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Bicarbonate and chloride transport across rat ileal basolateral membrane

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Abstract. The mechanisms of HCO_3^- and Cl^- transport across basolateral membranes from rat ileum were investigated in isolated vesicles by means of uptake experiments. Neither Cl^-/HCO_3^- exchanger nor Na^+-$(HCO_3^-)_n$ cotransport seem to be present in ileal basolateral membranes. Moreover Cl^- uptake is unaffected by *cis* Na^+ and/or K^+ gradients, indicating the absence of Na^+-Cl^-, K^+-Cl^- and Na^+-K^+-$2Cl^-$ symport activity. An electrically conductive pathway seems to be responsible for both HCO_3^- and Cl^- fluxes.
Evidence is also given for the presence of a Na^+/H^+ exchanger at the basolateral pole of ileal enterocytes.
Key words. Rat ileum; basolateral membrane vesicles; bicarbonate and chloride transport.

It has long been established that there is a great deal of heterogeneity of transport functions in different segments of the mammalian small intestine[1,2]. There is evidence that the absorption of sodium is obligatorily linked to the absorption of bicarbonate in the jejunum[3], whereas in the ileum sodium and chloride are absorbed and HCO_3^- is secreted[4]. Bicarbonate absorption in the jejunum is actually the result of two steps: proton secretion by means of a Na^+/H^+ exchange system located at the apical pole of the enterocyte[5], and HCO_3^- exit across the basolateral membrane by means of a Cl^-/HCO_3^- antiporter[6–9]. In the ileum there is evidence for a Cl^-/HCO_3^- exchange mechanism, operating in parallel to the Na^+/H^+ exchanger in the brush border membrane. The operation of these two systems would result in NaCl absorption and HCO_3^- secretion[4,10–12].
The heterogeneity of the mammalian small intestine with regard to functional differences between different regions (e.g. ileum versus jejunum) may depend on the transport properties of the apical and basolateral membranes. This study was designed to characterize the transport mechanisms mediating Cl^- and HCO_3^- movements in rat ileal basolateral membranes, in order to explore the contribution of these membranes to Cl^- absorption and HCO_3^- secretion processes.

Materials and methods

Basolateral membrane isolation. Two male albino rats (Wistar strain, Charles River Italia, Calco) weighing 250–300 g (about two months old), fed on rodent laboratory chow and tap water, were used for each experiment. Ileal enterocytes were collected by scraping off the mucosal layer, and suspended in 250 mM sucrose, 0.2 mM phenylmethanesulphonylfluoride (PMSF), 0.01% (v/v) ethanol, 10 mM Hepes/Tris buffer, pH 7.5. Basolateral plasma membranes were isolated and purified as described previously[13]. 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 individual experiments). 7 mM $CaCl_2$, which preferentially aggregates all membranes except brush border ones, was added. The collected pellets (basolateral membrane fraction) were washed and used for analysis and for uptake experiments. To ensure that the intravesicular space was loaded with the appropriate buffer, the collected pellets were further incubated in the same buffer at room temperature (gassed with the appropriate CO_2 tension when $NaHCO_3$ was present) and used after that for investigation of uptake by the rapid micro-filtration technique.

Enzyme activities. The activity of (Na, K)-ATPase (a marker enzyme for basolateral membrane) was estimated by the method of Schoner et al.[14], slightly modified. In this method the resynthesis of the ATP split by the ATPase is coupled via the 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 pre-incubated at room temperature for 20 min with the ionic detergent sodiumdodecylsulphate (SDS) at different concentrations, keeping the ratio mg protein:mg SDS = 1:0.1–0.05. Then both total ATPase and (Na, K)-ATPase

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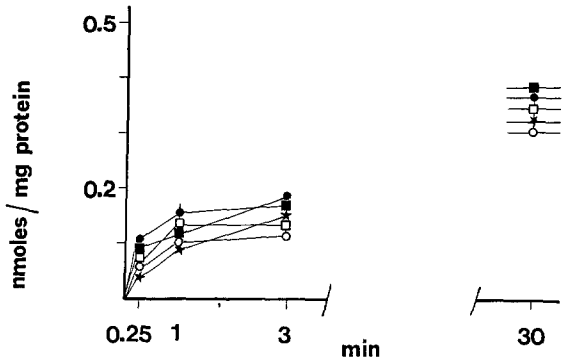

