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$Cl^- - HCO_3^-$ exchange in rat renal basolateral membrane vesicles

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Pathways for HCO_3^- transport across the basolateral membrane were investigated using membrane vesicles isolated from rat renal cortex. The presence of $Cl^- - HCO_3^-$ exchange was assessed directly by $^{36}Cl^-$ tracer flux measurements and indirectly by determinations of acridine orange absorbance changes. Under 10% CO_2/90% N_2 the imposition of an outwardly directed HCO_3^- concentration gradient (pH_o 6/pH_i 7.5) stimulated Cl^- uptake compared to Cl^- uptake under 100% N_2 in the presence of a pH gradient alone. Mediated exchange of Cl^- for HCO_3^- was suggested by the HCO_3^- gradient-induced concentrative accumulation of intravesicular Cl^-. Maneuvers designed to offset the development of ion-gradient-induced diffusion potentials had no significant effect on the magnitude of HCO_3^- gradient-driven Cl^- uptake further suggesting chemical as opposed to electrical $Cl^- - HCO_3^-$ exchange coupling. Although basolateral membrane vesicle Cl^- uptake was observed to be voltage sensitive, the DIDS insensitivity of the Cl^- conductive pathway served to distinguish this mode of Cl^- translocation from HCO_3^- gradient-driven Cl^- uptake. No evidence for K^+/Cl^- cotransport was obtained. As determined by acridine orange absorbance measurements in the presence of an imposed pH gradient (pH_o 7.5/pH_i 6), a HCO_3^- dependent increase in the rate of intravesicular alkalinization was observed in response to an outwardly directed Cl^- concentration gradient. The basolateral membrane vesicle origin of the observed $Cl^- - HCO_3^-$ exchange activity was verified by experiments performed with purified brush-border membrane vesicles. In contrast to our previous observations of the effect of Cl^- on HCO_3^- gradient-driven Na^+ uptake suggesting a basolateral membrane $Na^+ - HCO_3^-$ for Cl^- exchange mechanism, no effect of Na^+ on $Cl - HCO_3^-$ exchange was observed in the present study.

Introduction

The renal proximal tubule performs a critical function in maintaining acid-base balance by returning most of the filtered HCO_3^- to the circulation. The reabsorption of filtered HCO_3^- is achieved by proximal tubular acidification which occurs as a result of H^+ secretion across the luminal membrane. The carbonic acid formed by titration of filtered HCO_3^- is subsequently dehydrated by luminal membrane carbonic anhydrase with the evolving CO_2 moving into the cell where carbonic acid is reformed by the action of cytoplasmic carbonic anhydrase. The protons arising

Abbreviations: TMA, tetramethylammonium; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; Val, valinomycin; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; DIDS 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid.

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from intracellular carbonic acid are recycled back across the luminal membrane and HCO_3^- exits the cell across the basolateral membrane.

The predominant mechanism of H^+ secretion across the luminal membrane of the proximal tubule is by Na^+-H^+ exchange which couples the downhill influx of Na^+ to the uphill efflux of H^+. The evidence for this H^+ secretory pathway is widespread as its existence has been demonstrated in microperfusion experiments in vivo [1–4], in isolated tubules [5–8] and brush border membrane vesicles [9–11]. In contrast, only recently have the mechanisms mediating HCO_3^- efflux across the basolateral membrane been elucidated. Studies of mammalian and nonmammalian proximal tubules perfused in vivo [12,13] and in vitro [14–16] suggest an electrogenic Na^+/HCO_3^- cotransport mechanism as an exit pathway for basolateral membrane HCO_3^- efflux. This finding has been further supported by the demonstration of electrogenic Na^+/HCO_3^- cotransport in preparations of purified basolateral membrane vesicles [17–19]. In addition to renal proximal tubule cells this ion-coupled HCO_3^- transport pathway has also been identified in cultured cells derived from monkey kidney and bovine corneal endothelium [20,21].

