purina rat chow and tap water ad libitum. The National Council's guidelines for the care and use of laboratory animals were followed. Transport studies were performed in adolescent (42–43-days-old), weanling (21-day-old), and suckling (14-day-old) rats.

Preparation of Brush-Border Membrane Vesicles

Brush-border membrane vesicles were prepared using a Mg^{2+}/EGTA precipitation method from scraped rat ileal mucosa (Hopfer et al., 1973). The animals were killed by cervical dislocation. The distal one-third of the small intestine was removed, everted and scraped with a glass slide. All steps in this preparation were conducted at approximately 4°C. Using a Waring blender at maximal speed, the mucosal scrapings were homogenized for 3 min in 30 ml of 300 mM mannitol, 5 mM EGTA and 12 mM Tris HCl (pH 7.1). Ice-cold, distilled water (120 ml) was added. The homogenate was treated with 1.5 ml of 1 M $MgCl_2$ and centrifuged in a rotor (model J2-21; Beckman Instruments, Fullerton, CA) at 5,000 rpm for 15 min. The supernatant was then centrifuged at 15,000 rpm for 30 min. The resulting pellet was resuspended in 30 ml of 60 mM mannitol, 5 mM EGTA and 12 mM Tris HCl (pH 7.1), and homogenized in a Potter-Elvehjem tube for 10 strokes at the highest speed. The homogenate was treated with 0.3 ml of 1 M $MgCl_2$ and centrifuged at 5,000 rpm for 15 min. The supernatant was spun at 15,000 rpm for 30 min. The pellet was resuspended in 30 ml of 250 mM mannitol and 20 mM HEPES Tris (pH 7.4) and centrifuged at 20,000 rpm for 30 min. Using a tuberculin syringe with a 25-gauge needle, the pellet was resuspended in the desired volume of preincubation solution, the composition of which is described in each figure legend. The protein concentration was measured by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Purity of the Brush-Border Membrane Vesicle Preparation

Leucine aminopeptidase was measured by a Boehringer kit (no. 124869; Boehringer Mannheim Biochemicals, Indianapolis, IN). Na^+/K^+-ATPase activity was measured by the method of Scharschmidt et al. (1979). Cytochrome-*c* oxidase and NADPH cytochrome-*c* reductase were measured by the method of Beaufay et al. (1974).

Transport Measurements

Uptake of radiolabeled sodium was measured by a rapid filtration technique (Hopfer et al.,1973). All incubations were done at 25°C and were initiated by addition of 20 μl of vesicle suspension to 80 μl of incubation solution. The composition of the incubation medium is noted in the legend of each figure. At each desired incubation time interval, 1 ml of ice-cold stop solution, which consisted of 185 mM K-gluconate, 10 mM Tris, 16 mM HEPES and 0.1 mM amiloride, was added to the reaction mixture. The cold, diluted reaction mixture was immediately pipetted onto a prewetted filter (cellulose nitrate, 0.45-μm pore size; Sartorius Filters, Hayward, CA) and kept under suction. The filter was rinsed with 5 ml of ice-cold stop solution and then dissolved in Bray's solution. Radioactivity was counted in a scintillation counter (model LS 4000: Beckman Instruments, Palo Alto, CA) as a liquid scintillant. Radioactivity remaining in the filters after pipetting incubation medium into the radioactive substrate in the absence of vesicles was considered as background and was accounted for in the calculations.

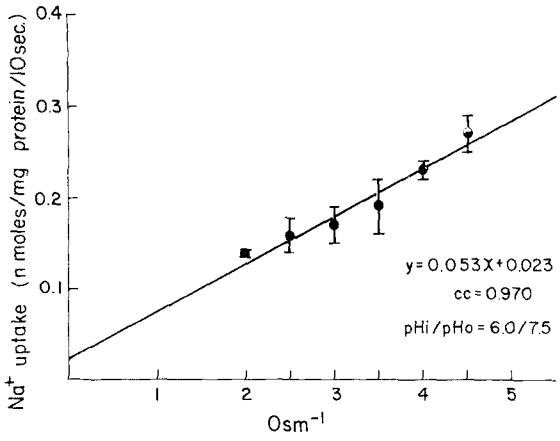

Fig. 1. Effect of media osmolality on 180-min uptake of 1 mM sodium. BBMV's were prepared in 100 mM TMA gluconate, 40 mM HEPES, 90 mM MES buffer, pH 5.2. The reaction was started by addition of 20 μl of membrane vesicles to a different media (incubation buffer) containing (in mM): 100 TMA gluconate, 85 HEPES, 45 Tris, pH 7.5, with 1 sodium and tracer ^{22}Na. Osmolality was altered by varying mannitol concentration to achieve osmolalities between 200–250 mOsm. The reaction was stopped at 180 min. Each point represents the mean ± SE of three separate experiments on different membrane preparations

