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Rat Jejunal Basolateral Membrane Cl/HCO_3 Exchanger is Modulated by a Na-sensitive Modifier Site

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Abstract. A Cl/HCO_3 exchanger mediates HCO_3 extrusion across rat jejunal basolateral membrane. Previous studies demonstrated that anion antiport activity is positively affected by Na, but evidence was given that this cation is not translocated by the carrier protein. Basolateral membranes isolated from rat jejunum were used to give more insight on Na effect. Uptake studies, performed together with vesicle sidedness determinations, indicated that the greatest stimulation of Cl-dependent HCO_3 uptake occurs when Na is present at both vesicle surfaces. The kinetic dependence of Cl/HCO_3 exchange on equal intra- and extravesicular Na concentration showed a hyperbolic relationship, and the calculated kinetic parameters were $V_{max} = 0.153 \pm 0.006$ nmol mg protein^{-1} sec^{-1}, $K_m = 23.0$ mM. Ion replacement studies indicated that Na can be partially substituted only by Li and not by other monovalent cations. Results of this study suggest that Na could act as a nonessential activator of the Cl/HCO_3 exchanger. A possible role of the Na-sensitive modifier site in the physiology of jejunal enterocyte is suggested.

Key words: Rat jejunum — Basolateral membrane vesicles — Cl/HCO_3 exchange — Na effect — Allosteric modifier site

Introduction

In recent years it has become obvious that there is a great deal of segmental heterogeneity of transport functions in the gut. Differences between jejunum and ileum have long been recognized; these comprise the transepithelial transport of HCO_3, which is absorbed in the jejunum and secreted in the ileum [31].

The mechanism by which HCO_3 leaves the jejunal enterocyte across the basolateral membrane could be represented by a Cl/HCO_3 exchanger, recently evidenced [28, 29, 39]. The two anions may be transported via one or more coupled carrier mechanisms located in the same membrane, namely a cation-independent antiport [1, 8, 11, 25, 32] and a Na-dependent exchanger, which generally catalyzes the exchange of Na and HCO_3 for Cl and a proton equivalent [1, 3, 9, 10, 14, 19, 32, 34]. However, while the former process was demonstrated in the basolateral membrane of jejunal enterocyte, the presence of the latter is ambiguous, in that Na ion increases the antiporter activity without being transported [39]. The aim of this work was to get more insight on sodium effect, for a more complete picture of bicarbonate transport in the physiology of jejunal enterocyte.

Materials and Methods

Basolateral Membrane Separation

Basolateral plasma membranes from rat jejunum enterocytes were isolated and purified as described [27]. Briefly, 7 mM $CaCl_2$, which preferentially aggregates all membranes except the brush border, 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). To ensure that the intravesicular space was loaded with the appropriate buffer, we ran the last centrifugation at 20°C. The collected pellets (3–7 mg protein/ml) were then incubated in the same buffer at room temperature for 30 min (gassed with the appropriate CO_2 tension when HCO_3 was present), and equilibrated with 0.2 mM EGTA and 25 μM valinomycin. After that, vesicles were used for either HCO_3 or Cl uptake by the rapid micro filtration technique.

Enzyme Activities

Total protein and γ-glutamyltransferase (γ-GT, a marker enzyme for brush border membrane) were determined as published [24]. The activity of (Na,K)-ATPase (a marker enzyme for basolateral membrane)

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was estimated by the slightly modified method of Schoner et al. [35], in which the resynthesis of the ATP split by the ATPase is coupled via PK-LDH reaction to NADH oxidation. In the same sample, the kinetics in the absence (total ATPase) and in the presence of ouabain (ouabain-insensitive ATPase) were recorded. The difference in the slope between the two straight lines represents a measure of the (Na,K)-ATPase activity. The final concentration of reagents was: 3.1 mM $MgCl_2$, 82 mM NH_4Cl, 103 mM NaCl, 70 mM imidazole buffer (pH 7.3), 2 mM phosphoenolpyruvate, 2.5 mM Na_2-ATP, 0.3 mM NADH, 1.9 U/ml PK, 1.7 U/ml LDH, 2.5 mM ouabain. For orientation studies, basolateral membrane vesicles were preincubated at room temperature for 20 min with the ionic detergent sodium dodecyl sulfate (SDS) at different concentrations, keeping the ratio mg protein : mg SDS = 1 : 0.1–0.2. Both total ATPase and (Na,K)-ATPase were then assayed and orientation was evaluated according to Boumendil-Podevin and Podevin [4].

Uptake Experiments

Uptake studies, performed at 28°C, were started by diluting the vesicle suspension into an incubation medium containing either $H^{14}CO_3$ or ^{36}Cl. The composition of the final resuspension solutions and incubation media is given in the figure legends. Samples were withdrawn at selected times. Special precautions were taken to perform HCO_3 uptake, as previously described [29]. In particular, experiments lasting not longer than 30 min were performed and, to take into account the decrease of specific activity of labeled bicarbonate (always about 10% of the end of the experiment), samples of the reaction mixture were withdrawn at the same selected times and used as standards. Samples were diluted with 0.8 ml ice-cold reaction-stopping solution (200 mM K acetate, 0.2 mM PMSF, 0.01% (v/v) ethanol, 100 mM HEPES/Tris buffer at pH 8.2; K acetate concentration was varied according to the osmolarity of the incubating solutions), 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, mod. 1600 TR). All experiments were performed in voltage-clamp conditions; intra- and extravesicular solutions were always isotonic. All the solutions used were prefiltered 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.