Figure 1. 1 mM HCO_3^- uptake into vesicles preloaded with different anions. 24 µl basolateral membrane vesicles, prepared either in 100 mM NaCl (filled circles), or in 100 mM NaBr (filled squares), or in 100 mM $NaNO_3$ (filled stars), or in 100 mM Na acetate (open squares), or in 186 mM sorbitol (open circles) were incubated in 456 µl of 1 mM $H^{14}CO_3^-$ and 186 mM sorbitol. All solutions contained 100 mM Hepes/Tris buffer (pH 8.2), 100 mM K gluconate, 0.2 mM PMSF and 0.01% (v/v) ethanol. All vesicles were pre-incubated with 25 µM valinomycin. Ordinate: HCO_3^- uptake, mean values ±SE (=vertical bars, absent if less than symbol height). Abscissa: incubation time.

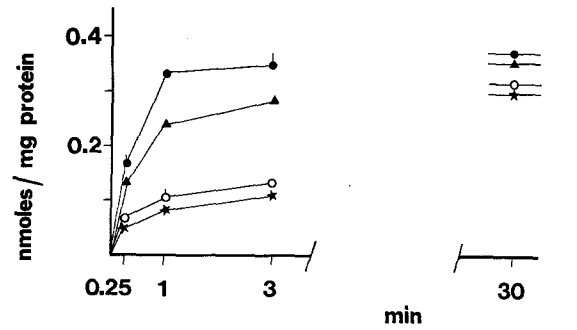

Figure 2. Effect of membrane potential on 1 mM HCO_3^- uptake. 20 µl basolateral membrane vesicles prepared in 220 mM sorbitol and 1 mM K_2SO_4 were incubated in 380 µl of either 100 mM K_2SO_4 (filled circles), or 10 mM K_2SO_4 and 200 mM sorbitol (triangles), or 1 mM K_2SO_4 and 220 mM sorbitol (open circles), or 0.1 mM K_2SO_4 and 222 mM sorbitol (stars). 1 mM $H^{14}CO_3^-$ was in all incubating solutions. All solutions contained 100 mM Hepes/Tris buffer (pH 8.2), 0.2 mM PMSF and 0.01% (v/v) ethanol. Vesicles were pre-incubated with 25 µM valinomycin. Ordinate: HCO_3 uptake, mean values ±SE (=vertical bars, absent if less than symbol height). Abscissa: incubation time.

were assayed and orientation was evaluated according to Boumendil-Podevin and Podevin[15].

To control the purity of the basolateral membrane fraction, total protein, γ-glutamyltransferase (γ-GT, a marker enzyme for brush border membrane), KCN-resistant NADH oxidoreductase (a marker enzyme for endoplasmic reticulum) and cytochrome c oxidase (a marker enzyme for mitochondria) were also determined as published[13].

Uptake experiments. Chloride, bicarbonate, sodium and D-glucose uptakes were measured. A volume of basolateral membrane suspension containing 2–4 mg protein/ml, equilibrated with 0.2 mM EGTA, was mixed at 28 °C with the appropriate incubation solution. The composition of the resuspension buffers and incubation media are given in the legends of the figures. Samples were taken at selected times and diluted with 0.8 ml ice-cold reaction-stopping solution (20 mM Hepes/Tris buffer pH either 7.5 or 8.2, 0.2 mM PMSF, 0.01% (v/v) ethanol, made isoosmotic with either CH_3COONa, or CH_3COOK, or KCl, according to the experiment), 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 under voltage-clamp conditions, except in the case reported in figure 2, where a diffusion potential was superimposed. The solutions used were pre-filtered through 0.22 µm pore size filters. Individual uptake experiments in triplicate, representing more than three repetitions with qualitatively identical results, are presented throughout the paper. Details of experiments are reported in the legends to the figures. Special precautions were taken when assessing HCO_3^- uptake, as previously described[7].

