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pH dependence of Cl/HCO_3 exchanger in the rat jejunal enterocyte

Marisa Tosco [a,*], Maria Novella Orsenigo [a], Giulia Gastaldi [b], Alide Faelli [a]

[a] *Dipartimento di Fisiologia e Biochimica Generali, Università di Milano, via Celoria 26, I-20133 Milan, Italy*
[b] *Istituto di Fisiologia Umana, Università di Pavia, via Forlanini 6, I-27100 Pavia, Italy*

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Abstract

During bicarbonate absorption in rat jejunum, a Cl/HCO_3 exchanger mediates bicarbonate extrusion across the basolateral membrane of the enterocyte. Previous studies demonstrated that anion antiport exhibits a particular behaviour: its activity is positively affected by the presence of sodium, but the cation is not translocated by the carrier protein. In view of the particular features of the jejunal Cl/HCO_3 antiporter, first we performed a pharmacological characterisation of the transport protein using various Cl channels blockers. Then, since it is well known that anion exchangers play a substantial role in cell pH regulation, we investigated the possible involvement of jejunal basolateral Cl/HCO_3 antiporter in intracellular pH maintenance. The sensitivity of the exchanger to pH was investigated by measuring ^{36}Cl uptake into basolateral membrane vesicles either varying simultaneously intra- and extravesicular pH, or presetting at 7.4 external pH and varying only the internal one. Experiments were performed both in the absence and in the presence of Na. In all the tested conditions, uptake peaked at pH of about 7.3–7.4 and then decreased, suggesting that the main function of Cl/HCO_3 exchanger is related to HCO_3 absorption rather than to intracellular pH control. Since pH-regulating mechanisms counteracting acidification are well known in the jejunal enterocyte, we investigated how it regulates pH after alkalinisation of the cytosol. We tested both basolateral and brush border membrane vesicles for the presence of a K/H exchanger, but we could not give evidence for its presence by means of ^{86}Rb uptake experiments. In conclusion, the jejunal enterocyte seems to lack a mechanism counteracting cellular alkalinisation: the main purpose of pH homeostasis might be to hinder acidification of the cytosol due to influx of protons and production of acid by the metabolism.

Keywords: Membrane vesicle; pH regulation; Cl/HCO_3 exchanger; Cl channel blocker; K/H exchanger

1. Introduction

The jejunal tract of rat intestine absorbs bicarbonate. A Cl/HCO_3 exchanger, evidenced in basolateral membranes isolated from the rat jejunal enterocyte [1,2] could account for HCO_3 efflux from the cell during its absorption. Up to now, two main types of anion exchange mechanisms have been reported: a cation-independent antiport [3–6] and a Na-dependent one that couples Na and HCO_3 fluxes [3,6–9]. These two systems coexist in several cell types [4,6,7,9].

The jejunal Cl/HCO_3 exchanger exhibits a particular behaviour: the presence of Na positively affects the rate of anion antiport, but Na is not transported [10]. Experiments performed to investigate the side of Na action [11] suggested that two regulatory Na sites, located on the inner and outer membrane surfaces, are involved in the protein carrier activation, but the intracellular Na-sensitive modifier site seems

* Corresponding author. Fax: +39 (2) 7064-4702; E-mail: marisa.tosco@unimi.it

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to be mainly responsible for the modulation of Cl/HCO_3 exchange activity [12]. The characteristic interaction of the jejunal anion antiporter with Na ion was confirmed by using intact isolated enterocytes [13] and was functionally expressed in *Xenopus laevis* oocytes [14].

In mammalian cells, the level of intracellular pH (pH_i) is regulated within the narrow boundaries that are compatible with normal cellular function. At least three membrane-based transport systems are known to be involved in the regulation of pH_i: the Na/H exchanger is a ubiquitous protein that, under physiological conditions, operates to export protons from the cell [15–17]; anion exchange also contributes to the regulation of pH_i and at levels of pH_i close to neutrality may be more active than Na/H exchange in some cell lines [15]: the Na-independent Cl/HCO_3 antiport extrudes HCO_3 from the cells in exchange for Cl ions and thereby reduces pH_i [15–18]; the Na-linked Cl/HCO_3 antiport exchanges extracellular Na and HCO_3 for intracellular Cl and thereby alkalinizes the cytosol [3,6–9]. Other transport mechanisms as well may be involved in the regulation of pH_i, e.g. the electrogenic Na-$(HCO_3)_n$ cotransport [19] and K/H exchange [20–24], besides ATP-driven proton pumps [25,26].