Analysis of Data

All values were expressed as nanomoles of sodium uptake per milligram of vesicle protein, and expressed graphically as the mean ± 1 SE. ANOVA test was used to evaluate the statistical significance of differences between the groups. A probability value of <0.05 was considered statistically significant.

Results

Purity of the Brush-Border Membrane Vesicle Preparation

Table 1 depicts the specific activities of marker enzymes in intestinal homogenate and brush-border

Table 1. Specific activity of marker enzymes in BBMV's and mucosal homogenate in the rats studied

	Suckling		Weanling		Adolescent	
	BBMV	Mucosa	BBMV	Mucosa	BBMV	Mucosa
Leucine aminopeptidase (μmole β NPHA hr^{-1} mg $protein^{-1}$)	2464 ± 125	320 ± 35 (8)	4344 ± 230	420 ± 29 (10)	9319 ± 375	920 ± 220 (10)
NADH cytochrome-*c* reductase (μmole min^{-1} mg $protein^{-1}$)	2.8 ± 0.5	5.1 ± 1.2 (0.55)	2.9 ± 0.4	6.2 ± 1.2 (0.46)	4 ± 0.5	8.2 ± 2 (0.39)
Cytochrome-*c* oxidase (μmole min^{-1} mg $protein^{-1}$)	1.5 ± 0.2	4.8 ± 1.1 (0.31)	3.0 ± 0.3	6.9 ± 1 (0.43)	3 ± 0.4	6.4 ± 1 (0.46)
Na^+-K^+-ATPase (μmol/P_i/hr^{-1}/mg $protein^{-1}$)	1.60 ± 0.18	2.10 ± 0.42 (0.61)	2.4 ± 0.1	4.6 ± 0.5 (0.52)	6.9 ± 0.4	15 ± 0.4 (0.46)

Values are mean ± SE (n = 6).
Number in parentheses indicates enrichment or impoverishment factor.

Table 2. Initial rate uptake of ^{22}Na by brush border membrane vesicles of adolescent, weanling and suckling rats

Time (sec)	Na^+ uptake (nmol/mg protein)		
	Adolescent	Weanling	Suckling
3	0.09 ± 0.05	0.06 ± 0.05	0.04 ± 0.03
5	0.16 ± 0.07	0.10 ± 0.04	0.07 ± 0.02
7	0.22 ± 0.08	0.16 ± 0.03	0.11 ± 0.03
10	0.28 ± 0.07	0.22 ± 0.06	0.17 ± 0.04
12	0.32 ± 0.06	0.26 ± 0.05	0.2 ± 0.02
15	0.34 ± 0.05	0.28 ± 0.06	0.22 ± 0.04
20	0.4 ± 0.1	0.32 ± 0.04	0.24 ± 0.04
Linear regression	$Y = 0.025\,x + 0.028$ cc = 0.98	$Y = 0.02\,x + 0.02$ cc = 0.98	$Y = 0.01\,x + 0.015$ cc = 0.99

membrane vesicles. Leucine aminopeptidase, a specific marker enzyme for brush-border membranes was enriched 8- to 10-fold as compared with total mucosal homogenate. Marker enzymes for basolateral membranes (Na^+-K^+-ATPase), mitochondria (cytochrome-*c* oxidase) and endoplasmic reticulum (NADPH cytochrome-*c* reductase) were all impoverished.

Binding *Versus* Uptake into the Intravesicular Space

To determine whether Na^+ uptake is into an osmotically sensitive intravesicular space or mere binding, we performed an osmolality study in which Na^+ uptake under outwardly directed pH gradient condition was studied at 180 min. Figure 1 depicts that the relationship between Na^+ uptake and liter/osm was linear as expressed by the formula $y = 0.053\,x + 0.023$, correlation coefficient (cc) was 0.97 suggesting a minor binding component. These observations were similar in all age groups studied (adolescent, weanling and suckling rats).

