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Characterization of basolateral membrane Na/H antiport in rat jejunum

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Na uptake studies were performed in order to examine the activity of a Na/H exchanger in basolateral membrane vesicles isolated from rat jejunum. Experiments were carried out under voltage-clamped conditions in order to avoid electrodiffusional ionic movements. 1 mM Na uptake was found to be enhanced by an outward proton gradient and its initial rate was further increased by the presence of monensin or nigericin. The pH gradient-driven Na uptake was inhibited by 2 mM amiloride and unaffected by 0.1 mM 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid. The initial rate of the proton gradient-induced Na uptake was saturable with respect to external Na, with a K_m of 13.6 ± 1.4 mM and a V_{max} of 35.4 ± 2.2 nmol/mg protein per min. Li competed with Na for the exchange process, whereas K, Rb, Cs, tetramethylammonium had no effect. We conclude that rat jejunal basolateral membrane contains a Na/H exchanger whose properties are similar to those of the antiporter identified in the brush-border membrane.

Introduction

The plasma membrane Na/H exchanger seems to be present in most cell types and it appears to play a key role in a number of diverse cellular functions, such as the regulation of intracellular pH, the control of cell volume and the proliferation in response to growth factors and mitogens [1–3] The antiporter activity is inhibited by the potassium-sparing diuretic amiloride and its analogs, which seem to compete with Na for its binding site [2,4] The exchange mechanism is energetically driven by the Na gradient across the membrane and, thus, is indirectly fueled by the Na/K pump, which ensures the maintenance of the Na gradient

The Na/H antiporter in intestinal and kidney epithelial cell brush-border membranes has been well documented since 1976 [5], recently, the existence of the exchanger was also assessed in the basolateral membrane of many epithelial tissues [6–20] In a previous paper, we provided evidence for the presence of the Na/H antiporter at the basolateral pole of rat enterocyte [21], in this current study, we have further characterized the antiport in order to check whether this mechanism shares some features with that present in the brush border, or whether it has different properties corresponding to the functional polarization of the enterocyte plasma membrane

Materials and Methods

Basolateral membrane separation

Basolateral plasma membranes from rat jejunum enterocytes were isolated and purified as described [22]. Briefly, 5 mM $MgCl_2$, which preferentially aggregates all membranes except the brush-border membrane, was added to basolateral membranes collected by self-orienting Percoll-gradient centrifugation (Kontron, Centrikon mod T2070 ultracentrifuge, Haake-Buchler, Auto Densi-Flow II C apparatus) Mg was used instead of Ca, since proton conductance can possibly be increased by Ca [23] To ensure that the intravesicular space was loaded with the appropriate buffer, the last centrifugation was run at 20 °C The collected pellets (3–7 mg protein/ml) were then incubated in the same buffer at room temperature for 30 min, equilibrated with 0 2 mM EDTA and subsequently used for Na uptake by the rapid micro-filtration technique

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Abbreviation DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid

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To control the purity of the basolateral membrane fraction, total protein, γ-glutamyltransferase (γ-GT, a marker enzyme for brush-border membrane), cytochrome-*c* oxidase (a marker enzyme for mitochondria) and Na/K-ATPase (a marker enzyme for basolateral membrane) were determined as published [22] To exclude rigorously mitochondrial contamination, succinic dehydrogenase activity was also assayed, according to the method of Pennington [24] with modifications following Porteous and Clark [25]

Sodium uptake

Uptake studies, carried out under voltage-clamped conditions at 28° C, were started by diluting the vesicle suspension into an incubation medium containing trace amounts of ^{22}Na and unlabelled NaCl The composition of the final resuspension solution and incubation media is given in the figure legends Samples were diluted with 0 8 ml ice-cold reaction-stopping solution (135 mM KCl, 20 mM Hepes-Tris buffer, pH 7 5), filtered on wet cellulose nitrate filters (0 45 μm pore size) and immediately rinsed with 5 ml of the stop solution The radioactivity of the filters was counted by liquid scintillation spectrometry (Tri-Carb, Packard, mod 300) All the solutions used were prefiltered through 0 22 μm pore size filters and their osmolarity was checked using an osmometer (Halbmikro, Knauer)

Individual uptake experiments performed in triplicate, representative of more than three repetitions with qualitatively identical results, are presented throughout the paper In view of the significant variations in intravesicular space between preparations, the effects of all tested substances were always checked with a single basolateral membrane preparation