In view of the particular features of the jejunal Cl/HCO_3 exchanger, aim of this work was to perform a pharmacological characterization of the transport protein and to assess its putative involvement in steady state intracellular pH maintenance.

2. Materials and methods

2.1. Basolateral membrane isolation

Two male albino rats (Wistar strain, Charles River, Italy) weighing 250–300 g (about 2 months age), fed a rodent laboratory chow and tap water, were used for each experiment. Jejunal enterocytes were collected by scraping off the mucosal layer and diluted in 250 mM sucrose, 1 mM dithiothreitol, 0.2 mM phenylmethanesulphonilfluoride (PMSF), 0.01% (v/v) ethanol, 20 mM HEPES/Tris buffer, pH 7.5. Basolateral plasma membranes were isolated and purified as described previously [27]. Briefly, basolateral membranes collected by self-orienting Percoll-gradient centrifugation (Kontron, Centrikon mod. T 2070 ultracentrifuge; Haake-Buchler, Auto Densi-Flow IIC apparatus), were suspended in the appropriate buffer (see single experiment). Seven mM $CaCl_2$, which aggregates preferentially all membranes except brush border, was added. Collected pellets (basolateral membrane fraction) were washed and used for analysis and for uptake experiments by a rapid microfiltration technique.

2.2. Brush border membrane isolation

Brush border membrane vesicles were isolated from enterocytes by the Ca^{2+} precipitation method first described by Schmitz et al. [28]. Mucosal scrapings, homogenized in hypotonic solution (50 mM sorbitol and 2 mM Tris-Cl, pH 7.1) and incubated with 10 mM $MgCl_2$ for 15 min at O°C, were centrifuged at $3000 \times g$ for 15 min. The supernatant was centrifuged at $27\,000 \times g$ for 30 min. The pellet was resuspended in the appropriate buffer and centrifuged at $43\,000 \times g$ for 20 min. The final pellet was resuspended again in the above solution.

To control the purity of the membrane fractions, as a rule total protein, γ-glutamyltransferase (γ-GT, a marker enzyme for brush border membrane) and Na,K-ATPase (a marker enzyme for basolateral membranes) were determined as published [27]

2.3. Uptake experiments

Transport of either 1 mM ^{36}Cl (0.51 MBq/mg Cl, Amersham, Little Chalfont, UK), or 0.1 mM ^{86}Rb (22.15 MBq/mg Rb, Amersham, Little Chalfont, UK), or 1 mM $H^{14}CO_3$ (0.2–0.3 MBq/mmol, crystalline solid, N.E.N., Boston, MA, USA) into vesicles was measured at 28°C by a rapid microfiltration technique. Due to the low specific activity of both ^{36}Cl and $H^{14}CO_3$, the unlabelled forms were omitted from the incubation medium. A volume of membrane suspension (2–4 mg protein/ml) equilibrated with 0.2 mM EGTA, was mixed with the proper incubation solution (zero time). The composition of the resuspension buffers and incubation media are given in the legends of the figures. Samples were removed at selected times and diluted with 0.8 ml of ice-cold reaction stopping solution (122 mM CH_3COONa, 0.2 mM PMSF, 0.01% (v/v) ethanol,

20 mM either HEPES/Tris or MES/Tris buffer at the same pH of the incubating solution), 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 1600 TR).

All experiments were performed under voltage-clamp conditions, either by means of valinomycin and equal intra- and extravesicular K concentrations or by means of equimolar NO_3^-. The solutions used were prefiltered through a 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. Special precautions were taken to perform $H^{14}CO_3$ uptake, as previously described [2]: in particular, transport was measured at pH 8.2 and experiments lasting not more than 30 min were performed; to take into account the decrease of specific activity of labelled bicarbonate (always about 10% at the end of the experiment), samples of the reaction mixture were withdrawn at the same selected times and used as standards.

