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Na^+-H^+ exchange activity in brush-border membrane vesicles isolated from chick small intestine

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Abstract

This study was undertaken to investigate the presence of a Na^+-H^+ antiporter in brush-border membrane vesicles (BBMV) isolated from chick small intestine. An outwardly directed proton gradient (pH 5.5 inside, 7.5 outside) stimulated Na^+ uptake into BBMV and resulted in a transient accumulation. No accumulation was observed in the absence of a proton gradient. Voltage clamping the membrane with K^+ and valinomycin decreased the Na^+ overshoot. Amiloride inhibited pH gradient-driven Na^+ uptake in a dose-dependent manner with an IC_{50} of 44 μM. The relationship between pH gradient-driven Na^+ uptake and external Na^+ concentration followed simple, saturating Michaelis-Menten kinetics. Eadie-Hofstee analysis of the pH gradient-driven Na^+ uptake indicated a single transport system with a V_{max} of 33 nmol/mg protein per 15 s and a K_m for Na^+ of 12 mM. The initial rate of pH-driven Na^+ uptake increased as the intravesicular pH decreased, with a Hill coefficient close to 1. These findings indicate that BBMV isolated from chicken small intestine posses a Na^+-H^+ exchanger. This exchanger does not appear to be the one involved in cell pH regulation.

Keywords: BBMV; Sodium ion–proton exchange; Kinetics

1. Introduction

The Na^+-H^+ exchanger is an ubiquitous membrane transport protein that has been implicated in diverse physiological functions including the control of cell volume and pH [1–4].

We have reported [5] that chicken enterocytes present a Na^+-H^+ exchanger that controls resting pH_i and helps to recover pH_i from an acid load. Isolated enterocytes lose their polarity and Na^+-H^+ exchanger is present in the apical and basolateral membrane domains of intestinal epithelial cells (see [3] for a recent review). Several lines of evidence suggest that the apical and basolateral Na^+-H^+ exchanger are two distinct forms with different cellular functions. The basolateral exchanger may represent a 'house-keeping' antiporter used for cell pH regulation and the apical may be involved in the transepithelial transport of salt and fluid (see [3] for a recent review).

In this study we have investigated the presence of the Na^+-H^+ exchanger in brush-border membrane vesicles (BBMV) isolated from chicken small intestine.

2. Materials and methods

2.1. Brush-border membrane vesicles preparation

BBMV were isolated from the small intestine of 4- to 6-week-old Hubbard chickens, by double Mg^{2+} precipitation method as described in Ref. [6]. Purification of the BBMV preparation was assessed as previously described [6]. Unless otherwise stated the BBMV were loaded with a pH 5.5 buffer consisting in 300 mM mannitol, 50 mM potassium gluconate and 20 mM Mes-Tris.

2.2. $^{22}Na^+$ uptake studies

$^{22}Na^+$ uptake was measured at 25° C by a rapid filtration technique as described by Cano et al. [6] for Cl^- uptake measurements. Except where indicated otherwise, the uptake buffer consisted in 300 mM mannitol, 50 mM potassium gluconate, 0.1 mM sodium gluconate, tracers of $^{22}Na^+$ and either 20 Mes-Tris (pH 5.5) or 20 mM Hepes-Tris (pH 7.5). The amount of protein in the assay tube ranged from 100 to 150 μg/100 μl of uptake buffer.

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2.3. Materials

$^{22}Na^+$ (carrier free) was purchased from Amersham. Valinomycin, amiloride and all the salts used in the current study were obtained from Sigma, St. Louis, MO.

2.4. Statistical analysis

Individual experiments were carried out in triplicate. Data are presented as mean ± S.E. In the figures vertical bars, that represent the S.E., are absent when they are less than symbol height. Comparison between different experimental groups was evaluated by the two-tailed Student's *t*-test.

3. Results and discussion

3.1. Binding vs. transport of Na^+

Nonspecific binding of a solute to the vesicle surface would lead to an overestimate of transport into the vesicle. Na^+ uptake at equilibrium (1 h) was decreased with increasing medium osmolarity (Fig. 1), indicating that Na^+ was taken into a closed intravesicular space. The relationship between the uptake and the reciprocal of osmolarity was linear and extrapolation of the line to infinite osmolarity (where intravesicular volume is zero) reveals that binding represents 55% of uptake at 440 mosmol/l. Binding was not affected by the presence or absence of a transmembrane pH gradient

3.2. Na^+ uptake into BBMV

The following observations are consistent with the presence of a tightly coupled electroneutral Na^+-H^+ exchanger at the brush-border membrane of chicken enterocytes and rule out an electrically coupled diffusion:

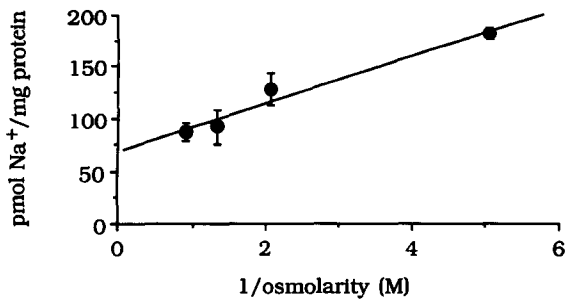

Fig. 1. Effect of external osmolarity on sodium uptake into BBMV isolated from chicken small intestine. Medium osmolarity was increased by addition of either sorbitol or mannitol. The composition of the intra- and extravesicular buffers are given in Materials and methods. Uptake of 0.1 mM Na^+ was measured during 1 h. Line was calculated by linear regression analysis, $y = 64.4 + 22.86x$, $r = 0.965$. Each point represents the mean value ± S.E. of triplicate assays using five different vesicle preparations.

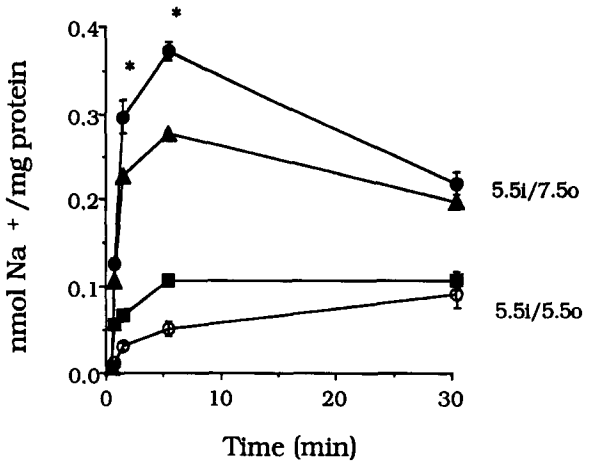

Fig. 2. Time-course of Na^+ uptake by intestinal BBMV. Na^+ uptake was measured in the presence (●, ▲, ■) and absence (○) of a proton gradient. (▲), 45 μM valinomycin or (■), 45 μM valinomycin and 1 mM amiloride were present in uptake buffer, pH 7.5. Other details as in Fig. 1. Each point represents the mean value ± S.E. of triplicate assays using six separate membrane preparations. * $P < 0.001$ as compared with the values obtained with either valinomycin or amiloride.

(i) In the presence of a proton gradient (pH 5.5 inside/pH 7.5 outside) Na^+ uptake overshoots the final steady-state equilibrium value by a factor of approx. 4. No overshoot was observed in the absence of a pH gradient (Fig. 2).

(ii) As Na^+ uptake might be stimulated by an accumulation of negative charges inside the vesicles as the pH gradient dissipates, the measurements were repeated with the voltage across the membrane brought to zero by equal internal and external K^+ concentrations in the presence of valinomycin. These voltage-clamped conditions caused a decrease in pH gradient-dependent Na^+ uptake after 1 min incubation (Fig. 2). Electrodiffusional coupling was thus excluded as the cause for the remaining pH gradient-driven Na^+ uptake. The rest of the experiments in this paper were carried out under voltage-clamped conditions.

(iii) pH-driven, membrane voltage-independent Na^+ uptake was inhibited by amiloride (Fig 2). The log-dose response curve of amiloride inhibition of Na^+ uptake in the presence of a pH gradient (5.5 inside, 7.5 outside) is given in Fig. 3. Apparent half maximal inhibition (IC_{50}) was achieved at 44 μM amiloride under the conditions tested (Fig. 3B). The IC_{50} value for amiloride inhibition is comparable to that reported for the Na^+-H^+ exchanger located in the apical membrane of other epithelial cell types and much higher than the values reported for the basolateral Na^+-H^+ isoform [3]. In the presence of 2.5 mM amiloride Na^+ uptake was equal to that observed in the absence of proton gradient.