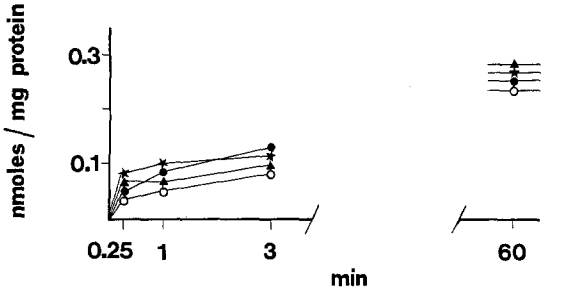

Figure 3. 1 mM $^{36}Cl^-$ uptake in the presence of sodium and/or potassium. 44 µl basolateral membrane vesicles obtained in 110 mM sorbitol were incubated in 396 µl of either 25 mM Na_2SO_4 and 54 mM sorbitol (triangles), or 25 mM K_2SO_4 and 54 mM sorbitol (stars), or 25 mM Na_2SO_4 and 25 mM K_2SO_4 (filled circles), or 110 mM sorbitol (open circles). All incubating solutions contained 1 mM $^{36}Cl^-$. All solutions contained 100 mM $LiNO_3$, 100 mM Hepes/Tris buffer (pH 7.5), 0.2 mM PMSF and 0.01% (v/v) ethanol. Ordinate: Cl^- uptake, mean values ±SE (=vertical bars, absent if less than symbol height). Abscissa: incubation time.

Results

The purity of the basolateral membranes was checked by measuring various marker enzyme activities. (Na, K)-ATPase was 8 times enriched while γ-GT and all other marker enzymes tested are drastically reduced. The sidedness of our membrane preparation was determined by measuring the latency of (Na, K)-ATPase in another group of experiments. We calculated that the ratio of unsealed to sealed vesicles is 2:1; sealed vesicles are about 65% right-side-out (RSO) and 35% inside out (IO) oriented. Similar results were obtained using fresh and frozen (−80 °C) membranes.

Even if enzymatic analysis gave evidence that there was no significant brush border cross-contamination, to rule out definitely the possibility that our results were affected by the presence of apical membrane vesicles, the ability of our preparation to accumulate D-glucose against a concentration gradient was tested: the Na-glucose cotransport system is known to be localized in the apical membrane of the enterocytes[16]. D-glucose uptake did not increase when a Na^+ gradient instead of a K^+ gradient was imposed across the membrane vesicles and was unaffected by membrane potential (data not shown). The lack of effect suggests the absence of apical contamination of the basolateral membranes.

Firstly, we demonstrated that 1 mM HCO_3^- uptake at 30 min is inversely proportional to the osmolarity of the incubation medium (data not shown); evidence was also given that HCO_3^- binds to a certain extent on vesicle surface.

The subsequent experiments were designed to determine the effect of outwardly-directed anion gradients on HCO_3^- uptake. HCO_3^- was used since in previous work[7] we gave evidence that results obtained either with $H^{14}CO_3^-$ or with $^{36}Cl^-$ were overlapping. To exclude electrodiffusional coupling between ionic movements, this experiment (as well as the following ones) was performed under short-circuiting conditions, with equilibrated K^+ and valinomycin. As shown in figure 1, intravesicular Cl^-, Br^-, NO_3^-, CH_3COO^- are not capable of influencing HCO_3^- uptake, neither do SCN^-, NO_2^-, nor lactate (data not shown). These findings do not support the hypothesis that an anion exchanger is present at the basolateral pole of ileal enterocyte.

To study whether a Na^+-$(HCO_3^-)_n$ symporter exists in ileal basolateral membranes we performed: 1) HCO_3^- uptake experiments in the presence of inwardly directed Na^+, K^+ and Li^+ gradients; 2) Na^+ uptake experiments in the presence of an inwardly directed HCO_3^- gradient. The latter case was also carried out by superimposing an inside positive membrane potential. Our findings (not reported) indicate the absence of both Na-dependent HCO_3^- transport and HCO_3^--dependent Na^+ transport. Thus we can rule out the existence of a cation-HCO_3^- cotransport mechanism.

Taken together, these results indicate the absence of any transport system for bicarbonate in ileal basolateral membrane. To investigate the presence of a rheogenic pathway, the effect of a superimposed membrane potential on HCO_3^- uptake was evaluated. Results are reported in figure 2 and give evidence for a conductive bicarbonate influx. From the data of figure 2 it seems that uptake data after 30 min incubation are not well equilibrated: however, experiments lasting longer than 30 min are not feasible due to HCO_3^- instability, as already reported[7].