The possible existence of a basolateral membrane Cl^--HCO_3^- exchange mechanism may represent an alternate HCO_3^- efflux pathway in addition to Na^+/HCO_3^- cotransport. The presence of basolateral membrane Cl^--HCO_3^- exchange has been suggested from both mammalian [14,22–24] and nonmammalian [25,26] tubule perfusion studies. Indirect evidence for basolateral membrane Cl^--HCO_3^- exchange has also been obtained from investigations of sulfate transport using isolated basolateral membrane vesicles [27].

In this report the possible presence of a basolateral membrane Cl^--HCO_3^- exchange mechanism was assessed by both $^{36}Cl^-$ tracer uptake and acridine orange absorbance measurements using preparations of purified membrane vesicles isolated from rat renal cortex. Evidence supporting the existence of Cl^--HCO_3^- exchange was obtained which is consistent with our previous observation of anion exchange mechanisms in rabbit basolateral membrane vesicles [28]. These findings suggest an alternate ion-coupled HCO_3^- transport pathway in addition to Na^+/HCO_3^- cotransport which may mediate HCO_3^- efflux across the proximal tubule cell basolateral membrane.

Materials and Methods

Membrane preparations

Rat renal basolateral membrane vesicles were prepared by differential and Percoll density gradient centrifugation as previously described [18]. The basolateral membrane fraction was enriched 9–12-fold compared to the homogenate in specific activity of the basolateral membrane marker ($Na^+ + K^+$)-ATPase and represented 9–14% of the total homogenate ($Na^+ + K^+$)-ATPase activity [29]. The specific activity of maltase, a luminal membrane marker enzyme, was enriched 1–2-fold and represented 1–2% of the homogenate maltase activity [30]. Both freshly prepared and frozen basolateral membrane preparations were used in this study as preliminary experiments indicated freezing ($-70\,^\circ C$) had no effect on Cl^--HCO_3^- exchange activity. Brush-border membrane vesicles were isolated from rat renal cortex by the divalent cation aggregation method described previously [31] and stored frozen at $-70\,^\circ C$ in 200 mM mannitol, 50 mM potassium gluconate, 10 mM Hepes-KOH (pH 7.4) until used. Membrane protein was determined by a sodium dodecyl sulfate-Lowry assay with bovine serum albumin as the standard [32].

Isotopic flux measurements

Thawed (20–25 °C) or freshly prepared aliquots of membrane vesicles were washed twice in 100 mM TMA gluconate, 86 mM Hepes, 43 mM TMA(OH) (pH 7.5) by centrifuging 30 min at $44\,000 \times g$. The second pellet was resuspended to 10–30 mg of protein/ml in the same media and isosmotic solutions of appropriate ionic composition were added to obtain the desired intravesicular solution described for each experiment in the figure legends. The membrane suspension was incubated for 120 min at room temperature to insure complete transmembrane equilibration of the added media. During the pre-equilibration period the membranes were gassed continuously with humidified 100% N_2 or 90% N_2/10% CO_2. The extravesicular media were prepared similarly and

the final composition for each experiment is given in the figure legends. Intravesicular $^{36}Cl^-$ content was assayed in triplicate at 37 °C in the continued presence of either 100% N_2 or 90% N_2/10% CO_2 by a rapid filtration technique previously described [18,33]. The uptake reaction was quenched by the rapid addition of 213 mM potassium gluconate, 2 mM probenecid, 10 mM Hepes-TMA (pH 7.5) kept at 4 °C. The diluted membrane suspension was passed through a 0.65 μm Millipore filter (DAWP) and washed with an additional 9 ml of the quench buffer. The filters were dissolved in 3 ml of Ready-Solv HP (Beckman) and counted by scintillation spectroscopy. The process of quenching, filtration and washing occurred within a 15-s period. The timed uptake values obtained were corrected for the nonspecific retention of isotope by the filters.