3.3. Kinetics study of the Na^+-H^+ exchanger

Na^+-uptake into BBMV was measured, in the presence and absence of an outwardly directed transmembrane pH

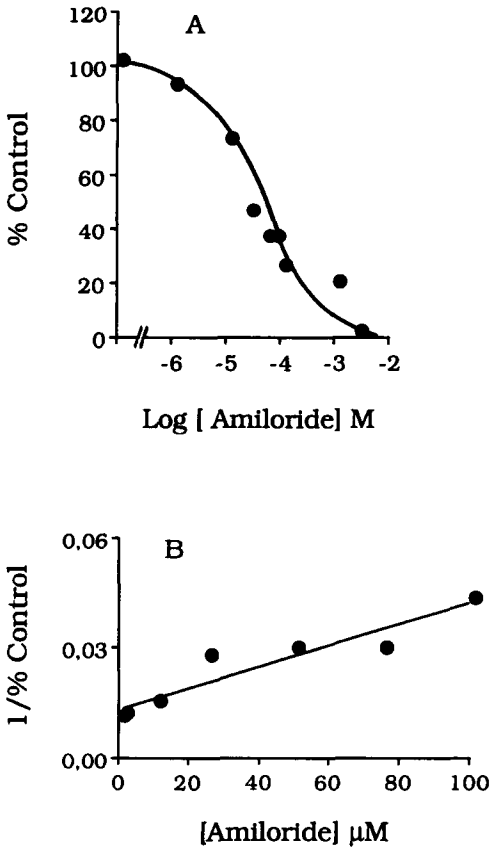

Fig. 3. Effect of amiloride on Na^+ uptake by intestinal BBMV. (A) Uptake of Na^+ was measured during 15 s in the presence and absence of a pH gradient. Uptake buffer, pH 7.5, contained 45 μM valinomycin and increasing concentrations of amiloride. (B) Dixon plot of the amiloride dose–response curve. These data were obtained by subtracting pH-independent Na^+ uptake from Na^+ uptake measured in the presence of a pH gradient. IC_{50} of amiloride was calculated using linear regression analysis ($y = 0.0123 - (2.8 \cdot 10^{-4})x$, $r = 0.90$). Each point represents the mean value ± S.E. of triplicate assays using seven separate membrane vesicle preparations.

gradient, at different external Na^+ concentrations. In the presence of a pH gradient Na^+ uptake plotted against its concentration shows an inflexion at low Na^+ concentration and it becomes a straight line at higher concentrations, not yielding a saturation curve (Fig. 4A). In the absence of a pH gradient Na^+ uptake showed a linear relationship with its extravesicular concentration. The difference between total Na^+ uptake and that observed in the absence of pH gradient follows first-order kinetics. Kinetic analysis with an Eadie-Hofstee plot (Fig. 4B) yielded a linear relationship, consistent with the existence of a single Na^+-H^+ exchanger. The apparent transport constants, K_m, and maximal rates of transport for Na^+, V_{max}, were 12 mM and 33 nmol/mg protein per 15 s, respectively. The apparent K_m for external Na^+ is similar to that reported for the apical Na^+-H^+ antiporter of rat colon [7] and renal [8] epithelial cells, and lower than that reported for rabbit ileum [9].

3.4. Na^+-H^+ activity and intravesicular pH

In a variety of cell types the Na^+-H^+ antiporter is virtually silent near physiological pH_i, but becomes activated below a certain threshold pH_i and the rate of exchange increases steeply with increasing pH_i (see [3] and [4] for reviews). So far an exception is the apical Na^+-H^+ antiporter of rat colon which is not modulated by pH_i [7]. The sensitivity of the apical exchanger of chicken small intestine to pH was investigated by measuring Na^+ uptake into BBMV with the intravesicular pH preset to various values, and at two different external pH (pH_o) 5.5 and 8. At pH_o 8 Na^+ uptake was stimulated by decreasing internal pH (Fig. 5A). The increase was completely inhibited at pH_o 5.5. The Hill plot of the relationship between