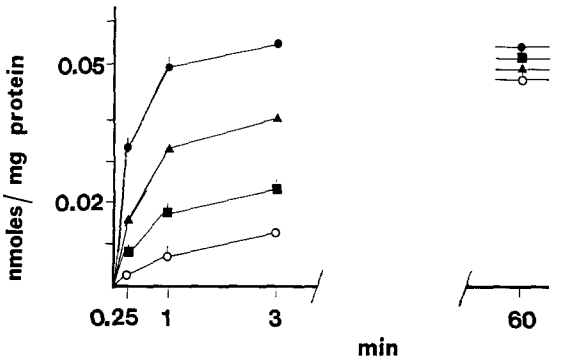

Figure 4. 0.1 mM Na^+ uptake in basolateral membrane vesicles. 25 µl basolateral membrane vesicles obtained in 100 mM Mes/Tris buffer, pH 5.5 and pre-equilibrated with 25 µM valinomycin were incubated in 225 µl of either 100 mM Hepes/Tris buffer, pH 7.5 (filled circles), or 100 mM Hepes/Tris buffer, pH 7.5 and 2 mM amiloride (squares), or 100 mM Hepes/Tris buffer, pH 7.5 and 50 µM FCCP (carbonylcyanide p-trifluoromethoxyphenylhydrazone, triangles), or 100 mM Mes/Tris buffer, pH 5.5 (open circles). 0.1 mM $^{22}Na^+$ was in all incubating solutions. All solutions contained 100 mM KCl, 0.2 mM PMSF and 0.11% (v/v) ethanol. Ordinate: Na^+ uptake, mean values ±SE (=vertical bars, absent if less than symbol height). Abscissa: incubation time.

We next set out to focus on the mechanism underlying the exit of Cl^- across the basolateral membrane. Since the results shown in figure 1 indicate that Cl^-/anion exchange plays no role in Cl^- movement, we undertook additional experiments to test for the existence of either Na^+-Cl^-, or K^+-Cl^-, or Na^+-K^+-$2Cl^-$ cotransport. The results, illustrated in figure 3, do not suggest any interaction between the fluxes of Na^+, K^+ and Cl^- ions in basolateral membranes. Once again, a possible potential-sensitive leaky pathway can be postulated. As a matter of fact, a superimposed membrane potential does affect Cl^- movement to a great extent (data not shown). To investigate whether Cl^- and HCO_3^- share the same conductive pathway, competition experiments between Cl^- and HCO_3^- uptake were carried out. Results (data not shown) seem to indicate that potential-dependent 2 mM Cl^- uptake is markedly (62%) inhibited by 20 mM extravesicular HCO_3^-, whereas potential-driven 2 mM HCO_3^- uptake is reduced to a lesser extent (12%) in the presence of external 20 mM Cl^-.

Since in previous work[17,18] we gave evidence for the presence of a Na^+/H^+ exchanger in the basolateral membrane of rat jejunal enterocyte[5], we addressed the question whether or not this transport protein works also in the ileal basolateral membrane. In order to do this, we investigated the effect of an outwardly directed proton gradient on Na^+ uptake. Results reported in figure 4 strongly suggest that basolateral membrane vesicles from ileal enterocyte possess a Na^+/H^+ antiport, which is affected by its specific inhibitor amiloride; moreover pH gradient-dependent Na^+ uptake is significantly reduced by the protonophore FCCP.

Discussion

The Percoll gradient technique of basolateral membrane vesicle preparation, applied to ileal enterocytes, yielded a membrane fraction enriched 8 times in (Na^+, K^+)-ATPase activity with a low contamination from non-basolateral sources. In previous work[9] a better basolateral membrane sample was obtained using the jejunal tract of rat intestine: the enrichment factor of (Na^+, K^+)-ATPase was higher and, moreover, the vesicles were mostly tight. This discrepancy is puzzling since, to our knowledge, there are no major structural differences at the basolateral pole of jejunal and ileal enterocytes: however, we did not succeed in improving the quality of this fraction. As a matter of fact, the relatively low percentage of sealed vesicles makes ileal basolateral membranes far from ideal for uptake studies: actually, we found low equilibrium uptake values in all experimental protocols.

In order to exclude definitely any contribution of brush border transport systems to our results, basolateral membrane vesicles were tested for their ability to accumulate D-glucose in the presence of an inward Na^+ gradient. The lack of effect is in agreement with the absence of brush border contamination.