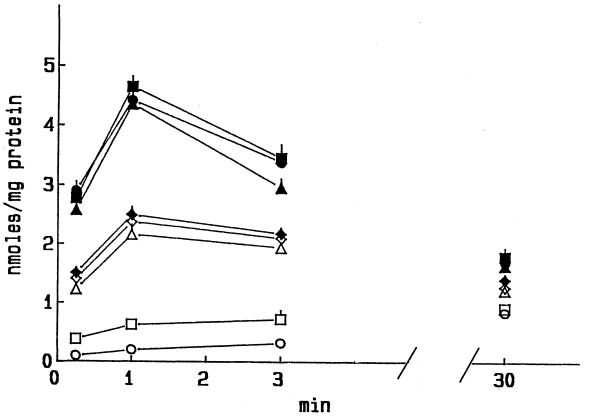

Fig. 1. Effects of different inhibitors on Cl/HCO_3 exchange. Twenty μl jejunal basolateral membrane vesicles containing either 196 mM sorbitol (open circles) or 100 mM NaCl (all other symbols) was incubated in 380 μl of 196 mM sorbitol alone (open and filled circles), or added with either 0.1 mM DIDS (open squares), or 0.1 mM NPPB (open triangles), or 0.2 mM flufenamic acid (open diamonds), or 0.2 mM niflumic acid (filled diamonds), or 0.2 mM DPC (filled triangles) or 0.2 mM 9AC (filled squares). All incubating solutions contained 1 mM $H^{14}CO_3^-$ and 0.1% (v/v) ethanol. All solutions contained 20 mM HEPES/KOH buffer (pH 8.2), 30 mM K-gluconate, 0.2 mM PMSF and 0.01% (v/v) ethanol. Vesicles were preincubated with 25 μM valinomycin. Ordinate; HCO_3^- uptake, mean values ± S.E. (vertical bars, absent if less than symbol height). Abscissa: incubation time.

3. Results

Basolateral membrane fraction is well purified from other membraneous components: from the Na,K-ATPase and γ-GT determinations, we calculated that the basolateral membranes are enriched about 12 times over the initial homogenate, whilst brush border is reduced about to a half. Moreover, in a previous work [1], we demonstrated that Cl/HCO_3 exchanger is indeed basolateral, since in jejunal brush border membrane this mechanism, if present, has a very low transport rate.

In spite of the particular interaction with Na ion, jejunal Cl/HCO_3 exchanger shares various properties with other anion antiporters, among which the stilbene disulphonate sensitivity [1,2]. Because DIDS is a blocker of some Cl channels [29] and because various anion exchangers are sensitive to inhibition by some channel blockers [30], a series of these drugs has been tested for the effectiveness on jejunal Cl/HCO_3 antiport. Anion antiporter activity has been evaluated by measuring $H^{14}CO_3$ uptake driven by an outwardly directed Cl gradient [2]. Data of Fig. 1 show that 0.2 mM 9-anthracene carboxylic acid (9AC) and 0.2 mM diphenylcarboxylic acid (DPC) have practically no inhibitory activity, whereas 0.2 mM niflumic acid, 0.2 mM flufenamic acid and 0.1 mM 5-nitro-2′-(3-phenylpropylamino) benzoic acid (NPPB) show roughly comparable inhibitory potency against jejunal antiporter. Obviously such an effect cannot be ascribed to the inhibition of Cl channels, since this reduces Cl efflux from the vesicles, thus enhancing the driving force for HCO_3 uptake. None of the tested drugs was more potent than DIDS.

Since in a previous study evidence was given that Cl/HCO_3 antiport can perform Cl/Cl self exchange [1], in the subsequent proceeding anion exchange activity has been evaluated measuring Cl uptake driven by an outwardly directed Cl gradient. At this purpose vesicles were incubated for 10 s either in the presence (effect) or in the absence (control) of an outwardly directed Cl gradient. Fig. 2 depicts the Cl gradient-driven Cl uptake (obtained by subtract-