Initial Rate Uptake of Na^+-H^+ Exchange

Na^+ uptake at 1 mM Na^+ concentration was determined under outwardly directed pH gradient condition (pH_i/pH_o = 5.2/7.5) at all age groups studied. Na^+ uptake was determined at 3, 5, 7, 10, 12, 15 and 20 sec. Na^+ uptake was linear up to 12 sec in all age groups as depicted by the formula $Y = 0.025\,x + 0.028$, cc = 0.98, $Y = 0.02\,x + 0.02$, cc = 0.98 and $Y = 0.01\,x + 0.015$, cc = 0.99 for adolescent, weanling and suckling rats, respectively. The positive nonzero intercept on the ordinate is due to binding component (Nord et al., 1984). Table 2 depicts the initial uptake values in the three age groups.

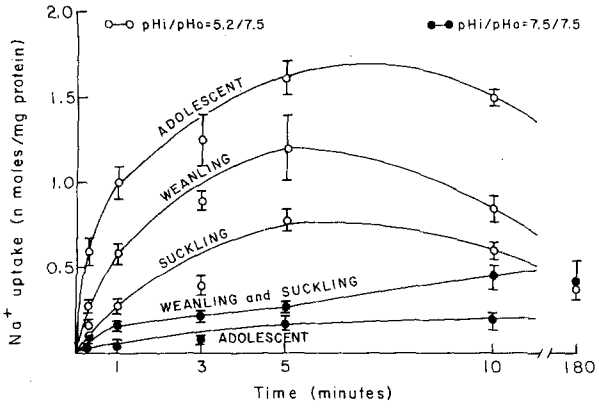

Fig. 2. Effect of age on 1 mM Na^+ uptake under outwardly directed pH gradient (pH_i/pH_o = 5.2/7.5) and no pH gradient (7.5/7.5). Vesicles were preincubated for 1 hr at 25°C with various combinations of Tris, HEPES, and MES (130 mM total) to bring intravesicular pH to 5.2 or 7.5. The reaction was started the addition of 20 μl of vesicles to a media containing (in mM): 100 TMA gluconate, 85 HEPES, 45 Tris, pH 7.5, with 1 Na^+ and tracer ^{22}Na. Each point represents the mean ± SE of three separate experiments on different membrane preparations. Peak "overshoot" uptake in adolescent rats was significantly greater than corresponding values in suckling rats ($P < 0.01$)

Effect of Age on Na^+-H^+ Exchange by Ileal BBMV's

To determine the maturational aspects of Na^+-H^+ exchange, we studied Na^+ uptake under outwardly directed pH gradient condition pH_i/pH_o = 5.2/7.5 and compared uptake values to those under no pH gradient condition pH_i/pH_o = 7.5/7.5.

Figure 2 depicts the presence of Na^+-H^+ exchanger at all age groups studied, as evident by marked stimulation of Na^+ uptake and the presence of an "overshoot" phenomena under pH gradient condition compared with no pH gradient condition. However, two important observations emerged, first the magnitude of "overshoot" was greater in adolescent and weanling rats BBMV's (fourfold increase) compared with suckling rat BBMV's (1.5-fold increase). Mean values at the peak of "overshoot" were significantly different between suckling and adolescent rats ($P < 0.001$). Second, Na^+ uptake under no pH gradient conditions was higher in suckling rat BBMV's compared to values obtained with adolescent BBMV's ($P < 0.05$ at 1, 3, 5 and 10 min). It is also important to note that equilibrium values were similar in all age groups. The vesicular size using L-glucose in all age groups was approximately 1–1.2 μl/mg protein.

Effect of Varying Intravesicular pH on Na^+-H^+ Exchange by Ileal BBMV's

To further explore the findings of Fig. 2, we performed extensive studies to determine the effect of varying outwardly directed pH gradients on Na^+ uptake. Under these experimental conditions, the pH of the intravesicular compartment was varied from 5.2 to 7.5 while the incubation media pH was 7.5. Na^+ uptake was determined at 5 sec well within the linear line of uptake. Figure 3 depicts the effect of varying intravesicular pH on Na^+ uptake on the three groups of rats studied. At all age groups, decreasing the outwardly directed pH gradient resulted in a decrease in Na^+ uptake. The magnitude of the decrease was most marked in the adolescent rats. Moreover, Na^+ uptake under maximal pH gradient condition was 0.32 ± 0.01, 0.2 ± 0.01 and 0.095 ± 0.01 nmol/mg protein/5 sec in adolescent, weanling and suckling rats, respectively ($P < 0.001$ suckling *versus* weanling and adolescent rats). Under no pH gradient condition, Na^+ uptake was 0.07 ± 0.005 compared to 0.04 ± 0.002 nmol mg protein/5 sec, in suckling and adolescent rats, respectively ($P < 0.05$). These findings suggest that the activity of the Na^+-H^+ exchanger is greater in adolescent rats compared with suckling rats.