Results

The purity of the basolateral membranes was checked by measuring various marker enzyme activities. The recovered fraction was enriched 11-fold compared to the homogenate in specific activity of (Na,K)-ATPase, while the enrichment factor of γ-GT was 0.5.

The sidedness of our membrane preparation was determined with the latency of (Na,K)-ATPase (Table). After activation of (Na,K)-ATPase with SDS, we calculated that the ratio of unsealed to sealed vesicles is 1:2; sealed vesicles are about 90% right-side-out (RSO) and 10% inside-out (IO) oriented. Overlapping results were obtained using both fresh and frozen (−80°C) membranes; the presence of NaCl during the separation procedure did not influence vesicle sidedness.

The first group of experiments was performed to investigate at which membrane surface sodium binds to the carrier protein. Therefore, HCO_3 uptake driven by an outwardly directed Cl gradient was evaluated in the presence and in the absence of intra- and/or extravesicular Na. Results, depicted in Fig. 1, show that the presence of either internal or external Na increases HCO_3 uptake; a further increase occurs when Na is present at both vesicle surfaces. Since we have determined that vesicles are 90% right side out and 10% inside out, we can hypothesize that Na action is exerted at both sides of the basolateral membrane.

To investigate the effect of increasing $Na_{in} = Na_{out}$ concentrations, we examined the time course of Cl-dependent HCO_3 uptake in the presence of Na 0, 10, 50 and 100 mM. Results, depicted in Fig. 2, demonstrate that the increase of Na concentration enhances Cl/HCO_3 activity without altering the equilibrium value, that is apparent vesicle volume.

In the presence of 100 mM intra- and extravesicular Na, the Cl-coupled HCO_3 uptake was determined as a function of short incubation times (*data not shown*), resulting in a linear response for at least 6 sec. Thus, a 6 sec uptake can be reasonably considered as an estimate of the initial uptake rate of 1 mM HCO_3. Figure 3 shows the results of an experiment in which Cl-driven HCO_3 unidirectional flux was measured as a function of equal intra- and extravesicular Na concentration. The hyperbolic relationship obtained, corrected for the Na-independent component, was linearized by the Eadie-Hofstee transformation of the data and kinetic parameters were calculated (J_{max} = 0.153 ± 0.006 nmol mg protein^{-1} sec^{-1}, K_m = 23.0 mM, correlation coefficient = 0.99). By Hill plot analysis of the same data, the Hill coefficient resulted about 1. The experiment illustrated in Fig. 3 was repeated with Na only on the outside (*data not shown*). Kinetic parameters were calculated both by Eadie Hofstee and Hill plots (J_{max} = 0.033 ± 0.002 nmol mg protein^{-1} sec^{-1}, K_m = 1.87 mM, correlation coefficient = 0.91, Hill number = 0.98 ± 0.07).

To see whether other alkali metal ions behave like sodium in stimulating Cl/HCO_3 antiport, we performed the experiment whose results are illustrated in Fig. 4. As shown, only Li can cause an increase of HCO_3 uptake to a similar level, whereas Rb, Cs and K are poor substitutes for Na.

The initial uptake rate of 3 mM Cl uptake driven by 30 mM HCO_3 in the presence and in the absence of 70 mM intra- and extravesicular Na is reported in Fig. 5. Since under these experimental conditions Cl uptake was linear for at least 13 sec (*data not shown*), this incubation time was selected as a measure of unidirec-

Table. Leakiness and orientation of basolateral membrane vesicles

Experimental condition	Total ATPase		RSO	(Na,K)-ATPase		Leaky	IO
		+SDS	(%)		+SDS	(%)	(%)
No NaCl during separation							
Fresh vesicles (6)	815 ± 90	1,998 ± 231	59	645 ± 95	1,848 ± 212	35	6
Frozen vesicles (6)	773 ± 75	2,025 ± 188	62	605 ± 54	1,902 ± 171	32	7
100 mM NaCl during separation							
Fresh vesicles (7)	951 ± 166	2,265 ± 307	58	732 ± 129	2,048 ± 276	36	6
Frozen vesicles (6)	827 ± 80	2,182 ± 202	62	666 ± 64	2,066 ± 190	32	6

Total and (Na,K)-ATPase activities are expressed as nmol P_i/min per mg protein. Number of experiments in parentheses. RSO = right-side-out orientation. IO = inside-out orientation.