D-Glucose uptake

60 μl vesicles obtained in 250 mM sorbitol were incubated in 180 μl of 65 mM sorbitol, 0 1 mM D-glucose plus trace amounts of D-[^{14}C]glucose and either 100 mM NaCl or 100 mM KCl All solutions contained 10 mM Hepes-Tris buffer (pH 7 5), 0 2 mM phenylmethylsulfonyl fluoride (PMSF) and 0 11% (v/v) ethanol At the selected times (10 s, 1 min, 2 min and 60 min) samples were taken and processed as described above

Materials and Statistics

^{22}NaCl (0 2 Ci mg^{-1} Na) was from Amersham International (Amersham, U K.) D-[^{14}C(U)]Glucose (329 mCi · mmol^{-1}) was from Du Pont De Nemours (F R G) Valinomycin, monensin, nigericin, amiloride and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) were purchased from Sigma (St Louis, MO, U S.A) Percoll was from Pharmacia Fine Chemicals (Uppsala, Sweden) All other chemicals were of reagent grade

All data are presented as means ± S E , statistical significance was determined by the Student's *t*-test

Results and Discussion

It is well known that the Na/H exchanger is present in the brush border of enterocyte [5] and in mitochondrial membranes [26] Therefore, in order to characterize the Na/H antiport in the basolateral membrane, a particularly well purified fraction is needed In our preparation the enrichment factor for the specific activity of the basolateral membrane marker enzyme, Na/K-ATPase, was 12 4 + 0 8 (n = 16) with respect to the starting homogenate, whereas the enrichment factor for γ-glutamyltransferase, brush-border marker enzyme, was 0.5 ± 0 07 and that for cytochrome-*c* oxidase, marker enzyme for mitochondria, was 0 4 ± 0 06 Furthermore, the specific activity of succinic dehydrogenase was significantly reduced in the basolateral membrane preparation as compared with the crude homogenate, yielding an enrichment factor of 0 35 ± 0 04 and, thus, excluding mitochondrial contamination

To rule out the possibility that our results were affected by the presence of brush-border membrane vesicles, the ability of our preparation to accumulate D-glucose against a concentration gradient was tested the sodium–glucose cotransport system is known to be localized in the apical membrane of the enterocyte [27] D-Glucose uptake did not increase when a Na gradient, instead of a K gradient, was imposed across the membrane vesicles (data not reported) The lack of effect suggests the absence of apical contamination of the basolateral membranes

In a previous study [21], we reported that, in the presence of external KCl, nonspecific binding of Na to the membranes is minimal compared with transport Fig 1 depicts the effect of a pH gradient on Na uptake An outwardly directed proton gradient (pH_i = 5 5, pH_o = 7 5) enhances Na uptake over the equilibrium value, there is no stimulation of Na uptake when no Δ pH is present ($pH_i = pH_o$ = 5 5) To exclude electrodiffusional coupling between Na and H movements, Na uptake studies were performed in vesicles voltage-clamped with equal internal and external K concentrations and valinomycin The effects of nigericin and monensin on Na uptake in the presence of Δ pH are also reported in Fig 1 Monensin and nigericin are ionophores that facilitate electroneutral Na/H exchange across the membrane The results of this experiment show that monensin and nigericin stimulate the initial rate of Na uptake (uptake values at 15 s and at 1 min of incubation in the presence of the ionophores are statistically different from those obtained under other conditions), whereas the equilibrium value remains unaffected Moreover monensin, which is more specific for Na, causes the strongest enhancement of 15 s uptake

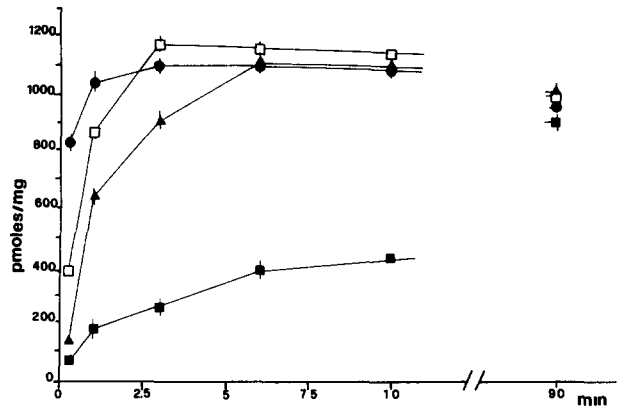

Fig 1 1 mM Na uptake in basolateral membrane vesicles 60 μl vesicles obtained in 100 mM Mes-Tris buffer (pH 5 5) and pre-equilibrated with 25 μM valinomycin, were incubated either in 180 μl of 100 mM Hepes-Tris buffer (pH 7 5) (▲) added with 10 μM monensin (●) or 10 μM nigericin (□), or in 180 μl of 100 mM Mes-Tris buffer (pH 5 5) (■) All solutions contained 80 mM potassium gluconate, 0 2 mM PMSF and 0 11% (v/v) ethanol Ordinate Na uptake, mean values ± S E (= vertical bars, absent if less than symbol height) Abscissa incubation time