Effect of Varying Amiloride Concentrations on Na^+-H^+ Exchange by Ileal BBMV's

Figure 4 depicts the effect of varying concentrations of amiloride (10^{-5}–10^{-2} M) on 1 mM Na^+ uptake under outwardly directed pH gradient (pH_i/pH_o = 5.2/7.5) in all age groups. At 1 mM amiloride concentration, Na^+ uptake was inhibited by 80, 45 and 20% in adolescent, weanling and suckling rats, respectively. The K_i for amiloride, calculated by Dixon plot, was 0.1, 0.05 and 0.06 mM for adolescent, weanling and suckling rats, respectively. Moreover, the relationship between Na^+ uptake and amiloride concentration was linear at all age groups, suggesting a single amiloride-inhibitory site at all age groups. Linear regression analysis of amiloride concentration *versus* Na^+ uptake showed a linear relationship as expressed by the formulas $Y = 5.5\,x + 0.129$, cc = 0.82, $Y = 5.4\,x + 0.2$, cc = 0.8 and $Y = 3\,x + 2.7$, cc = 0.92 for suckling, weanling and adolescent rats, respectively.

Effect of Membrane Potential on Na^+ Uptake by Ileal BBMV's

To determine whether Na^+ uptake represents a pH gradient-driven Na^+ uptake or is secondary to an

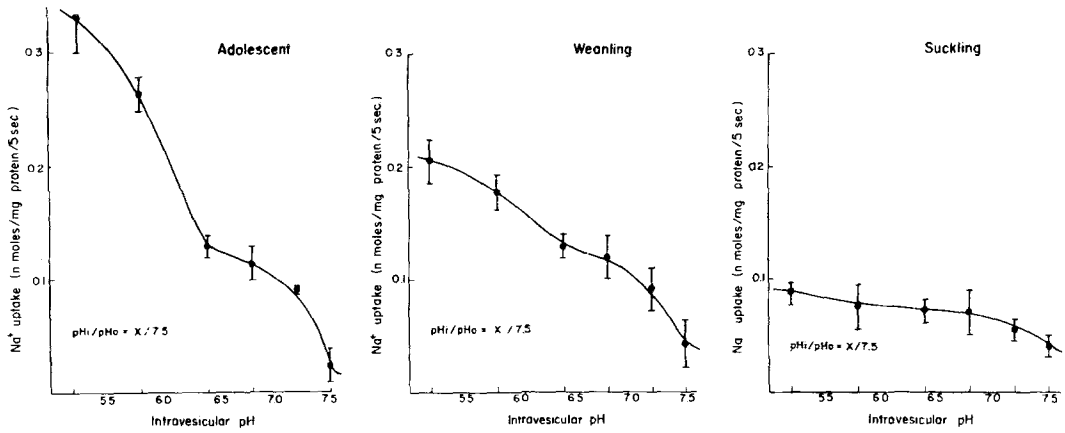

Fig. 3. Effect of varying intravesicular pH on Na^+-H^+ exchange by ileal BBMV's. BBMV's were prepared in 100 mM TMA gluconate and various combinations of Tris, HEPES and MES (130 mM total) to bring intravesicular pH from 5.2–7.5. The reaction was started by addition of 20 μl of vesicles to a media containing 100 mM TMA gluconate and HEPES/Tris buffer, pH 7.5. Each point represents three separate experiments on different membrane preparations

