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Na^+/H^+ exchange mechanism in the basolateral membrane of the rat enterocyte

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Basolateral membrane vesicles from rat jejunal enterocytes, especially purified of brush-border contamination, were used for Na^+ uptake. The basolateral membrane vesicles are osmotically active and under our experimental conditions Na^+ binding is much lower than transport. An outwardly directed proton gradient stimulates Na^+ uptake at both 5 μM and 5 mM concentrations. The proton gradient effect can be inhibited completely by 2 mM amiloride and partially by either FCCP or NH_4Cl (NH_3 diffusion). Membrane potential effects can be excluded by having valinomycin plus K^+ on both sides of the vesicles. These results suggest that there is an Na^+/H^+ exchanger in the basolateral membrane of rat enterocytes.

The plasma membrane protein that supports the carrier-mediated exchange of Na^+ for H^+ is a ubiquitous transport system that plays a critical role in the regulation of multiple cell functions, such as homeostasis of intracellular pH, control of cell volume, sodium and bicarbonate absorption [1,2]. The presence of the Na^+/H^+ exchanger, well documented in brush-border membranes of numerous epithelial tissues, was recently hypothesized in the basolateral membrane of mouse thick limbs [3] and evidenced with different techniques also in the basolateral membranes of various preparations, such as the proximal tubule of the tiger salamander [4], the rabbit cortical collecting tubule [5], the frog skin [6], the oxyntic cells [7] and the rabbit enterocytes [8]. It could not be found in rat renal basolateral membranes by quenching of acridine orange fluorescence [9,10].

Aim of our work was to evaluate the existence of an Na^+/H^+ antiporter in the basolateral membrane of the rat enterocyte. The presence of this mechanism in the brush-border membrane [11–15] and in mitochondria makes it necessary to have particularly well-purified basolateral membranes and we obtained this goal by our separation procedure in which enriched basolateral membranes, particularly free of mitochondria, were purified in a subsequent step by brush-border cross-contamination.

Materials and Methods

Basolateral plasma membranes from jejunum enterocytes were isolated and purified as described [16] with minor modifications. Two albino male rats (Wistar strain, Charles River Italiana) weighing 250–300 g were used for each experi-

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ment. The homogenate was suspended and centrifuged in 250 mM sucrose, 1 mM 1,4-dithiothreitol, 0.2 mM phenylmethanesulfonyl fluoride (PMSF), 0.01% (v/v) ethanol, 10 mM Hepes-Tris buffer (pH 7.5). 5 mM $MgCl_2$, which preferentially aggregates all membranes except the brush border, was added to basolateral membranes collected by self-orienting Percoll-gradient centrifugation (Kontron, Centrikon Model T 2070 ultracentrifuge; Haake-Buchler, Auto Densi-Flow II C apparatus). Mg^{2+} was used instead of Ca^{2+}, since proton conductance can possibly be increased by Ca^{2+} [17]. According to the experiment, basolateral membrane vesicles were suspended in the appropriate solution (see figures). To ensure that the intravesicular space was loaded with the appropriate solution, the last centrifugation was run at 20°C. The collected pellets were then incubated in the same solution at room temperature for 30 min, and used after that for Na^+ uptake by the rapid microfiltration technique.

To control the purity of the basolateral membrane fraction, as a rule total protein, γ-glutamyltransferase (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 [16].

Unless otherwise stated, 25 μl of basolateral membrane suspension (3–7 mg protein/ml), equilibrated with 25 μM valinomycin and 0.2 mM EGTA, were mixed at zero time with 225 μl of the appropriate incubation solution (see figures) containg trace amounts of ^{22}Na and unlabeled NaCl to a final concentration of 5 μM or 5 mM Na, according to the experiment (briefly, 5 μM ^{22}Na or 5 mM ^{22}Na). Membrane potential was shunted by addition of 80 mM KCl to the internal and external solutions. After 15 s, 1 min, 3 min and 90 min incubation at 28°C, 50 μl samples were taken and diluted with 0.8 ml ice-cold reaction-stopping solution (135 mM KCl/20 mM Hepes-Tris buffer (pH 7.5)), filtered on wetted 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, Model 300). All the solutions used were pre-filtered through 0.22 μm pore size filters. Individual uptake experiments in triplicate, representative of more than three repetitions with qualitatively identical results, are presented throughout the paper. Since uptake equilibria values differ from different membrane preparations, the effects of all the tested substances were always checked with a single basolateral membrane preparation.