To confirm that the pH stimulation of Na uptake is due to a Na/H exchanger, the effects of amiloride and DIDS on proton gradient-dependent Na uptake were tested (Fig 2) 2 mM amiloride blocks the enhanced Na uptake, in agreement with previous data showing complete inhibition [21] 0 1 mM DIDS had no effect on ΔpH-dependent Na uptake this result argues against the presence of Na–OH (HCO_3) cotransport in the basolateral membrane of rat enterocyte, as already suggested by Hagenbuch et al [28]

Fig 3 shows the effect of varying the Na concentration on the initial rate of Na uptake in voltage-clamped

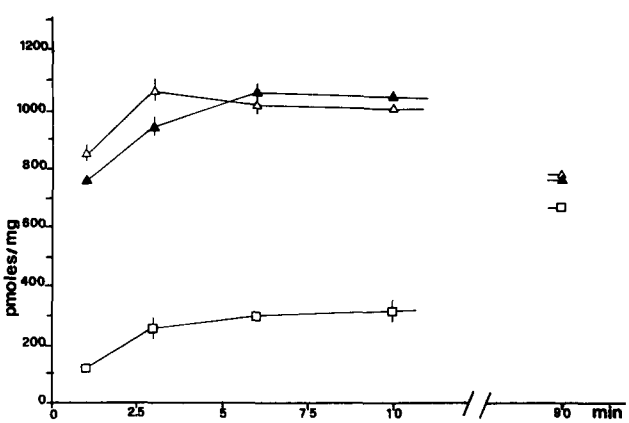

Fig 2 1 mM Na uptake in basolateral membrane vesicles 60 μl vesicles obtained in 100 mM Mes-KOH buffer (pH 5 5) were incubated in 180 μl of 100 mM Hepes-KOH buffer (pH 7 5) (▲), added with 0 1 mM DIDS (△), or 2 mM amiloride (□) All solutions contained 150 mM sorbitol, 0 2 mM PMSF and 0 01% (v/v) ethanol Since DIDS formed a precipitate when added to the solution containing amiloride, studies using amiloride and DIDS were performed separately Ordinate Na uptake, mean values ± S E (= vertical bars, absent if less than symbol height) Abscissa incubation time

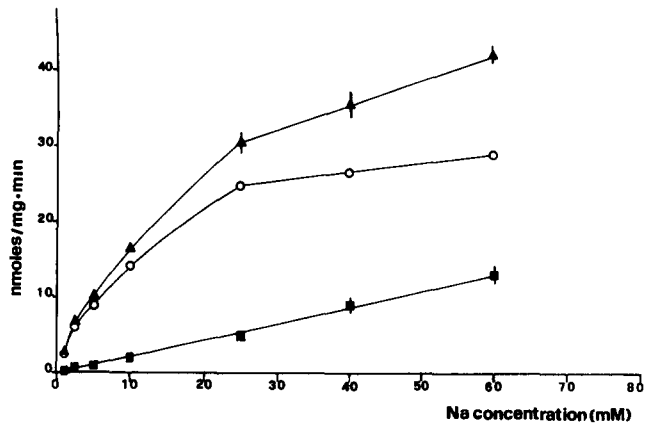

Fig 3 Effect of increasing concentrations of external Na on the initial rate of Na uptake (1 min incubation) 10 μl vesicles obtained in 113 mM sorbitol, 100 mM Mes-Tris buffer (pH 5 5) and pre-equilibrated with 25 μM valinomycin, were incubated in 30 μl of 100 mM Hepes-Tris buffer (pH 7 5) (▲) or in 30 μl of 100 mM Mes-Tris buffer (pH 5 5) (■) Incubating solution contained increasing concentrations of NaCl isoosmotically substituted with sorbitol All solutions contained 30 mM potassium gluconate, 0 2 mM PMSF and 0 11% (v/v) ethanol The difference between the two curves (○) represents the carrier-mediated saturable Na uptake Ordinate Na uptake, mean values ± S E (= vertical bars, absent if less than symbol height) Abscissa Na concentration, mM

vesicles (1 min incubation), in the presence or in the absence of an outward proton gradient The difference between the two curves evidences a saturable process Kinetic analysis using an Eadie-Hofstee plot (Fig 4) indicates that the data are well fit by the Michaelis-Menten equation ($r = 0\ 97$) and, thus, are consistent with the existence of a single pH gradient-dependent Na transport system The K_m for Na is 13 60 ± 1 39 mM and the V_{max} is 35 42 ± 2 23 nmol/mg protein per min