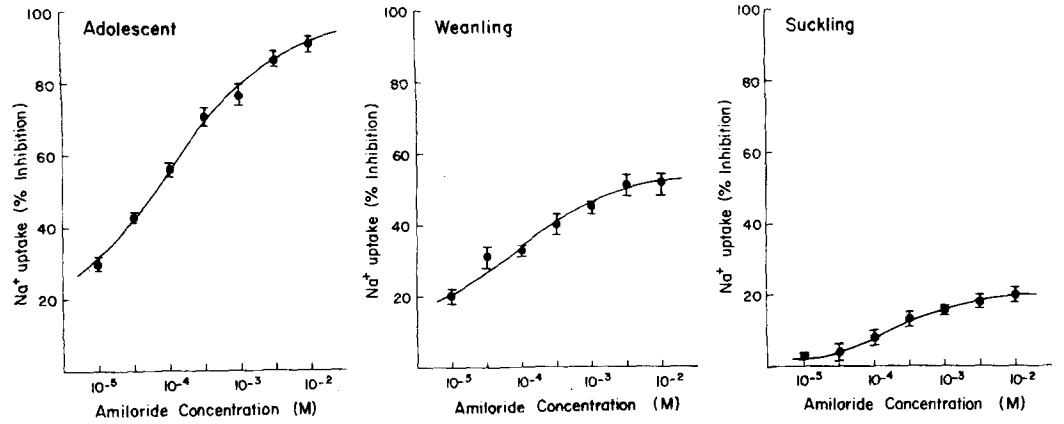

Fig. 4. Effect of varying concentrations of amiloride (10^{-5}–10^{-2} M) on Na^+-H^+ exchange by ileal BBMV's. BBMV's were prepared in 100 mM TMA gluconate, 40 mM HEPES, 90 mM MES buffer, pH 5.2. The reaction was started by the addition of 20 μl of vesicles to a media containing (in mM): 100 TMA gluconate, 85 HEPES, 45 Tris buffer, pH 7.5, 1.0 Na^+ gluconate, tracer ^{22}Na and various concentrations of amiloride (10^{-5}–10^{-2} M). Reaction was stopped at 5 sec. Data were expressed as percent inhibition of uptake without amiloride. Values are mean ± SE of three separate experiments on different membrane preparations

inside-negative electrical potential stimulating Na^+ uptake (due to H^+ diffusion), the effect of electrical potential on Na^+ uptake was determined. Vesicles were made inside-negative by loading the vesicle with 100 mM TMA gluconate, 40 mM HEPES, 90 mM MES buffer, pH 5.3. The vesicles were then added to a media containing (in mM) 100 TMA, 85 HEPES, 45 Tris, pH 7.5, 1 Na^+ gluconate with or without 80 μM FCCP (Kinsella & Aronson, 1980). As for glucose studies, the vesicles were added to a media containing (in mM) 100 NaCl, 85 HEPES, 45 Tris, pH 7.5, 0.1 D-glucose with or without 80 μM FCCP. Table 3 depicts that Na^+ uptake was not different with or without negative membrane potential induced by FCCP. In contrast, D-glucose uptake was significantly enhanced with negative membrane potential induced by FCCP in the presence of Na^+ gradient compared to Na^+ gradient alone. Similar results (*not shown*) were obtained using valinomycin-induced negative membrane potential. These findings suggest that Na^+ uptake is secondary to pH gradient conditions, rather than by a negative membrane potential.

Kinetics of Na^+-H^+ Exchange in Ileal BBMV's

The kinetics of Na^+ uptake were investigated at 5 sec in the setting of outwardly directed pH gradient

Table 3. Effect of membrane potential induced by FCCP on D-glucose uptake and on Na^+-H^+ exchanger

Time	D-Glucose uptake (nmol/mg protein)			
	−FCCP		+FCCP	
	Suckling	Adolescent	Suckling	Adolescent
20 sec	0.8 ± 0.1	1.2 ± 0.2	1.6 ± 0.2	2.8 ± 0.4
1 min	0.52 ± 0.04	0.8 ± 0.15	0.75 ± 0.1	1.4 ± 0.3
60 min	0.15 ± 0.03	0.17 ± 0.03	0.16 ± 0.64	0.17 ± 0.03
Time	Na^+ uptake (nmol/mg protein)			
	−FCCP		+FCCP	
	Suckling	Adolescent	Suckling	Adolescent
10 sec	0.21 ± 0.02	0.54 ± 0.04	0.22 ± 0.01	0.6 ± 0.06
1 min	0.32 ± 0.01	1.35 ± 0.12	0.31 ± 0.02	1.3 ± 0.1
180 min	0.41 ± 0.02	0.45 ± 0.03	0.4 ± 0.02	0.43 ± 0.02

Brush-border membranes were preincubated with 100 mM TMA gluconate, 40 mM HEPES, 90 mM MES buffer, pH 5.2. The reaction was started by the addition of 20 μl of vesicles to a media containing (in mM): 100 NaCl, 85 HEPES, 45 Tris, pH 7.5, 0.1 D-glucose with or without FCCP (for glucose studies), or to a media containing (in mM): 100 TMA gluconate, 85 HEPES, 45 Tris, pH 7.5, and 1 Na^+ gluconate, with or without 80 μM FCCP (for Na^+ studies). The reaction was stopped at desired time intervals using a cold stop solution containing (in mM): 185 K gluconate, 10 Tris, 16 HEPES and 0.1 amiloride (for Na^+ studies) and 100 NaCl, 100 mannitol, 20 HEPES/Tris and 0.2 phlorizin (for D-glucose studies).