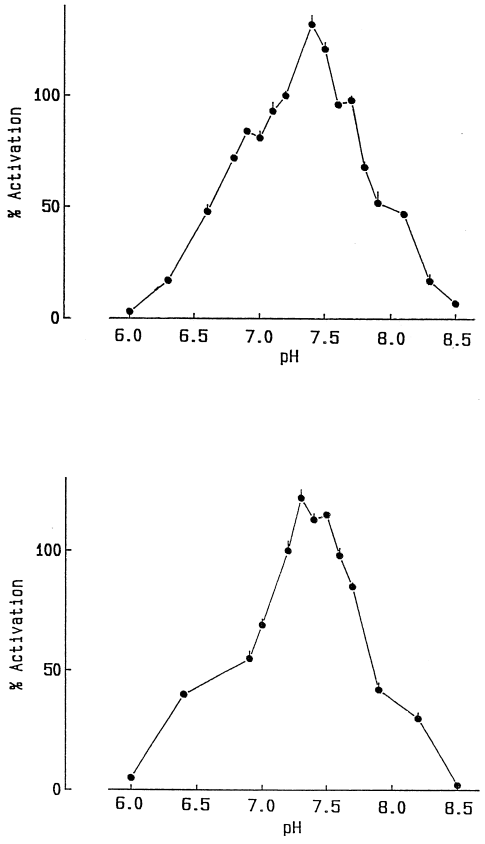

Fig. 2. Effect of pH ($pH_{in} = pH_{out}$) on $Cl/^{36}Cl$ exchange. Forty μl jejunal basolateral membrane vesicles was incubated for 10 s in 360 μl of incubating solution either in the presence (effect) or in the absence (control) of an outwardly directed Cl gradient. The Cl gradient-driven uptake was obtained by subtracting the latter value from the former one. Data are reported as percentages of the value obtained at pH 7.2. All incubating solutions contained 1 mM ^{36}Cl. All solutions contained 100 mM Tris/HEPES/MES buffer at various pH values (6–8.5), 0.2 mM PMSF and 0.01% ethanol (v/v). Intra- and extravesicular solutions were always buffered at the same pH. Vesicles were preincubated with 25 μM valinomycin. Upper panel: uptakes performed in the absence of Na. The intravesicular solutions contained either 50 mM KCl and 33 mM sorbitol (effect) or 25 mM K_2SO_4 and 70 mM sorbitol (control); the incubating solutions contained either 25 mM K_2SO_4 and 70 mM sorbitol (effect) or 25 mM K_2SO_4, 5 mM KCl and 66 mM sorbitol (control). Lower panel: uptakes performed in the presence of 20 mM intravesicular and 100 mM extravesicular Na. The intravesicular solutions contained either 20 mM NaCl, 30 mM KCl, 10 mM K_2SO_4 and 60 mM sorbitol (effect) or 25 mM K_2SO_4, 10 mM Na_2SO_4 and 87 mM sorbitol (control) and the incubating solutions contained either 50 mM Na_2SO_4 and 25 mM K_2SO_4 (effect) or 47.5 mM Na_2SO_4, 5 mM NaCl and 25 mM K_2SO_4 (control).

ing the control data from the effect ones) as a function of equal intra- and extravesicular pH. Uptakes are expressed as percentages of the value obtained at pH 7.2, that should correspond to the resting intracellular pH in intestinal epithelial cells [36,37].

In the same figure, results obtained both in the absence (upper panel) and in the presence of Na (lower panel) are reported. In the latter case, intra- and extravesicular Na concentrations mimicked the intra- and extracellular ones (20 and 100 mM respectively): this was consistent with the previous finding that basolateral membrane vesicles are 90% right side out oriented [11]. All uptake values were higher in the presence of sodium, as previously reported [10–12], but in Fig. 2 they are expressed as percentages of the value obtained at pH 7.2. From data reported in the figure, it is evident that there is a very strong pH dependence of anion exchange activity, but no sig-