Spectrophotometric determinations of ΔpH

The rate of change of intravesicular pH in response to an imposed pH gradient (pH_o 7.5/pH_i 6) was monitored by acridine orange absorbance spectroscopy using an SLM-Aminco DW2 spectrometer. In the dual wavelength mode the time-dependent change in the absorbance difference, $A_{492-546}$, was monitored using 546 nm as a reference wavelength (slit width 5 nm) [34]. Equal aliquots of thawed (20–25 °C) or freshly prepared membranes were washed twice into either 165 mM KCl, 5 mM Mes-TMA (pH 6) or 165 mM potassium gluconate, 5 mM Mes-TMA (pH 6) by centrifuging 30 min at 44000 × *g*. Each pellet was resuspended in its respective media and solutions of appropriate ionic composition were added to obtain the desired intravesicular solution as described in the figure legend. The membrane suspension was incubated for 120 min at room temperature under continuous gassing with either humidified 100% N_2 or 95% N_2, 5% CO_2. The extravesicular or cuvette solutions were prepared similarly and their ionic composition is given in the figure legend. Experiments were initiated by the rapid addition of 10 μl membranes (50–80 μg/protein) to a 2.5 ml stirred cuvette solution thermostatically maintained at 25 °C. Upon addition of the vesicles to the cuvette buffer an immediate quench of absorbance ('pH jump') was noted in response to the imposed pH gradient. Among the various transmembrane ionic conditions tested no significant difference in the magnitude of the 'pH jump' was observed which indicates the dye response and pH gradient was unaffected by manipulating the intra- and extravesicular ionic composition. The initial rate of change of intravesicular pH was determined from the linear segment of the absorbance change occurring immediately after the 'pH jump'.

Materials

Acridine orange, Percoll, probenecid, valinomycin and DIDS were purchased from Sigma (St. Louis, MO). Furosemide was generously supplied by Hoechst-Roussel (Somerville, NJ). ^{36}Cl was obtained from New England Nuclear (Boston, MA). Valinomycin was dissolved in 95% ethanol and was added to the membrane suspension in a 1 : 100 dilution. Equivalent volumes of ethanol were added to control aliquots of membranes. All solutions were prepared with distilled-deionized water and passed through a 0.22 μm Millipore filter.

Results

HCO_3^- gradient-driven Cl^- influx

The presence of a Cl-HCO_3^- exchange mechanism in rat renal basolateral membrane vesicles would be suggested by the ability of a HCO_3^- concentration gradient to serve as a driving force for intravesiclar concentrative Cl^- accumulation. The time course of intravesicular Cl^- accumulation (Cl_o = 5 mM) is illustrated in Fig. 1 as a function of imposed transmembrane pH and HCO_3 gradients. When intra- and extravesicular pH was equal at pH 6 Cl^- uptake was low and unaffected by the presence (pH_o 6/pH_i 6 + CO_2/HCO_3) or nominal absence (pH_o 6/pH_i 6 − CO_2/HCO_3) of CO_2/HCO_3^-. The imposition of an inside alkaline pH gradient in the nominal absence of CO_2/HCO_3 (pH_o 6/pH_i 7.5 − CO_2/HCO_3) induced a modest stimulation of Cl^- uptake which was further increased in the presence of an outwardly directed HCO_3^- concentration gradient (pH_o 6/pH_i 7.5 + CO_2/HCO_3). The observation of concentrative Cl^- uptake in the presence but not the absence of an outwardly