A certain $H^{14}CO_3^-$ binding to vesicle membranes was evidenced (data not shown). Similar exerimental findings were obtained when $^{36}Cl^-$ was used instead of $H^{14}CO_3^-$: therefore we can hypothesize that positive charges are present on ileal membrane surfaces, which is a difference from jejunal basolateral membranes[6,7]. Cl^- binding was also found in basolateral membranes from lobster hepatopancreas[19].

The lack of a Cl^-/HCO_3^- antiport in the basolateral membrane domain of the ileal enterocyte (fig. 1) could be related to the HCO_3^- secreting function developed by this intestinal tract. As a matter of fact a different HCO_3^- transport polarity characterizes jejunum and ileum: in the former, in which HCO_3^- absorption occurs (at least in rat, dog and man[20,21]), the Cl^-/HCO_3^- exchanger is selectively localized in the basolateral membrane[6], whereas in the latter, which secretes HCO_3^-, the Cl^-/HCO_3^- exchanger seems to be confined to the apical pole[4,10,11]. Actually, in the duodenum and proximal colon HCO_3^- secretion is also related to a Cl^-/HCO_3^- antiport located in the brush border[22,2], but no definitive data are available about its presence in the basolateral membrane. Other authors[23,24] have also failed to find a Cl^-/HCO_3^- antiport in basolateral membranes from rabbit ileum.

A Cl^--independent Na^+-$(HCO_3^-)_n$ cotransport system effectuates the greater part of basolateral HCO_3^- efflux in the mammalian kidney. This cotransporter has a widespread distribution in the basolateral membrane of several epithelial tissues, where it relates not only to the absorptive processes as it happens in the proximal tubule, but also to secretive functions, as it was reported for rat duodenum[22], rat distal colon[25] and the oxyntic cells of the frog gastric fundus[26]. We failed to demonstrate the presence of Na^+-$(HCO_3^-)_n$ cotransport in rat ileal basolateral membranes: similarly this transport mechanism is reported to be absent from rabbit ileum[23]. Moreover Na^+-$(HCO_3^-)_n$ cotransport does not seem to work at the rat jejunal basolateral pole[6,7]. To sum up, it would be likely that HCO_3^- crosses ileal basolateral membranes only by means of conductive pathway (fig. 2).

In the ileal tract of the small intestine, Na^+ absorption is linked to Cl^- transport. Since Cl^-/HCO_3^- exchange was found to be missing from the ileal basolateral membrane (fig. 1), another kind of transport mechanism for Cl^- must be taken into account at the antiluminal surface. In various epithelial cell membranes Cl^- movement occurs via a cotransport with cations, namely K^+-Cl^- [27-29], Na^+-Cl^- [30,31], and Na^+-K^+-$2Cl^-$ [32-35]. As already evidenced in jejunal basolateral membrane[6], none of these cotransport systems seems to exist in rat ileal basolateral membrane (fig. 3). Thus, simple electrodiffusion of Cl^- through Cl^- conductance would provide a means of basolateral exit. Because other Cl^- channels have been found to have a significant permeability to HCO_3^- [36], we investigated whether Cl^- and HCO_3^- uptake might be mediated by the same conductive pathway. Competition experiments would suggest that a single conductance could serve as a route for both HCO_3^- and Cl^-, exhibiting however a higher permeability for HCO_3^- than for Cl^-.

In order to complete the comparison between ileal and jejunal basolateral membranes, we looked for the presence of a Na^+/H^+ antiport system, which in jejunal enterocytes was found to be symmetrically distributed through the entire plasma membrane, in spite of the functional differentiation between brush border and basolateral membrane[17,18]. The results shown in figure 4 consistently establish the presence of a Na^+/H^+ antiport system also in ileal basolateral membranes: this Na^+/H^+ exchange may fulfill cellular functions other than transport, including regulation of cell pH, control of cellular volume, modulation of cell growth and proliferation in response to mitogenic stimuli.

Together, the results of this work and those reported in the literature seem to indicate that the dominate mechanism responsible for HCO_3^- movement across ileal and jejunal enterocyte, e.g. Cl^-/HCO_3^- exchange, is located either at the apical pole (ileum) or at the basolateral one (jejunum), depending on the direction of HCO_3^- flux.

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