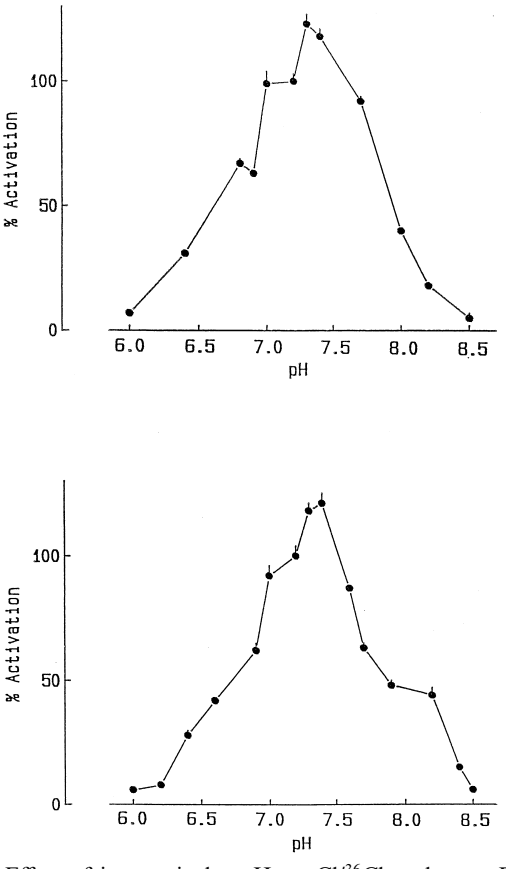

Fig. 3. Effect of intravesicular pH on $Cl/^{36}Cl$ exchange. Experimental protocol as in Fig. 2. Incubating solutions were always buffered with 100 mM HEPES/Tris pH 7.4, while intravesicular pH was varied (6–8.5) using Tris/HEPES/MES buffer.

nificant difference is apparent in the presence and absence of Na ion and the maximal transport rate occurs in both cases at pH of about 7.3–7.4. Since the extent of activation decreases at both acidic and alkaline pHs, the base movement generated by the anion exchanger does not seem to be switched by pH shifts.

Because anion antiport activities in other cells have been reported to vary with cytoplasmic pH, it was of interest to determine the effect of clamping intravesicular pH at several values, keeping constant the external solution at pH 7.4. Fig. 3 illustrates the results obtained using this experimental protocol, carried out both in the absence (upper panel) and in the presence of Na (lower panel), as in Fig. 2. Data reported in Fig. 3 demonstrate that the anion exchange activity is also sensitive to changes in intravesicular pH only, but results are similar to those reported in Fig. 2 as to the maximally activating pH and the ineffectiveness of Na ion on the shape of the curve. Thus, from these data it seems reasonable to conclude that jejunal anion exchanger does

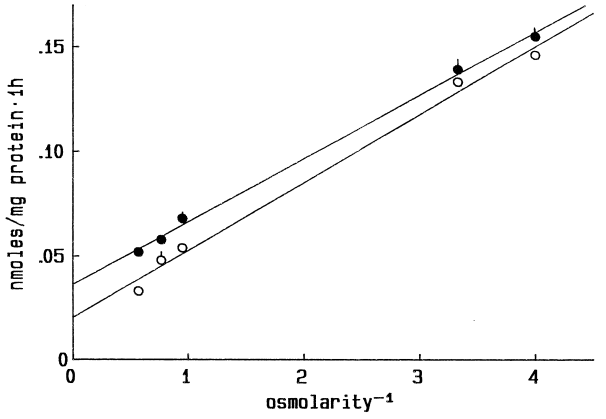

Fig. 4. Effect of extravesicular osmolarity on the uptake of 0.1 mM ^{86}Rb. One hundred and fifty μl jejunal basolateral membrane vesicles (0.5–1 mg protein/ml) were incubated with 300 μl of incubation medium. Vesicles contained 24 mM sucrose and 100 mM HEPES/MES/Tris buffer either at pH 8.0 (filled circles) or at pH 6.0 (open circles). The incubation medium contained 100 mM HEPES/MES/Tris buffer either at pH 8.0 (filled circles) or at pH 6.0 (open circles), $RbCl_2$ (0.1 mM) plus trace amounts of ^{86}Rb and 24, 74, 824, 1074 or 1524 mM sucrose. All solutions contained 70 mM $NaNO_3$, 0.2 mM PMSF and 0.01% ethanol. In both experiments, after 60 min incubation, 100 μl samples were processed as described. Ordinate: Rb uptake, mean values ± S.E. (vertical bars, absent if less than symbol height). Abscissa: 1/osmolarity.