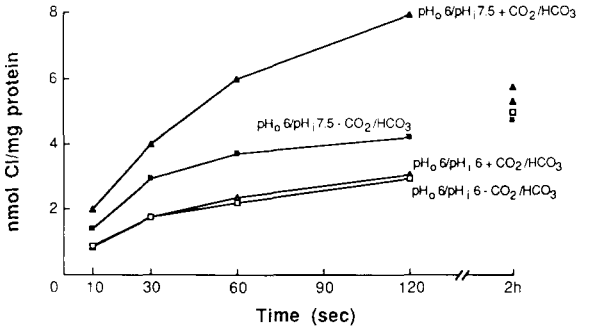

Fig. 1. Effect of HCO_3^- on Cl^- influx. Basolateral membrane vesicles were pre-equilibrated with: (pH_o 6/pH_i 6 − CO_2): 110 mM TMA gluconate, 57.3 mM potassium gluconate, 52 mM Mes, 45.3 mM Hepes, 23 mM TMA(OH) under 100% N_2; (pH_o 6/pH_i 6 + CO_2): 110 mM TMA gluconate, 57.3 mM potassium gluconate, 52 mM Mes, 45.3 mM Hepes, 23 mM TMA(OH), 1.8 mM HCO_3^-; (pH_o 6/pH_i 7.5 − CO_2): 110 mM TMA gluconate, 57.3 mM potassium gluconate, 52 mM mannitol, 45.3 mM Hepes, 23 mM TMA(OH) under 100% N_2; (pH_o 6/pH_i 7.5 + CO_2): 110 mM TNA gluconate, 57.3 mM $KHCO_3$, 52 mM mannitol, 45.3 mM Hepes, 23 mM TMA(OH) under 10% CO_2/90% N_2. Uptake of $^{36}Cl^-$ (5 mM) occurred from extravesicular solutions containing: (pH_o 6/pH_i 6 − CO_2): 109 mM TMA gluconate, 57.4 mM potassium gluconate, 52 mM Mes, 44.3 mM Hepes, 27 mM TMA(OH) under 100% N_2; (pH_o 6/pH_i 6 + CO_2): 109 mM TMA gluconate, 57.4 mM potassim gluconate, 52 mM Mes, 44.3 mM Hepes, 27 mM TMA(OH), 1.8 mM HCO_3^- under 10% CO_2/90% N_2; (pH_o 6/pH_i 7.5 − CO_2): 109 mM TMA gluconate, 57.4 mM potassium gluconate, 47 mM Mes, 9 mM Hepes, 25 mM TMA(OH), 42 mM mannitol under 100% N_2; (pH_o 6/pH_i 7.5 + CO_2): 109 mM TMA gluconate, 57.4 mM K^+, 59 mM gluconate, 47 mM Mes, 9 mM Hepes, 25 mM TMA(OH), 29 mM mannitol, 2 mM HCO_3^- under 10% CO_2/90% N_2. A representative experiment of three independent observations is illustrated.

directed HCO_3^- gradient suggests the coupled exchange of Cl^- for HCO_3^- in the membranes.

The nature of HCO_3^- coupling to Cl^- uptake was next examined by evaluating the effect of maneuvers designed to minimize membrane potential development as is shown in Fig. 2. In the presence of a pH gradient (pH_o 6/pH_i 7.5 + CO_2/HCO_3) HCO_3^- gradient-induced Cl^- uptake was determined with $K_o = K_i$ in the presence (450 μM) or absence of valinomycin. The magnitude of HCO_3^- gradient induced Cl^- uptake was only slightly reduced in the presence of valinomycin which suggests a negligible contribution of ion-gradient (H^+, OH^-, HCO_3^-)-induced diffusion potentials to Cl uptake.