Results and Discussion

From the Na^+/K^+-ATPase, γ-glutamyltransferase and cytochrome *c* oxidase determinations, we calculated that the basolateral membranes were enriched 12.4-fold over the initial homogenate, whilst brush-border and mitochondria were reduced to a half (0.5 and 0.4, respectively). This means that the basolateral fraction was purified approx. 12.4/0.5 = 25-fold over brush-border membrane and 12.4/0.4 = 31-fold over mitochondria. In the small intestine, enrichment factors ranging from 6- to 15-fold are reported [8,18–26]. In cited studies basolateral membranes are purified 5- to 30-fold over brush border and 17- to 30-fold over mitochondria.

From both Figs. 1 and 2, it is evident that an outwardly directed proton gradient increases Na^+

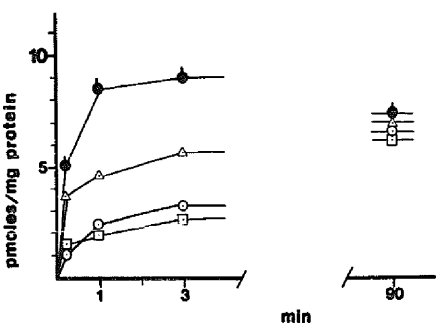

Fig. 1. 5 μM Na^+ uptake in four solutions of low buffer capacity. Basolateral membrane vesicles, obtained in 103 mM sorbitol/10 mM Mes-Tris buffer (pH 5.5) were incubated with 103 mM sorbitol/10 mM Hepes-Tris buffer (pH 7.5) with (□) or without (●) 2 mM amiloride (final concentration); or with 55 mM NH_4Cl/10 mM Hepes-Tris buffer (pH 7.5) (△); or with 103 mM sorbitol/10 mM Mes-Tris buffer (pH 5.5) (○) (control experiment without imposed pH gradient). All solutions contained 80 mM KCl, 0.2 mM phenylmethanesulfonyl fluoride (PMSF) and 0.11% (v/v) ethanol. All incubating solutions contained 5 μM ^{22}Na (final concentration). Ordinate: Na^+ uptake, mean values ± S.E. (= vertical bars, absent if less than symbol height). Abscissa: incubation time.

uptake at both 5 μM and 5 mM concentrations. Furthermore, in the presence of 5 μM Na^+, vesicles are able to accumulate Na^+ above the equilibrium value. If the imposed proton gradient is decreased by adding NH_4Cl (NH_3 diffusion into vesicles, Fig. 1) or the protonophore FCCP (Fig. 2), Na^+ uptake is lowered. 2 mM amiloride completely inhibits the enhanced Na^+ uptake in the presence of 5 μM Na^+ and reduces it drastically in the presence of 5 mM Na^+. Valinomycin in the presence of 80 mM KCl on both sides of the vesicles short-circuits the membrane potential, thus excluding electrodiffusional coupling.

Fig. 3 shows that 5 μM Na^+ uptake at 30 min is inversely proportional to the osmolarity of the incubation medium. By extrapolating Na^+ uptake to infinite osmolarity it is evident that binding is lower in the presence of 80 mM KCl. With 5 mM Na^+, both in the presence and in the absence of 80 mM KCl (values not reported in Fig. 3), the binding value is lower than with 5 μM Na^+ and unaffected by KCl (11% of the isoosmotic uptake value = 7.9 ± 0.4 nmol/mg protein per 30 min in the presence of 80 mM KCl and 12% of the isoosmotic uptake value = 7.5 ± 0.3 nmol/mg protein per 30 min in the absence of 80 mM KCl). These results demonstrate that Na^+ is transported into an osmotically sensitive intravesicular space and in the presence of 80 mM KCl the binding is small compared with transport.

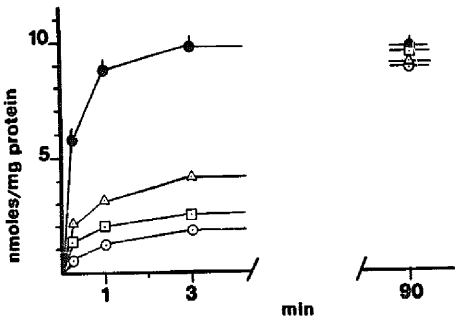

Fig. 2. 5 mM Na^+ uptake in solutions of high buffer capacity. Basolateral membrane vesicles, obtained in 100 mM Mes-Tris buffer (pH 5.5), were incubated with 100 mM Hepes-Tris buffer (pH 7.5) with (□) or without (●) 2 mM amiloride (final concentration); or with 100 mM Hepes-Tris buffer (pH 7.5)/50 μM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP, △) (in this case basolateral membrane vesicles were pre-equilibrated with 50 μM FCCP); or with 100 mM Mes-Tris buffer (pH 5.5) (○) (control experiment without imposed pH gradient). All solutions contained 80 mM KCl, 0.2 mM PMSF, 0.11% (v/v) ethanol. All incubating solutions contained 5 mM ^{22}Na (final concentration). Ordinate: Na uptake, mean values ± S.E. (= vertical bars, absent if less than symbol height). Abscissa: incubation time.