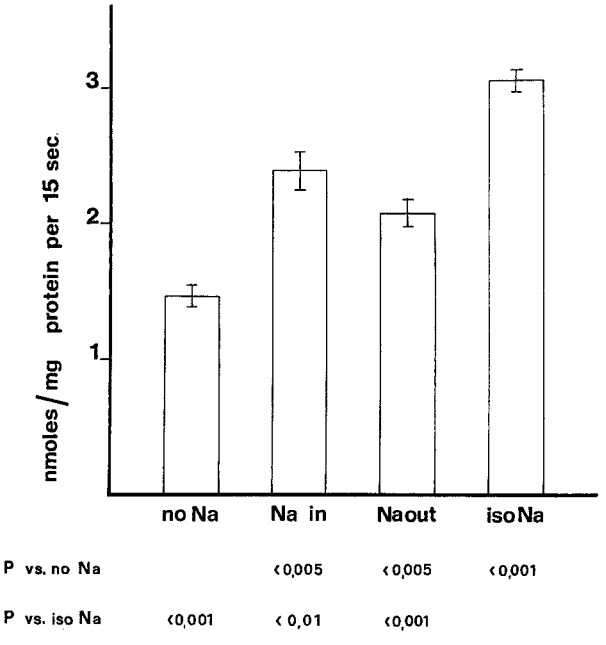

Fig. 1. Effect of intra- and/or extravesicular Na on Cl gradient-dependent HCO_3 uptake. Vesicles obtained either in 100 mM KCl and 182 mM sorbitol (*no Na* and Na_{out}), or in 100 mM NaCl and 100 mM K gluconate (Na_{in} and *iso Na*) were incubated either in 100 mM K gluconate and 186 mM sorbitol (*no Na* and Na_{in}), or in 100 mM Na gluconate, 100 mM K gluconate and 4 mM sorbitol (Na_{out} and *iso Na*). All solutions contained 100 mM HEPES/Tris buffer at pH 8.2, 0.2 mM PMSF and 0.01% (v/v) ethanol. All incubating solutions contained 1 mM $H^{14}CO_3$. Vesicles were preincubated with 25 μM valinomycin. Mean values of HCO_3 uptake ± SE (bars) are represented. *P* values with respect to "*no Na*" and "*iso Na*" conditions are reported. *P* value between Na_{in} and Na_{out} conditions = NS.

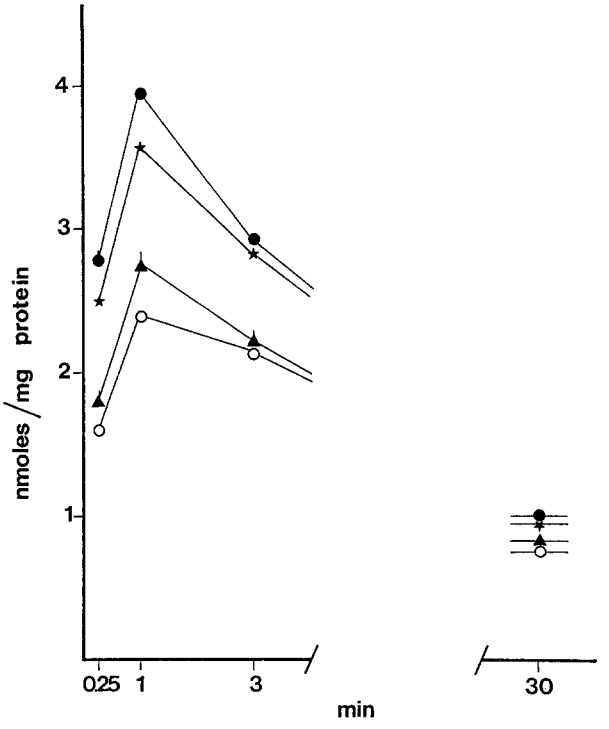

Fig. 2. Effect of 0, 10, 50 and 100 mM Na on Cl-dependent HCO_3 uptake. Basolateral membrane vesicles (20 μl), obtained either in 182 mM sorbitol (open circles), or in 10 mM Na gluconate and 164 mM sorbitol (triangles), or in 50 mM Na gluconate and 91 mM sorbitol (stars), or in 100 mM Na gluconate (filled circles) were incubated in 380 μl of either 186 mM sorbitol (open circles), or 10 mM Na gluconate and 168 mM sorbitol (triangles), or 50 mM Na gluconate and 95 mM sorbitol (stars), or 100 mM Na gluconate and 4 mM sorbitol (filled circles). KCl (100 mM) was present in all basolateral membrane vesicles. $H^{14}CO_3$ (1 mM) and 100 mM K gluconate were present in all incubating solutions. All solutions contained 100 mM HEPES/Tris buffer at pH 8.2, 0.2 mM PMSF and 0.01% (v/v) ethanol. Vesicles were preincubated with 25 μM valinomycin. Ordinate: HCO_3 uptake, mean values ± SE (vertical bars, absent if less than symbol height). Abscissa: incubation time.

tional flux. The antiporter activities result from the difference between Cl uptake values evaluated in the absence and in the presence of the disulfonic stilbene DIDS, a well-known inhibitor of anion exchangers. The