(pH_i/pH_o = 5.2/7.5) using Na^+ concentration between 1–50 mM in the presence and absence of 1 mM amiloride. The amiloride-sensitive component (total uptake − uptake in the presence of 1 mM amiloride) was analyzed using a computerized model of Michaelis-Menten kinetics. The binding component, as determined from the nonzero intercept of the initial rate, was also substrated. Figure 5 depicts a plot of amiloride-sensitive Na^+ uptake *versus* Na^+ concentration in suckling, weanling and adolescent rats. V_{max} was significantly greater in adolescent and weanling rats compared with mean values of suckling rats ($P < 0.001$). K_m values were not significantly different. These results suggest the affinity of the Na^+-H^+ exchanger is similar at all age groups; however, the capacity of antiporter increases with advancing age.

Dissipation of ΔpH in Brush-Border Membranes of the Rats Studied

To determine whether membranes from the suckling, weanling and adolescent rats maintain similar

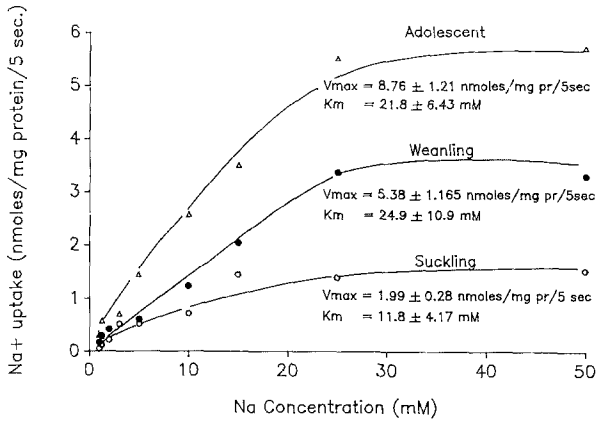

Fig. 5. Effect of age on kinetics of Na^+-H^+ exchanger by rat ileal brush-border membrane vesicles. BBMV's were prepared in 100 mM TMA gluconate, 40 mM HEPES, 90 mM MES buffer, pH 5.2. The reaction was started by the addition of 20 μl of vesicles to a media containing (in mM): 100 TMA gluconate, 85 HEPES, 45 Tris buffer, pH 7.5, and various concentrations of Na^+ gluconate (1–50 mM) and tracer ^{22}Na in the presence and absence of amiloride. TMA gluconate concentration was decreased with increasing Na^+ to maintain equal of molality across the membranes. Amiloride-sensitive component was plotted against Na^+ concentration. Reaction was stopped at 5 sec. Each point represents mean of three experiments on different membrane preparations. K_m and V_{max} values were analyzed using a computerized model of the Michaelis-Menten kinetics

pH gradient across their membranes, two studies were conducted. First, dissipation of ΔpH was studied using the fluorescence quenching of acridine orange as described previously (Kleinman et al., 1988). Brush-border membrane vesicles from suckling, weanling and adolescent rats were preincubated in 100 mM TMA gluconate, 50 mM K gluconate, 40 mM HEPES and 90 mM MES, pH 5.2. Vesicles were then diluted in a media containing (in mM): 100 TMA gluconate, 50 K gluconate, 85 HEPES and 45 Tris, pH 7.5 and 6 μM acridine orange. Under this setting, the acridine orange fluorescence quenching occurred with spontaneous dissipation of the pH gradient. Monensin 10^{-2} M totally collapsed the pH gradient. Figure 6, shows that the rate of dissipation of pH was similar in all age groups. Second, the effect of FCCP-induced negative membrane was studied on glucose uptake by suckling and adolescent rats. Table 2 depicts that negative membrane potential induced by FCCP resulted in twofold greater uptake compared to Na^+ gradient alone in both age groups, again indicating the maintenance of an H^+ gradient.