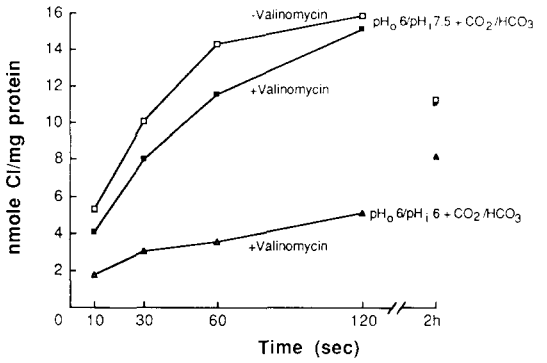

Fig. 2. Effect of valinomycin on HCO_3^- gradient-driven Cl^- influx. Basolateral membrane vesicles were pre-equilibrated as described in the legend to Fig. 1. The timed uptake of $^{36}Cl^-$ (5 mM) occurred from solutions described in the legend to Fig. 1. Where indicated membranes were preincubated with valinomycin (450 μM) or an equivalent volume of ethanol (1%) for a minimum of 30 min. Results of a representative experiment performed in triplicate are illustrated.

Conductive Cl^- uptake

Possible indirect electrical coupling between an outwardly directed HCO_3^- gradient and Cl^- uptake was further evaluated by testing for a basolateral membrane Cl^- conductive pathway. Cl^- uptake was measured with $K_o > K_i$ in the presence (225 μM) and absence of valinomycin. As shown in Fig. 3, a valinomycin-induced inside positive K diffusion potential markedly stimulated Cl^- uptake compared to control indicating the presence of a conductive pathway for Cl^- translocation. This voltage-dependent stimulation of Cl^- uptake was virtually abolished when the protonophore FCCP was present in addition to valinomycin. With $K_o > K_i$ in the absence of valinomycin Cl^- uptake was similar to control which suggests a lack of K^+/Cl^- cotransport in these membranes.

In an attempt to distinguish the Cl^- conductive pathway from Cl^--HCO_3^- exchange each was tested for sensitivity to DIDS inhibition. As shown on the left of Fig. 4 the conductive uptake of Cl^- was insensitive to DIDS (10 μM–250 μM). In contrast, as shown to the right of Fig. 4, HCO_3^- gradient stimulated Cl^- uptake was markedly inhibited by DIDS (I_{50} 20 μM). The observed difference in sensitivity to DIDS inhibition suggests HCO_3^- gradient-induced Cl^- uptake does not oc-

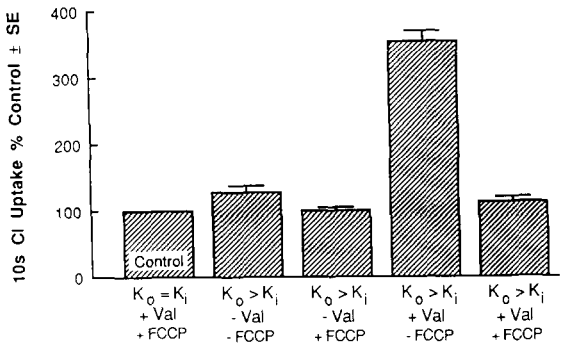

Fig. 3. Cl^- influx driven by a valinomycin-induced K^+ diffusion potential. Basolateral membrane vesicles were pre-equilibrated with: ($K_o = K_i$): 110 mM TMA gluconate, 57.3 mM potassium gluconate, 52 mM Mes, 45.3 mM Hepes, 23 mM TMA(OH) (pH 6); ($K_o > K_i$): 167.3 mM TMA gluconate, 52 mM Mes, 45.3 mM Hepes, 23 mM TMA(OH) (pH 6). The 10 s uptake of $^{36}Cl^-$ (5 mM) occurred from an extravesicular solution containing 110 mM TMA gluconate, 57.3 mM potassium gluconate, 52 mM Mes, 45.3 mM Hepes, 23 mM TMA(OH) (pH 6). Where indicated membranes were preincubated with valinomycin (225 μM) or an equivalent volume of ethanol (1%) for a minimum of 30 min. FCCP (65 μM) or an equivalent amount of ethanol (0.13%) was present in the extravesicular solution. Control Cl^- uptake was 1.113 ± 0.014 nmol/mg protein. The mean ± S.E. of five experiments, each performed with a different membrane preparation, is shown.