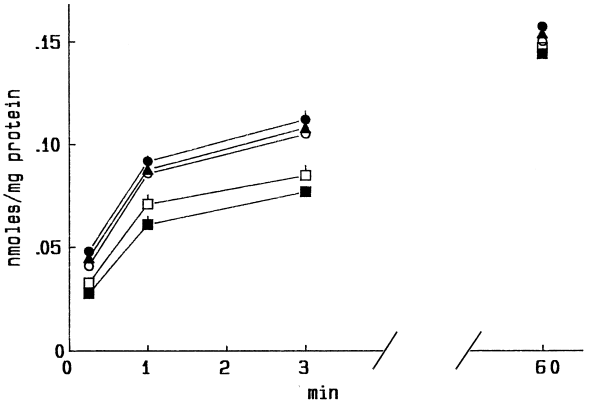

Fig. 5. Rb (0.1 mM) uptake into jejunal basolateral membrane vesicles. Forty μl vesicles containing 45 mM sorbitol and 100 mM MES/Tris buffer pH 6.0, pre-equilibrated (filled triangles) or not (all other symbols) with 50 μM FCCP, were incubated in 360 μl of either 45 mM sorbitol and 100 mM HEPES/Tris buffer at pH 8.0 (filled circles), or 45 mM sorbitol, 50 μM FCCP and 100 mM HEPES/Tris buffer at pH 8.0 (filled triangles), or 45 mM sorbitol and 100 mM MES/Tris buffer at pH 6.0 (open circles), or 35 mM sorbitol, 4 mM $BaCl_2$ and 100 mM HEPES/Tris buffer at pH 8.0 (open squares), or 25 mM K-gluconate and 100 mM HEPES/Tris buffer at pH 8.0 (filled squares). All incubating solutions contained 1 mM amiloride and 0.1 mM RbCl plus trace amounts of ^{86}Rb. All solutions contained 70 mM $NaNO_3$, 0.2 mM PMSF and 0.11% (v/v) ethanol. Ordinate: Rb uptake, mean values ± S.E. (vertical bars, absent if less than symbol height). Abscissa: incubation time.

not subserve a function such as cell pH defense, being probably mainly involved in HCO_3 absorption.

How does the jejunal enterocyte regulate its intracellular pH? It is well known that it possesses a mechanism counteracting cytosolic acidification, namely the housekeeping Na/H exchanger [15–17]. Na/H exchangers are located both in brush border [31] and in basolateral membrane [32]; however, the enterocyte should also defend cell pH against alkalinization and would require a transporter which acts as an acidifying mechanism in response to a basic load. Since Cl/HCO_3 does not seem to play this role and $Na\text{-}(HCO_3)_n$ is not present in the jejunal enterocyte [1], we tested for a possible K/H exchange activity, that has been reported in other intestinal tracts [20,24]. Using rubidium as a tracer for potassium, firstly we demonstrated that 0.1 mM Rb uptake at 60 min is inversely proportional to the osmolarity of the incubation medium (Fig. 4); a certain binding (23% at pH 8 and 13% at pH 6) is detectable in the presence of 70 mM Na. Rb uptake into baso-

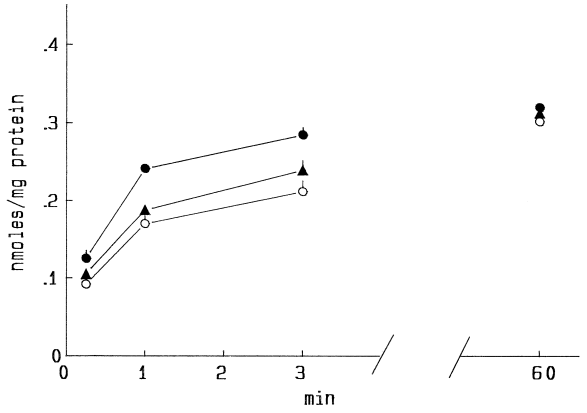

Fig. 6. Rb (0.1 mM) uptake into ileal brush border membrane vesicles. Forty μl vesicles containing 45 mM sorbitol and 100 mM MES/Tris buffer pH 6.0, pre-equilibrated (filled triangles) or not (open and filled circles) with 50 μM FCCP, were incubated in either 360 μl of 45 mM sorbitol and 100 mM HEPES/Tris buffer at pH 8.0 (filled circles), or 45 mM sorbitol, 50 μM FCCP and 100 mM HEPES/Tris buffer at pH 8.0 (filled triangles), or 45 mM sorbitol and 100 mM MES/Tris buffer at pH 6.0 (open circles). All incubating solutions contained 1 mM amiloride and 0.1 mM RbCl plus trace amounts of ^{86}Rb. All solutions contained 70 mM $NaNO_3$, 0.2 mM PMSF and 0.11% (v/v) ethanol. Ordinate: Rb uptake, mean values ± S.E. (vertical bars, absent if less than symbol height). Abscissa: incubation time.