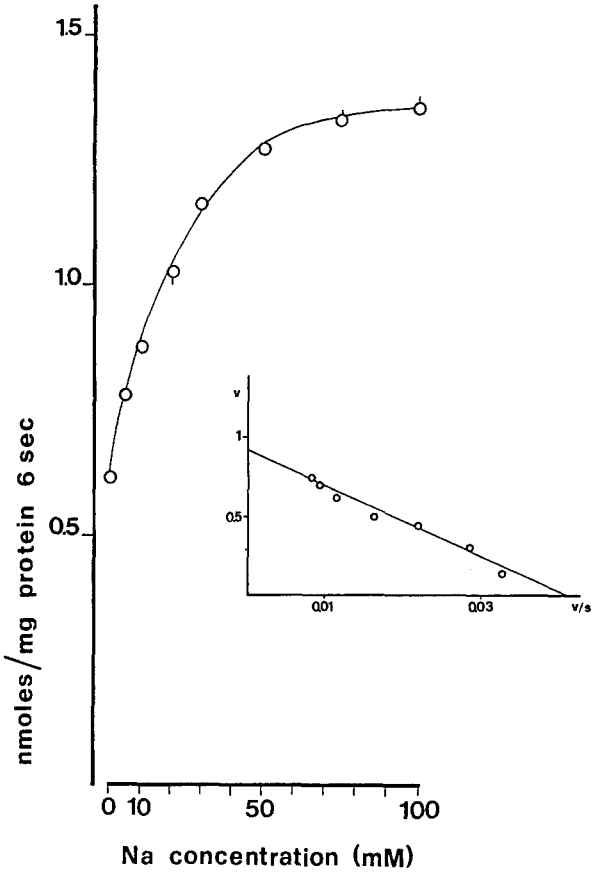

Fig. 3. Effect of different Na concentrations on the initial rate of Cl-dependent HCO_3 uptake. Basolateral membrane vesicles (5 µl), obtained in 100 mM KCl and various concentrations of Na gluconate from 5 to 100 mM, were incubated in 95 µl of 100 mM K gluconate, 1 mM $H^{14}CO_3$ and the same Na gluconate concentrations. All solutions contained 100 mM HEPES/Tris buffer at pH 8.2, 0.2 mM PMSF and 0.01% (v/v) ethanol. Vesicles were preincubated with 25 µM valinomycin. Ordinate: HCO_3 uptake, mean values ± SE (=vertical bars, absent if less than symbol height). Abscissa: Na concentration, mM. Inset: Eadie-Hofstee plot of the same data subtracted of the Na-independent component.

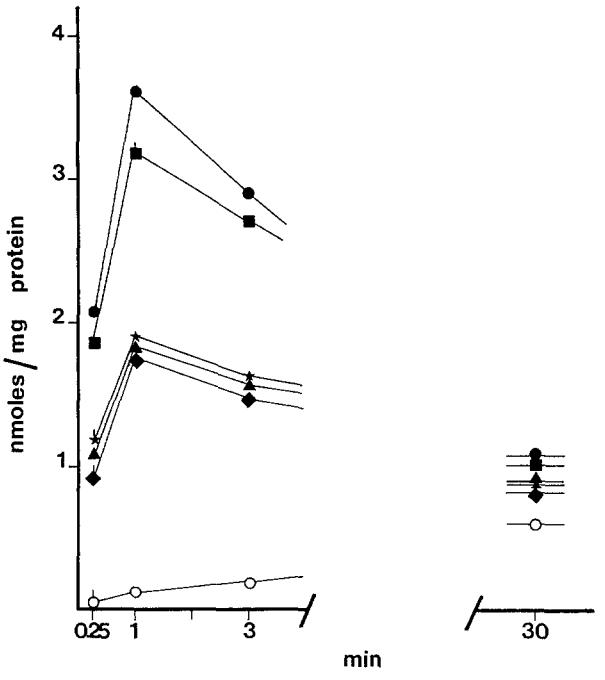

Fig. 4. Effect of alkaline monovalent cations on Cl-dependent HCO_3 uptake. Basolateral membrane vesicles (20 µl), obtained either in 100 mM NaCl and 100 mM K gluconate (filled circles), or in 100 mM LiCl and 100 mM K gluconate (squares), or in 100 mM RbCl, 100 mM K gluconate and 6 mM sorbitol (stars), or in 100 mM KCl and 182 mM sorbitol (triangles), or in 100 mM CsCl, 100 mM K gluconate and 6 mM sorbitol (diamonds), or in 100 mM K gluconate and 182 mM sorbitol (open circles), were incubated in 380 µl of either 50 mM Na_2SO_4, 100 mM K gluconate and 70 mM sorbitol (filled circles), or 50 mM Li_2SO_4, 100 mM K gluconate and 70 mM sorbitol (squares), or 50 mM Rb_2SO_4, 100 mM K gluconate and 70 mM sorbitol (stars), or 50 mM K_2SO_4 and 252 mM sorbitol (triangles), or 50 mM Cs_2SO_4, 100 mM K gluconate and 70 mM sorbitol (diamonds), or 50 mM K_2SO_4 and 252 mM sorbitol (open circles). $H^{14}CO_3$ (1 mM) was in all incubating solutions. All solutions contained 100 mM HEPES/Tris buffer at pH 8.2, 0.2 mM PMSF and 0.01% (v/v) ethanol. Vesicles were preincubated with 25 µM valinomycin. Ordinate: HCO_3 uptake, mean values ± SE (vertical bars, absent if less than symbol height). Abscissa: incubation time.

presence of Na produces approximately an 80% increase of the antiporter-mediated Cl influx.