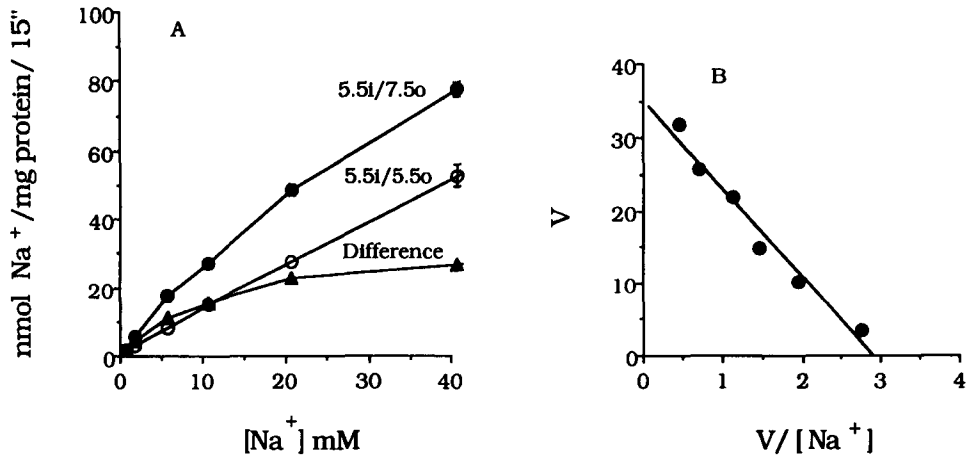

Fig. 4. Effect of increasing concentrations of external Na^+ on the initial rate of Na^+ uptake (15 s). Uptake buffer contained increasing concentrations of sodium gluconate isoosmotically substituted with mannitol. (A) Uptake in the presence and absence of proton gradient. Difference: total uptake minus that in the absence of a pH gradient. (B) Eadie-Hofstee plot of the difference data. Kinetic parameters (i.e., the apparent K_m and V_{max}) of the Na^+-H^+ exchanger were calculated using linear regression analysis ($y = 33.5 - 12.17x$, $r = 0.97$). Each point represents the mean value ± S.E. of triplicate assays using seven independent membrane vesicle preparations.

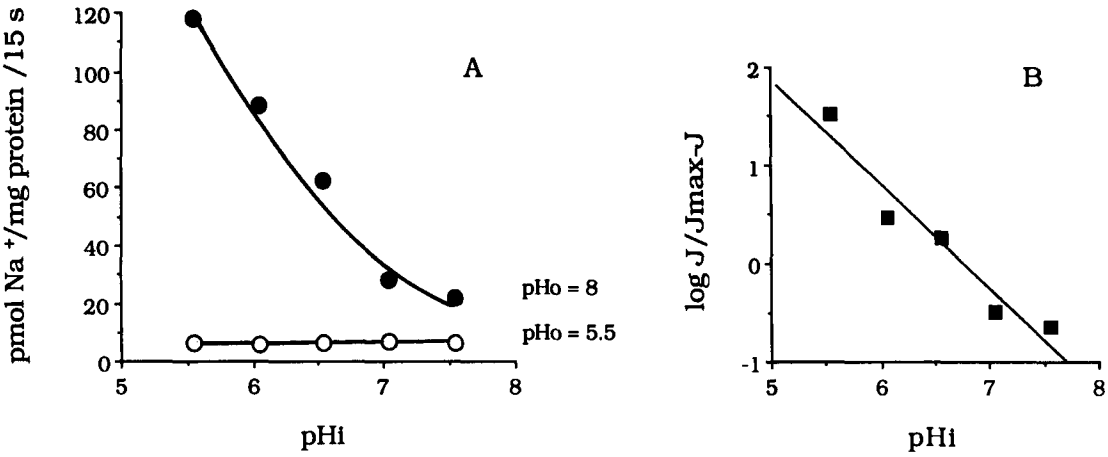

Fig. 5. Effect of intravesicular pH on Na^+ uptake. (A) Vesicles were loaded with 300 mM mannitol, 50 mM potassium gluconate and 20 mM of either Hepes or Mes buffered with Tris to the indicated intravesicular pH. 15 s uptake of 0.1 mM Na^+ was assayed in the presence of 300 mM mannitol, 50 mM potassium gluconate, tracers of $^{22}Na^+$ and either 20 Mes-Tris (pH_o 5.5, ○) or 20 mM Hepes-Tris (pH_o 8, ●). Each point represents the mean value ± S.E. of triplicate assays using three independent membrane vesicle preparations. Influx at pH_o 5.5 was subtracted from values at pH_o 8 and the Hill plot of data is given in B. J_{max} was calculated from the Lineweaver-Burk plot of the data. Line was calculated by linear regression analysis, $y = 7.03 - 1.06x$, $r = 0.93$.

pH-driven Na^+ uptake and intravesicular pH (Fig. 5B) gives an interaction coefficient (n) of 1 and a $[H^+]_{0.5}$ of 221 nM. This indicates that the apical Na^+-H^+ antiporter is not allosterically regulated by cytosolic proton. We have previously reported [5] that the activity of the Na^+-H^+ antiporter involved in pH_i regulation in chicken enterocytes is allosterically regulated by cytosolic protons.

The present results suggest that, as in other epithelial cells, chicken enterocytes may have at least two types of Na^+-H^+ antiporters with different sensitivity to amiloride and to regulation by protons, and with different functions.

Acknowledgements

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