lateral membrane vesicles was studied under different experimental conditions: data of Fig. 5 indicate that Rb uptake is inhibited by 4 mM Ba and by 25 mM K, but is unaffected by an outwardly directed proton gradient, with or without the protonophore FCCP. These findings do not support the presence of K/H antiport in basolateral membranes. Since this mechanism was detected in rat ileal brush border [20] and in brush border of chick intestine [24], we carried out the same experimental protocol using jejunal brush border membranes; although higher uptake values were obtained with brush border, due probably to the greater percentage of sealed vesicles [11,33], the pattern of the results (not reported) overlap the one depicted in Fig. 5.

Thus, there is no evidence for the presence of K/H antiport in the jejunal enterocyte. To better strengthen this conclusion, the presence of K/H exchanger was investigated in ileal brush border: actually, data reported in Fig. 6 show that Rb uptake is stimulated by the imposed pH gradient and the pH effect is reduced by FCCP. Thus, results of Fig. 6 are in agreement with those reported in the literature.

4. Discussion

In rat intestine, an Na-independent Cl/HCO_3 exchanger is located in ileum brush border membranes [34]; on the contrary, the anion exchanger is localized only at the basolateral pole of the enterocyte in the jejunal tract [1]. The particular features of its interaction with Na ion make jejunal anion antiporter different from those described in the literature [11].

None of the drugs tested in the current study (Fig. 1) was a potent inhibitor of anion exchanger and no one was more potent than DIDS. The tested substances are blockers of Cl channels and belong to a large group of arylaminoalkyl benzoates, originally derived from anthracene-9-carboxylate [30]. DPC is closely related to a group of non-steroidal anti-inflammatory drugs that were found to block anion exchanger in the red blood cells; it is apparent from Fig. 1 that it is ineffective on jejunal antiporter. NPPB exhibits a structural similarity with loop diuretics of the furosemide type and, as a matter of fact, its inhibitory action is comparable to the one exerted by furosemide on jejunal exchanger [10]. Flufenamic and niflumic acids display a similar inhibitory activity. The partial inhibition of Cl/HCO_3 antiport by Cl channels blockers differentiates jejunal antiporter from other anion exchangers; this may suggest that jejunal basolateral exchanger and Cl channels could share some common structural motifs.

The main objective of this study was to gain insight into the pH influence on the activity of the jejunal Cl/HCO_3 exchanger in light of its potential contribution to intracellular pH homeostasis. Since evidence was given that anion antiporter can perform Cl/Cl self-exchange [1], we measured only $^{36}Cl/Cl$ exchange, ruling out any interference with transport of HCO_3, the availability of which is strongly pH-dependent. Therefore we could measure the dependence of anion antiport rates on pH in the absence of significant changes in the driving force of exchangeable anions [18].

Results of Fig. 2 demonstrate that anion exchanger is inactive at pH 6, increases in activity as pH raises and once again becomes progressively inactive at high levels of pH. The presence of sodium, in concentrations mimicking the intra- and extracellular ones, increases the rate of operation of the antiporter

at all tested pH values, but no discrepancies are apparent concerning both the shape of the curve and the maximally stimulating pH.