Discussion

Marker enzyme analysis demonstrated that the basolateral membrane fraction is very well purified from brush border contamination. Moreover, in a recent work [28], evidence was provided that in rat jejunal brush border membrane Cl/HCO_3 exchange, if present, has a very low transport rate. Thus, the transport system we are dealing with is indeed basolateral.

Cl/HCO_3 exchangers have been identified in many different tissues [5, 12–14, 17, 19, 33, 40]. In general, two types have been described in cells from both invertebrates and vertebrates. The sodium-independent Cl/HCO_3 exchanger mediates the exchange of Cl for HCO_3 with a stoichiometry of 1:1 [1, 8, 11, 25, 32]. The best characterized Na-independent Cl/HCO_3 exchanger is the erythroid band 3; this and other band 3-like proteins have been cloned from various tissues [6]. The Na-dependent Cl/HCO_3 exchanger mediates the electroneutral transport of one Na and one HCO_3 in one direction for one Cl and one proton equivalent in the opposite direction [1, 3, 9, 10, 14, 19, 32, 34]. Various evidences indicate that these two systems coexist in several cell types [25, 32]. In previous papers [28, 29, 39], we identified an anion antiporter that is able to function as Cl/HCO_3 exchanger in rat jejunal basolat-

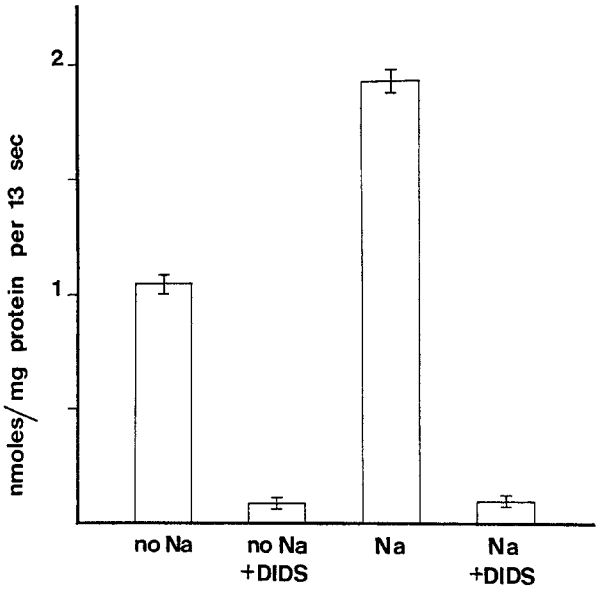

Fig. 5. Initial rate of HCO_3-dependent Cl uptake. Basolateral membrane vesicles (2 μl), obtained either in 30 mM $KHCO_3$, 70 mM K gluconate and 127 mM sorbitol (*no Na, no Na + DIDS*), or in 30 mM $NaHCO_3$, 40 mM Na gluconate and 100 mM K gluconate (*Na, Na + DIDS*), were incubated in 198 μl of either 100 mM K gluconate and 127 mM sorbitol (*no Na, no Na + DIDS*), or 70 mM Na gluconate and 100 mM K gluconate (*Na, Na + DIDS*). All solutions contained 100 mM HEPES/Tris buffer at pH 8.2, 0.2 mM PMSF and 0.01% (v/v) ethanol. All incubating solutions contained 3 mM ^{36}Cl and were added or not with 1 mM DIDS. Vesicles were preincubated with 25 μM valinomycin and gassed with 0.9% CO_2. Mean values of Cl uptake ± SE (bars) are represented.

eral membrane. We provided evidence that membrane vesicles mediate a Na-independent Cl/HCO_3 antiport which is positively affected by the presence of Na; however, neither HCO_3 nor Cl gradients can drive the countertransport of Na [39].

The object of this research was to test if Na could bind to a regulatory site located at one face of the basolateral membrane. Since vesicle population is not uniformly oriented, sidedness determination was necessary. Results of the sidedness studies (Table) are not in agreement with the ones we mentioned previously [28], as in the present paper unsealed vesicles are reduced and the right-side-out : inside-out ratio is increased. Variable data were reported also by other authors [4, 16, 20, 21] and the difference could be due to seasonal variations. Nevertheless, in the present work sidedness studies were carried out on the same membrane preparation used for the uptake experiments depicted in Fig. 1. Moreover, unlike data published by Mandla et al. [20], RSO percentage of a single membrane preparation did not vary in the long run; therefore, data concerning the side of sodium action are not questionable. Since results of Fig. 1 indicate that the greatest stimulation of Cl-dependent HCO_3 uptake occurs when Na is present at both vesicle surfaces, we can argue either that two regulatory sites are involved in the protein carrier activation, or that one site is translocated from the inner to the outer face of the membrane. However, the finding that Na is not transported by the anion exchanger makes the latter possibility unlikely to be true.