Discussion

Mammalian small intestine undergoes morphological, biochemical and functional changes during mat-

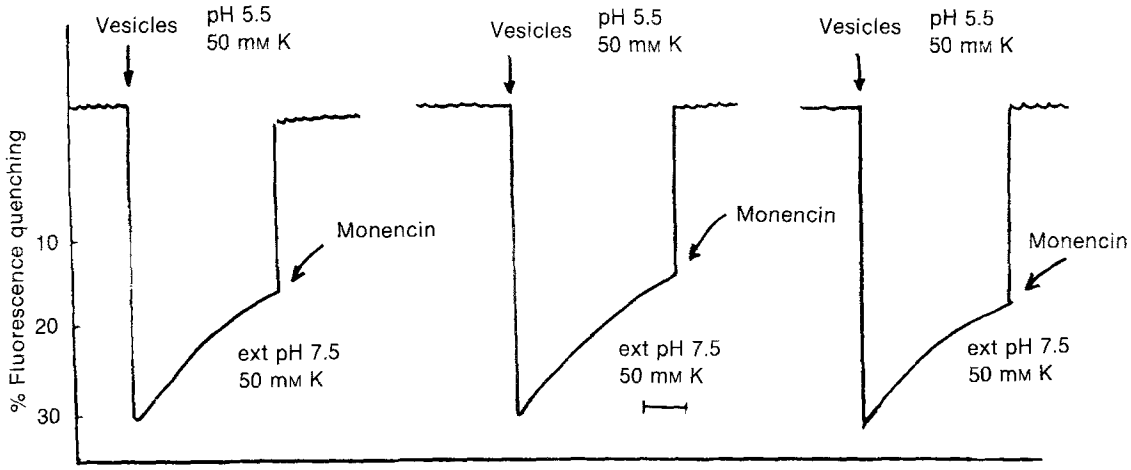

Fig. 6. Effect of age on dissipation of pH gradient. Membrane vesicles from adolescent, weanling and suckling rats were preincubated in 100 mM TMA gluconate, 50 mM K gluconate, 40 mM HEPES and 90 mM MES, pH 5.2. Vesicles were then diluted in a media containing (in mM): 100 TMA gluconate, 50 K gluconate, 85 HEPES and 45 Tris, pH 7.5, and 6 acridine orange. Changes in fluorescence quenching were monitored at room temperature using an amino spectroflurometer

uration. The mechanisms underlying these changes include genetic, hormonal and environmental factors. The functional studies in general lagged behind the morphological and biochemical changes. The available studies on ion transport during maturation are quite limited (Grand, Watkins & Torti, 1976). The intestinal mucosa of newborn and suckling rats has a very slow rate of cellular proliferation and migration as compared to adult animals (Koldovsky et al., 1966; Herbst & Sunshine, 1969). Therefore, it was suggested that this slow rate of cellular proliferation and migration could be a contributing factor in the pathogenesis of electrolyte imbalances and intestinal damage repair resulting from infant diarrheal states. Recent studies suggest a link between Na^+-H^+ exchanger and cell growth (L'Allemain et al., 1984). The present study describes for the first time the postnatal development of this novel and unique transport system, Na^+-H^+ exchanger, in ileal brush-border membranes. We used well-validated ileal BBMV's from animals during maturation to demonstrate the differences of ion transport in the animals during maturation. The purity of the ileal membranes was validated by morphological biochemical and functional criteria (Barnard, Ghishan & Wilson, 1985; Barnard & Ghishan, 1986). Brush-border enzyme markers were enriched several-fold over mucosal homogenate, however, the activity of a basolateral and subcellular organelles enzyme markers were all impoverished. D-glucose uptake studies showed a clear "overshoot" which is a well characteristic phenomena of brush-border membranes (Table 2). To determine whether sodium uptake into brush-border membrane vesicles from all age groups is into osmotically active space, vesicles were incubated in media with increasing osmolality and Na^+ uptake was determined at equilibrium. As seen in Fig. 1, binding component depicted by the intercept indicates a small binding component. Therefore, our studies provide evidence that BBMV's are suitable for the study of sodium transport processes in the rat during maturation.