The data in Fig. 2 were obtained by varying simultaneously the pH of intra- and extravesicular solutions, corresponding, respectively, to the cytosolic and extracellular environments due to the prevailing right side out orientation of membrane vesicles [11]. In a number of cell types, anion exchange is activated by shifts in intracellular pH [15,16,35–38]. However, all studies performed in intact cells have the problem that, because of the limited manipulability of the intracellular ionic environment, the transmembrane anion concentrations cannot be strictly controlled. Our membrane vesicle preparation allows to measure the pH_i dependence of Cl uptake into Cl-loaded vesicles, keeping constant pH_o. Indeed, evidence was given that transmembrane pH gradient per se cannot drive Cl movement since Cl/OH exchange process is missing in jejunal basolateral membranes [10]. Results, reported in Fig. 3, do not differ significantly from the ones of Fig. 2. Thus, the observed changes in activity of the exchanger could be due to a direct effect of pH_i on its rate of operation. We do not have any explanation for the effect of pH on the exchanger activity. It could be likely that it merely exhibits an optimum stimulating pH at 7.4, rather than being involved in pH homeostasis. Anyhow, in the whole cell an indirect regulation of Cl/HCO_3 antiporter activity is certainly accomplished by pH_i, since it strongly affects HCO_3 concentration.

In a number of cell types, an acidic pH activates Na-dependent Cl/HCO_3 exchange rate [3,4,6–9], whereas an alkaline pH increases Na-independent anion exchange activity [15,16,35–38]; in some tissues an allosteric activation of the exchanger by the pH_i takes place [35,36]. The pH dependency curve of basolateral membrane anion exchange rates indicates that Cl/HCO_3 antiporter is maximally activated at pH of at about 7.3–7.4. At pH 7.2, which should correspond to the resting intracellular value of intestinal epithelial cells [36,37], anion exchanger seems to be already activated to a great extent. As a matter of fact, in epithelia that effect transepithelial acid/base transport, membrane transporters must be active at resting values of cell pH [17,18], while in non-polar cells it is more energetically favourable for these transporters to be quiescent, being activated only when needed [39].

To sum up, we can exclude a major role for jejunal anion antiporter in contributing to the recovery of pH_i, ruling out also the presence of a pH-sensitive modifier site on the transport protein. The main function of the exchanger is probably linked to bicarbonate transport: this could account for the polarized localization of Cl/HCO_3 antiport in different intestinal tracts, e.g. basolaterally in the jejunum, where HCO_3 is absorbed, apically in ileum and duodenum, where HCO_3 is secreted.

Plasma membrane anion exchangers (AE) constitute a diverse family of transporters. Three members of this family, designated AE1, AE2 and AE3 have been cloned [40–42]; their physiological roles differ widely among the cell types in which they are expressed, however it is likely that all these proteins play a role in the regulation of intracellular pH in their respective tissue [40]. In the light of results of this study, jejunal basolateral anion exchanger seems to differ functionally from AE gene family both for its characteristic interaction with Na ion and for its relative ineffectiveness in pH_i control.

In this scenario, it remains obscure how the jejunal enterocyte could counteract cytosolic alkalinization, as $Na\text{-}(HCO_3)_n$ cotransport does not seem to be present in this section of the intestinal tract [1]. Therefore we tested for the existence of K/H exchanger, that has been detected in the inner membrane of mitochondria [43] and in the plasma membrane of different cell types [20–24] and that functions as a proton loader. Due to the negative charges of membrane surface, a considerable binding of ^{86}Rb on vesicles was detected (not reported data); nevertheless, binding was reduced to a great extent by adding 70 mM sodium in the incubating solution (Fig. 4). The presence of K/H exchange was tested both in basolateral (Fig. 5) and in brush border membrane vesicles (not reported data), but in both membrane preparations it was apparent that Rb moves through a Ba-sensitive K channel [44] and pH gradient is ineffective on its uptake. One mM amiloride was present in this experimental protocol to prevent pH gradient dissipation caused by Na/H exchange activity; however, given that amiloride might be inhibitory also on K/H antiporter [23], some experiments were performed in the absence of

the drug by substituting Na with tethramethylammonium, that cannot be a substrate for Na/H exchanger [32]. As a matter of fact, results did not change.

Thus, the jejunal enterocyte seem to lack an acidifying mechanism acting in response to a basic load. Two possibilities arise: either an alternative system is involved in this process, or such a mechanism is not necessary. Actually, it is known that the jejunal enterocyte itself produces large quantities of lactic acid, despite an adequate oxygen supply [45,46]: this would not allow the set up of alkaline conditions. In light of the above discussion, this point should be reconsidered.

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