The effect of increasing equal transmembrane Na concentrations has been tested both on the time course and on the initial rate of Cl-linked HCO_3 uptake. Results are illustrated in Figs. 2 and 3, respectively. As shown in Fig. 2, HCO_3 influx is enhanced by increasing equimolar transmembrane Na concentrations, whereas at a 30 min equilibrium time point, uptake values are practically the same. Thus, the stimulation of HCO_3 uptake by Na cannot be explained by increased intravesicular volume. When the initial rate of Cl gradient-dependent HCO_3 uptake was measured as a function of bilateral Na concentration (equal on both sides), a hyperbolic relationship was observed (Fig. 3). The Na-dependent Cl/HCO_3 transport was obtained after subtraction of the uptake value measured in the absence of Na and the kinetic parameters were calculated by means of both Eadie-Hofstee linearization of the data and Hill plot analysis. Presumably, the increase of transport velocity illustrated in Fig. 3 represents some allosteric interaction between Na and the carrier protein, although the nature of these putative sites and conformational changes remains obscure. As the kinetic dependence of Cl/HCO_3 exchange on Na concentration seems to obey a Michaelis-Menten equation, we can presume either that a single modifier site is involved in Na activatory effect (hence translocating across the membrane thickness but, as mentioned above, this seems unlikely to be true), or that more allosteric modifier sites might exist with no cooperativity [36]. The presence of Na only in the outer solution (*data not shown*) seems to induce a relatively small activation of the anion exchanger, which moreover seems to be saturated at a low Na concentration. Thus, the most effective site in modulating the exchanger activity should be the inner one. Reasonably, we can hypothesize that the Na affinity of the internal site is not very unlike from the one calculated with equal transmembrane Na concentrations. In fact, if one subtracts from the data of Fig. 3, the velocity curve obtained with Na only in the outside, the K_m value is affected just to a very small extent. Na ion could act as a nonessential activator of Cl/HCO_3 antiport. Such allosteric effects are not unprecedented, analogous modifier sites having been found in other transport proteins [2, 22, 23].

From the results of Fig. 4, it is evident that Na can be partially substituted only by Li. This finding is not amazing since Li can compete with Na in various transport mechanisms [7, 15, 30, 37].

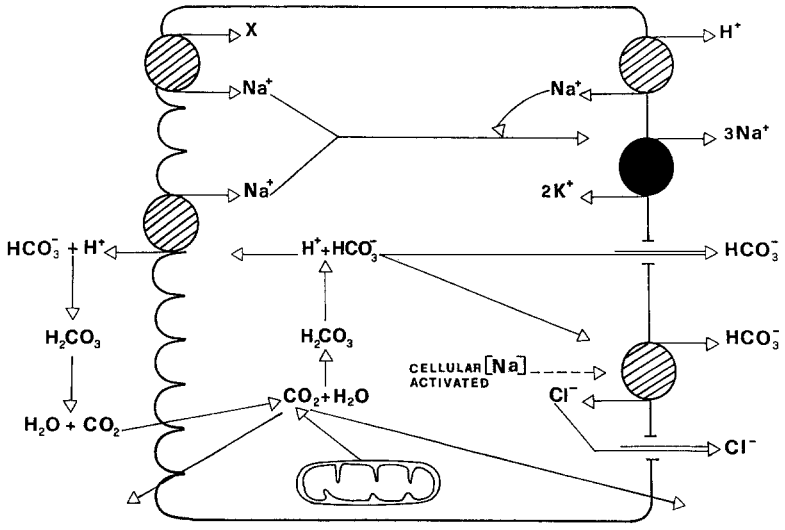

Fig. 6. Schematic drawing showing transport systems described in the text.

Figure 5 shows that 70 mM Na causes an 80% increase in Cl/HCO_3 exchanger activity. In this experimental condition, Cl uptake was evaluated, since a previous study [29] demonstrated that overlapping results are obtained using Cl and HCO_3. Moreover, this experiment was performed in the presence of a low HCO_3 gradient to reduce its concentration in the extravesicular medium and to minimize a possible HCO_3/HCO_3 exchange. Thus, in this experimental condition, Na effect is not underestimated.

The physiological significance of the Na stimulatory effect on the basolateral Cl/HCO_3 antiport of jejunal enterocyte can be envisioned in the light of the kinetic parameters determined. While in physiological conditions the Na concentration in the extracellular environment exerts the maximal activation of the antiporter, cytoplasmic Na concentration is closely related to the K_m value. Thus, small changes in cellular Na produce significant variations in Cl/HCO_3 exchange activity. This leads us to the teleological question: why is the HCO_3 transport step coupled to Na? Intracellular Na rise could be due to an increase in Na/H antiporter activity, which in jejunal enterocyte was evidenced both in brush border [18, 24] and in basolateral membrane [30, 38]. This in turns elevates cytoplasmic pH. It is well known that the induced alkalinization can indirectly increase the transmembrane exchange of extracellular Cl for internal HCO_3 by elevating the intracellular concentration of the latter HCO_3 [22, 26]. In addition, a direct stimulation of Cl/HCO_3 exchange could be accomplished by the increased cytoplasmic Na, counteracting the buildup of alkaline cellular pH. In other cell types, Cl/HCO_3 antiport was reported to exhibit a pH-sensitive cytoplasmic modifier site [22, 23, 26]. An increase in intracellular Na level (produced by Na-linked co- and countertransports) would also activate the Na pump, which removes Na ions at the expense of ATP consumption, with production of CO_2. It is likely that, given the relatively high permeability of cell membranes to CO_2, this will freely equilibrate between the lumen, cell and serosal compartment. However, part of the metabolic CO_2 could be hydrated to HCO_3 by means of carbonic anhydrase activity. Once again, a Na-sensitive modifier site promoting the exit of HCO_3 by stimulating basolateral Cl/HCO_3 exchange would represent the coupling factor modulating the magnitude of the homeostatic response. A schematic model of proposed mechanisms is illustrated in Fig. 6.