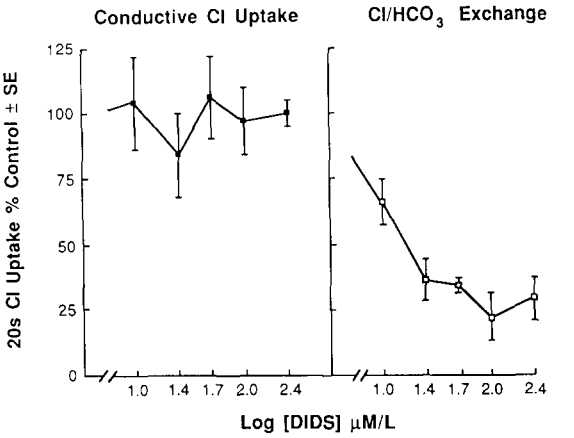

Fig. 4. Effect of DIDS on conductive Cl^- uptake vs. HCO_3^- gradient-driven Cl^- uptake. Conductive Cl^- uptake: the 20-s uptake of $^{36}Cl^-$ (5 mM) was assayed as described in the legend to Fig. 3 for $K_o > K_i$ + Val; HCO_3^- gradient driven Cl^- uptake: the 20-s uptake of $^{36}Cl^-$ (5 mM) was assayed as described in the legend to Fig. 1 for pH_o 6/pH_i 7.5 + CO_2. Membranes were preincubated with valinomycin (450 μM) for a minimum of 30 min. Control values for conductive and HCO_3^- gradient-driven Cl^- uptake were 1.59 ± 0.49 and 2.4 ± 0.23 nmol/mg protein, respectively. The data from four experiments, each performed with a different membrane preparation, are shown.

cur via the previously described Cl^- conductive pathway.

Cl^- gradient-dependent intravesicular alkalinization

If a Cl^--HCO_3^- exchange mechanism is present then not only should gradients of HCO_3^- drive Cl^- uptake as previously shown, but Cl^- gradients should drive intravesicular HCO_3^- accumulation. The latter was examined by determining the effect of outwardly directed Cl^- gradients on the rate of intravesicular pH change as shown in Fig. 5. In the presence of an imposed pH gradient (pH_o 7.5/pH_i 6) Cl^- gradient-induced alterations of the rate of intravesicular alkalinization was monitored by acridine orange absorbance measurements in the presence or absence of CO_2/HCO_3^-. As shown on the left in Fig. 5 the imposition of an outward Cl^- gradient had no effect on the rate of intravesicular alkalinization in the nominal absence of CO_2/HCO_3^-. The imposition of an inwardly directed HCO_3^- gradient significantly increased the rate of intravesicular alkalinization which was still further increased in the presence of an outwardly directed Cl^- gradient. Whereas the Cl^- gradient-induced increase in the rate of intravesicular alkalinization was significantly reduced by the red cell anion exchange inhibitor furosemide [35], no significant inhibition was observed in the absence of Cl^-. These results further suggest a flux coupling of Cl^- and HCO_3^- which is sensitive to furosemide.

Brush-border membrane HCO_3^- gradient-driven Cl^- influx

The presence of Cl^--HCO_3^- exchange in purified brush-border membrane vesicles was evaluated to determine the membrane origin the anion antiporter. As shown in Fig. 6 the imposition of an inside alkaline pH gradient in the nominal absence of CO_2/HCO_3^- (pH_o 6/pH_i 7.5, N_2) significantly stimulated brush-border membrane Cl^- uptake compared to the level observed in the absence of a pH gradient (pH_o 6/pH_i 6, CO_2/HCO_3). The same inside alkaline pH gradient in the presence of CO_2/HCO_3^- further stimu-