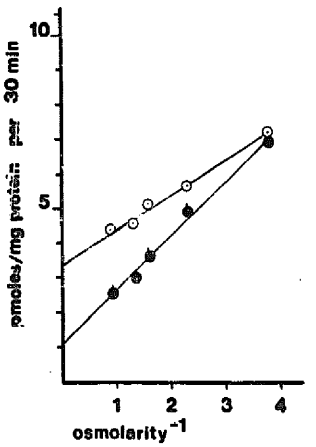

Fig. 3. Effects of extravesicular osmolarity (abscissa, 1/osmolarity) on the uptake of 5 μM Na^+ after 30 min equilibration. Mean values ± S.E. (= vertical bars, absent if less than symbol height) are reported in pmol/mg protein (ordinate). 90 μl basolateral membrane vesicle (1–2 mg protein/ml) were incubated for 30 min with 180 μl of incubation medium. Vesicles obtained in 250 mM sucrose/10 mM Hepes-Tris buffer (pH 7.5) were incubated in 10 mM Hepes-Tris buffer (pH 7.5), 5 μM ^{22}Na and 250, 500, 750, 1000 or 1500 mM sucrose (○). Vesicles obtained in 103 mM sucrose/80 mM KCl/10 mM Hepes-Tris buffer (pH 7.5) and pre-equilibrated with 25 μM valinomycin were incubated in 80 mM KCl, 10 mM Hepes-Tris buffer (pH 7.5), 5 μM ^{22}Na and 103, 353, 603, 853 or 1353 mM sucrose (●). All solutions contained 0.2 mM PMSF and 0.11% (v/v) ethanol. In both experiments, after 30 min incubation, 50 μl samples were processed as described above.

We can exclude any contribution of brush-border and mitochondrial Na^+/H^+ antiporters in the proton-gradient-stimulated Na^+ uptake, since the enzyme marker analysis shows that our basolateral membrane preparation is very well purified of these two membranes. The absence of brush border allowed us to use sucrose (a poorly permeating substance) to increase osmolarity.

The results of both Figs. 1 and 2 can be ex-

plained by the existence of the Na^+/H^+ exchanger in the basolateral membrane: in fact, when valinomycin is added in the presence of K^+ on both sides of the membrane, Na^+/H^+ exchange does not result from electrodiffusional coupling but from a directly coupled flux. Moreover, if the proton gradient is lowered by NH_3 diffusion (with low buffer capacity) or by the protonophore FCCP, the driving force for Na^+ uptake is reduced. Besides the ability of an inwardly directed NH_4 gradient to dissipate pH gradient, external NH_4 could inhibit Na^+ flux by competition for the same transport site on the Na^+/H^+ antiporter [2].

It is generally reported that Na^+/H^+ exchangers are maximally inhibited by relatively high concentrations of amiloride (0.1–2 mM), whilst only micromolar concentrations of the drug can completely block Na^+ channels in many tissues. The present study shows that the effect of amiloride is total at the lower Na^+ concentration and significant at the higher one.

Since membrane surfaces possess negative charges, previous incubation with another monovalent cation (K^+) drastically reduces 5 μM Na binding (Fig. 3): different binding values in different incubation solutions were also found by Takano et al. [27].

Therefore the Na^+/H^+ exchanger found in the basolateral membrane of other cells [4–8], appears also to be present in the basolateral membrane of rat enterocyte.

In physiological conditions there is a remarkable electrical and chemical Na^+ gradient directed inwardly across the basolateral plasma membrane. Moreover, intracellular H^+ concentration is generally equal to or slightly higher than extracellular H^+ concentration (with opposite electrical gradient): thus, the driving force favours the net entry of Na^+ (in exchange for internal H^+). Another physiological implication for the basolateral membrane Na^+/H^+ antiporter could be its involvement in the HCO_3 transport mechanism, perhaps by coupling with a $Cl^-/HCO_3^-(OH^-)$ exchanger. $Na^+/(HCO_3^-)_n$ cotransport does not seem to occur in the rat small intestinal basolateral membrane [28] and it is not clear whether the $Cl^-/HCO_3^-(OH^-)$ exchanger does. This mechanism requires further investigation.

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