Beck (1985) using BBMV's from the fetal rabbit kidney have demonstrated the presence of immaturity in the Na^+-H^+ exchange activity. However, at the time of six weeks after birth, there was an accumulation of Na^+ above the equilibrium value and 87% of the initial rate was amiloride sensitive (Beck, 1985). Our studies in suckling rat depicted the presence of immaturity in the Na^+-H^+ exchange system in the rat small intestine. Under the outwardly directed pH gradient condition, Na^+ uptake showed a 1.5-fold blunt "overshoot" with time and only 20% of the initial rate uptake was amiloride sensitive in the BBMV's of suckling rat. Whereas, in the adult rat, Na^+ uptake demonstrated over threefold clear "overshoot" phenomena and 80% of the initial uptake rate was amiloride sensitive. Furthermore, as seen in Fig. 2, the initial uptake rate at the varying outwardly directed pH gradient were always higher in the adolescent BBMV's especially at the gradient pH_i/pH_o = 6.5/7.5 or more. These data suggested that the response of the Na^+-H^+ exchanger to the driving forces made by pH gradient is more sensitive in adolescent rat small intestine as compared to the suckling rat small intestine.

The kinetics of the transport system were done at 5 sec, at a time when the transport is linear. The kinetic parameters were obtained by subtracting the

uptake of the amiloride-nonsensitive component and the binding component from the total Na^+ uptake under outwardly directed pH gradient. Values of V_{max} and K_m were obtained using a computerized model of the Michaelis-Menten kinetics (Vaughn, Neal & Anderson, 1976). As seen in Fig. 5, V_{max} was significantly greater in adolescent and weanling rats compared with mean values of suckling rats ($P < 0.001$). These kinetic parameters were quite similar to those of rabbit ileal BBMV's (Knickelbein et al., 1983), rabbit renal BBMV's (Warnock, Reenstra & Yee, 1982). These results suggest the affinity of the Na^+-H^+ exchanger is similar at all age groups, however, the capacity of antiporter increases with advancing age.

To determine whether the changes in the kinetic parameters are due to faster dissipation of pH gradient across the membranes of rats at different ages, acridine orange studies and FCCP-induced negative membrane potential studies were performed. Both studies indicate that the rate of pH dissipation and generation of negative membrane potential was similar in the age groups studied. Therefore, the change in V_{max} reflects a change in the activity of the transporter. Whether this change is secondary to increase in the number of transporter or represents an increase in the turnover rate of the transporter cannot be determined from these studies.

In the proximal tubular BBMV's of kidney, an electroneutral transport process of Na^+ has been revealed (Kinsella & Aronson, 1980). The importance of a membrane potential on Na^+ transport in rat small intestine is somewhat controversial. Some investigators suggest an electroneutral process (Knickelbein et al.,1983), while others suggest the presence of an electrogenic component for Na^+ transport (Ramaswamy, Harig & Kleinman, 1987). We created an "inside" diffusion potential using valinomycin, but no stimulation of Na^+ uptake occurred either when uptake was compared with a control incubation without valinomycin or a control incubation under voltage clamped condition. Our results, therefore, support the concept of an electroneutral transport process in all three age groups. Savolic and Burckhardt (1984) demonstrated that the rate of Na^+-H^+ exchange as well as the conductances for various ions in the isolated renal BBMV's depend on membrane preparation. The rates of ΔpH dissipation with K^+ gradients ($\pm$valinomycin) were higher by 50 to 150% in membranes prepared with $CaCl_2$ than in membranes isolated with $MgCl_2$, indicating much higher H^+ and K^+ conductances in membranes obtained with $CaCl_2$. This difference between the two preparation methods might be one possibility to explain the different results regarding the electrogenesis of Na^+ transport in intestinal BBMV's of rat small intestine.

In summary, the data reported in the present paper, provide direct evidence for localization of a Na^+-H^+ exchange mechanism on the brush-border membrane of rat ileum during maturation. The characteristic of the Na^+-H^+ exchanger are: (*i*) outwardly directed hydrogen gradients stimulated sodium uptake, which cannot be explained by the generation of a diffusion potential because sodium uptake was not potential sensitive; (*ii*) amiloride inhibited the pH gradient-stimulated sodium uptake; (*iii*) saturation kinetics were similar to the finding observed with Na^+-H^+ exchangers from other epithelia; (*iv*) magnitude of pH gradient-stimulated sodium uptake was greater in adolescent and weanling rat brush-border membrane vesicles compared with suckling rat brush-border membrane vesicles; (*v*) amiloride-sensitive Na^+ uptake was much greater in adult rat ileum than in weanling and suckling ileum; (*vi*) at all age groups studied, the affinity of the Na^+-H^+ exchanger was similar; however, the capacity of antiporter increased with advancing age. This increase in Na^+-H^+ activity, with age, is not related to differences in rate of dissipation of pH gradient across the different membrane preparation.

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