The ability of various monovalent cations to compete with Na for the uptake process was investigated The data of Table I show that Li can substitute for Na in the exchange process, whereas the other cations tested have less affinity NH_4, which can also be a substrate

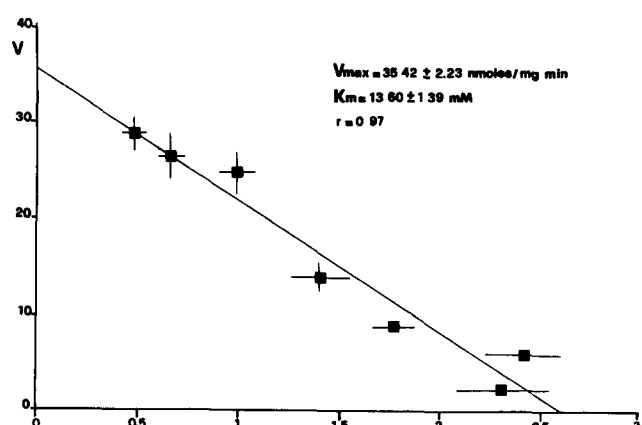

Fig 4 Eadie-Hofstee plot of data (○) shown in Fig 3

TABLE I

Percent cation inhibition of 1 mM Na uptake

10 μl vesicles obtained in 135 mM sorbitol, 100 mM Mes-Tris buffer (pH 5 5) were incubated for 1 min in 30 μl of 100 mM Hepes-Tris buffer (pH 7 5) added with 56–58 mM inhibitory cations (according to their osmotic coefficient, chloride salts) All solutions contained 30 mM KSCN, 0 2 mM PMSF and 0 01% (v/v) ethanol

Cation	% Inhibition
Na	80 5±3 5
Li	58 0±5 0
K	22 0±2 0
Rb	19 5±4 2
Cs	21 2±4 1
TMA	4 0±0 1

for the exchanger in other membranes [2], was not tested, as under our experimental conditions the inhibition of Na uptake could be attributed to the ability of an inward NH_4 gradient to dissipate an inside-acid ΔpH (NH_3 diffusion) without invoking direct interaction of NH_4 with the exchanger

Our study further demonstrates the presence of a Na/H exchange mechanism in the basolateral membrane of rat enterocyte and provides more insight on the principal features of this antiport, as summarized below

(i) An outwardly directed proton gradient increases Na uptake

(ii) Na/H exchange does not result from electrodiffusional coupling, but from a directly coupled flux

(iii) The effect of nigericin and monensin is consistent with an artificially increased number of functioning exchangers in the membranes

(iv) The Na/H antiporter is blocked by the diuretic drug amiloride and is insensitive to the stilbene derivative DIDS

(v) pH gradient-dependent Na uptake exhibits saturable kinetics, with an affinity value similar to those generally reported brush-border membranes from kidney and intestine show K_m values ranging from 4 5 to 30 mM [23,29–37], basolateral membranes from intestine, kidney, parotid and liver show K_m values ranging from 5 4 to 18 mM [9–11,13,17]

(vi) Cation specificity agrees well with values reported for other membranes, since only Li appears to have affinity for the transporter [1,2,26]

In conclusion, these results provide evidence that, in rat jejunum, the general characteristics of the basolateral membrane and brush-border Na/H exchangers are not different Thus, in jejunal enterocyte, the Na/H exchanger differs from other transport systems in that it seems to be symmetrically distributed throughout the entire plasma membrane, in spite of the functional differentiation between brush-border and basolateral membrane This homogeneous localization could permit a more efficient control of intracellular pH Actually, at the cell basolateral pole, H extrusion would not be hindered by the acid microclimate that is present at the mucosal side

Besides its implication in the control of many intracellular processes, such as pH homeostasis, the basolateral Na/H antiporter could be involved in the bicarbonate transport mechanism Since Na–$(HCO_3)_n$ cotransport seems to be absent in intestinal basolateral membrane [28], Na/H antiporter with coupling to a possible Cl/HCO_3 exchange system, could account for bicarbonate exit at the basolateral pole of jejunal enterocyte

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