Up to now there has been little information regarding the regulation of the anion antiport. Results of this work suggest that a Na-stimulated activatory site in the basolateral membrane of jejunal enterocyte could modulate Cl/HCO_3 activity in processes such as HCO_3 absorption and cell pH regulation.

References

1. Alpern, R.J., Chambers, M. 1987. Basolateral membrane Cl/HCO_3 exchange in the rat proximal convoluted tubule. Na-dependent and -independent modes. *J. Gen. Physiol.* **89:**581–598
2. Aronson, P.S. 1985. Kinetic properties of the plasma membrane Na^+-H^+ exchanger. *Annu. Rev. Physiol.* **47:**545–560
3. Boron, W.F. 1985. Intracellular pH-regulating mechanism of the squid axon. Relation between the external Na^+ and HCO_3^- dependences. *J. Gen. Physiol.* **85:**325–345
4. Boumendil-Podevin, E.F., Podevin, R.A. 1983. Isolation of basolateral and brush border membrane from the rabbit kidney cortex. Vesicle integrity and membrane sidedness of the basolateral fraction. *Biochim. Biophys. Acta* **735:**86–94
5. Cabantchik, Z.I., Knauf, P.A., Rothstein, A. 1978. The anion transport system of the red blood cell. The role of membrane protein evaluated by the use of probes. *Biochim. Biophys. Acta* **515:**239–302
6. Chow, A., Dobbins, J.W., Aronson, P.S., Igarashi, P. 1992.

cDNA cloning and localization of a band 3-related protein from ileum. *Am. J. Physiol.* **263:**G345–G352
7. Del Castillo, J.R., Marin, R., Proverbio, T., Proverbio, F. 1982. Partial characterization of the ouabain-insensitive, Na^+-stimulated ATPase activity of kidney basal-lateral plasma membranes. *Biochim. Biophys. Acta* **692:**61–68
8. Grassl, S.M., Holohan, P.D., Ross, C.R. 1987. Cl^--HCO_3^- exchange in rat renal basolateral membrane vesicles. *Biochim. Biophys. Acta* **905:**475–484
9. Grinstein, S., Rotin, D., Mason, M.J. 1989. Na^+/H^+ exchange and growth factor-induced cytosolic pH changes. Role in cellular proliferation. *Biochim. Biophys. Acta* **988:**73–97.
10. Guggino, W.B., London, R., Boulpaep, E.L., Giebisch, G. 1983. Chloride transport across the basolateral cell membrane of the *Necturus* proximal tubule: Dependence on bicarbonate and sodium. *J. Membrane Biol.* **71:**227–240
11. Halligan, R.D., Shelat, H., Kahn, A.M. 1991. Na^+-independent Cl^--HCO_3^- exchange in sarcolemmal vesicles from vascular smooth muscle. *Am. J. Physiol.* **260:**C347–C354
12. Hoffmann, E.K. 1986. Anion transport systems in the plasma membrane of vertebrate cells. *Biochim. Biophys. Acta* **864:**1–31
13. Hoffmann, E.K., Simonsen, L.O. 1989. Membrane mechanisms in volume and pH regulation in vertebrate cells. *Physiol. Rev.* **69:**315–382
14. Kahn, A.M., Cragoe, E.J., Jr., Allen, J.C., Halligan, R.D., Shelat, H. 1990. Na^+-H^+ and Na^+-dependent Cl^--HCO_3^- exchange control pH_i in vascular smooth muscle. *Am. J. Physiol.* **259:**C134–C143
15. Kinne, R., Hannafin, J.A., Konig, B. 1985. Role of the NaCl-KCl cotransport system in active chloride absorption and secretion. *Ann. NY Acad. Sci.* **456:**198–206
16. Kinsella, J.L., Holohan, P.D., Pessah, N.I., Ross, C.R. 1979. Isolation of luminal and antiluminal membranes from dog kidney cortex. *Biochim. Biophys. Acta* **552:**468–477
17. Knickelbein, R., Aronson, P.S., Schron, C.M., Seifter, J., Dobbins, J.W. 1985. Sodium and chloride transport across rabbit ileal brush border. II. Evidence for Cl-HCO_3 exchange and mechanism of coupling. *Am. J. Physiol.* **249:**G236–G245
18. Liedtke, C.M., Hopfer, U. 1977. Anion transport in brush border membranes isolated from rat small intestine. *Biochem. Biophys. Res. Commun.* **76:**579–585
19. Liu, S., Piwnica-Worms, D., Lieberman, M. 1990. Intracellular pH regulation in cultured embryonic chick heart cells. Na^+-dependent Cl^-/HCO_3^- exchange. *J. Gen. Physiol.* **96:**1247–1269
20. Mandla, S., Scriver, C.R., Tenenhouse, H.S. 1988. Decreased transport in renal basolateral membrane vesicles from hypertaurinuric mice. *Am. J. Physiol.* **255:**F88–F95
21. Marin, T., Proverbio, T., Proverbio, F. 1986. Inside-out basolateral plasma membrane vesicles from rat kidney proximal tubular cells. *Biochim. Biophys. Acta* **858:**195–201
22. Mason, M.J., Smith, J.D., De Jesus Garcia Soto, J., Grinstein, S. 1989. Internal pH-sensitive site couples Cl-HCO_3 exchange to Na-H antiport in lymphocytes. *Am. J. Physiol.* **256:**C428–C433
23. Mugharbil, A., Knickelbein, R.G., Aronson, P.S., Dobbins, J.W. 1990. Rabbit ileal brush-border membrane Cl-HCO_3 exchanger is activated by an internal pH-sensitive modifier site. *Am. J. Physiol.* **259:**G666–G670
24. Murer, H., Hopfer, U., Kinne, R. 1976. Sodium/proton antiport in brush-border-membrane vesicles isolated from rat small intestine and kidney. *Biochem. J.* **154:**597–604
25. Nakhoul, N.L., Chen, L.K., Boron, W.F. 1990. Intracellular pH regulation in rabbit S_3 proximal tubule: basolateral Cl-HCO_3 exchange and Na-HCO_3 cotransport. *Am. J. Physiol.* **258:**F371–F381
26. Olsnes, S., Tonnessen, T.I., Ludt, J., Sandvig, K. 1987. Effect of intracellular pH on the rate of Cl uptake and efflux in different mammalian cell lines. *Biochemistry* **26:**2778–2789
27. Orsenigo, M.N., Tosco, M., Esposito, G., Faelli, A. 1985. The basolateral membrane of rat enterocyte: its purification from brush border contamination. *Anal. Biochem.* **144:**577–583
28. Orsenigo, M.N., Tosco, M., Faelli, A. 1991. Cl/HCO_3 exchange in the basolateral membrane domain of rat jejunal enterocyte. *J. Membrane Biol.* **124:**13–19
29. Orsenigo, M.N., Tosco, M., Faelli, A. 1992. Basolateral Cl^-/HCO_3^--exchange in rat jejunum: evidence from $H^{14}CO_3^-$ uptake in membrane vesicles. *Biochim. Biophys. Acta* **1108:**140–144
30. Orsenigo, M.N., Tosco, M., Zoppi, S., Faelli, A. 1990. Characterization of basolateral membrane Na/H antiport in rat jejunum. *Biochim. Biophys. Acta* **1026:**64–68
31. Parsons, D.S. 1956. The absorption of bicarbonate-saline solutions by the small intestine and colon of the white rat. *Q. J. Exp. Physiol.* **41:**410–420
32. Preisig, P.A., Alpern, R.J. 1989. Basolateral membrane H-OH-HCO_3 transport in the proximal tubule. *Am. J. Physiol.* **256:**F751–F765
33. Putnam, R.W. 1990. pH regulatory transport systems in a smooth muscle-like cell line. *Am. J. Physiol.* **258:**C470–C479
34. Sasaki, S., Yoshiyama, N. 1988. Interaction of chloride and bicarbonate transport across the basolateral membrane of rabbit proximal straight tubule. Evidence for sodium coupled chloride-bicarbonate exchange. *J. Clin. Invest.* **81:**1004–1011
35. Schoner, W., von Ilberg, C., Kramer, R., Seubert, W. 1967. On the mechanism of Na- and K-stimulated hydrolysis of adenosine triphosphate. Purification and properties of a Na^+- and K^+-activated ATPase from ox brain. *Eur. J. Biochem.* **1:**334–343
36. Segel, I.H. 1975. Multisite and allosteric enzymes. *In:* Enzyme Kinetics. pp. 346–403. Wiley-Interscience, New York.
37. Skou, J.C. 1988. Overview: the Na,K-pump. *Methods Enzymol.* **156:**1–25
38. Tosco, M., Orsenigo, M.N., Esposito, G., Faelli, A. 1988. Na^+/H^+ exchange mechanism in the basolateral membrane of the rat enterocyte. *Biochim. Biophys. Acta* **944:**473–476
39. Tosco, M., Orsenigo, M.N., Faelli, A. 1993. Basolateral Cl/HCO_3 exchange in rat jejunum: the effect of sodium. *J. Membrane Biol.* **135:**129–135
40. Turner, R.J., George, J.N. 1988. Cl^--HCO_3^- exchange is present with Na^+-K^+-Cl^- cotransport in rabbit parotid acinar basolateral membranes. *Am. J. Physiol.* **254:**C391–C396