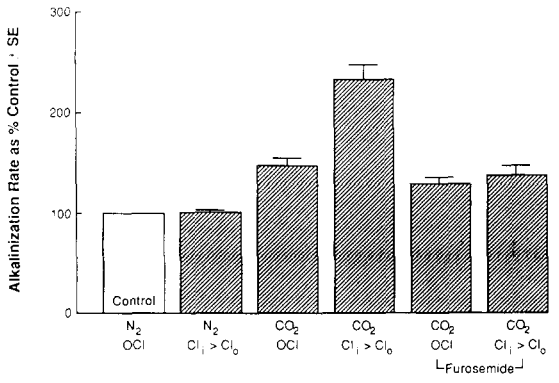

Fig. 5. Effect of Cl^- on the rate of intravesicular alkalinization. Basolateral membrane vesicles were pre-equilibrated with 165 mM K gluconate (0Cl) or 165 mM KCl ($Cl_i > Cl_o$), 5 mM Mes/TMA(OH) (pH 6), 20 μM acridine orange and 1.5 mM potassium gluconate when under 100% N_2. Membranes gassed with 5% CO_2/95% N_2 were similarly pre-equilibrated except substituting 1.5 mM $KHCO_3$ for 1.5 mM potassium gluconate and where indicated 0.5 mM furosemide was present. The extravesicular cuvette solutions were gassed in parallel with the membrane vesicles and consisted of 165 mM potassium gluconate, 5 mM Hepes/TMA(OH) (pH 7.5), 20 μM acridine orange under 100% N_2; 137 mM potassium gluconate, 28 mM $KHCO_3$, 5 mM Hepes/TMA(OH) (pH 7.5), 20 μM acridine orange and where indicated 0.5 mM furosemide under 5% CO_2/95% N_2. Membranes were preincubated with valinomycin (450 μM) for at least 30 min. The control absorbance change rate was 0.1455 ± 0.008 absorbance unit/min per mg protein. The data shown represents values obtained from nine experiments.

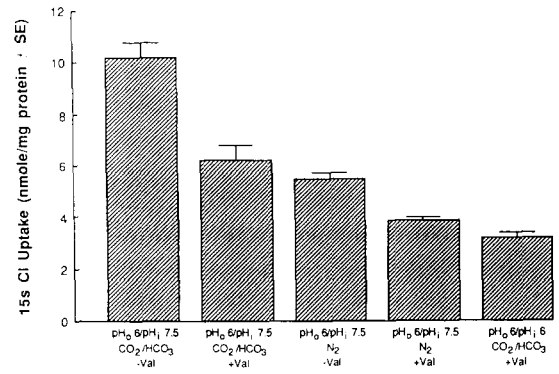

Fig. 6. HCO_3^- gradient-driven Cl^- uptake by brush border membrane vesicles. Brush-border membrane vesicles were pre-equilibrated as described in the legend to Fig. 1. The 15 s uptake of $^{36}Cl^-$ (5 mM) occurred from extravesicular solutions described in the legend to Fig. 1. Where indicated membranes were preincubated with valinomycin (450 μM) or an equivalent volume of ethanol (1%) for a minimum of 30 min. The data from three experiments are shown.

lated Cl^- uptake by these luminal membrane vesicles. However, the pH gradient induced stimulation of Cl^- uptake both in the presence and absence of CO_2/HCO_3^- was significantly reduced by maneuvers designed to offset membrane potential development (valinomycin + $K_o = K_i$). Although this maneuver abolished pH gradient-induced Cl^- uptake in the absence of CO_2/HCO_3^-, Cl^- uptake in the presence of CO_2/HCO_3^- remained above the control level (pH_o 6/pH_i 6) which may suggest the existence of luminal membrane Cl-HCO_3 exchange. In view of the low level of brush-border membrane contamination present in the basolateral membrane preparation used in these studies the magnitude of this putative luminal membrane Cl^--HCO_3^- exchanger is not sufficient to account for the Cl^--HCO_3^- exchange activity observed in basolateral membranes.

Na^+ coupling to Cl^--HCO_3^- exchange

Previously we presented evidence suggesting the

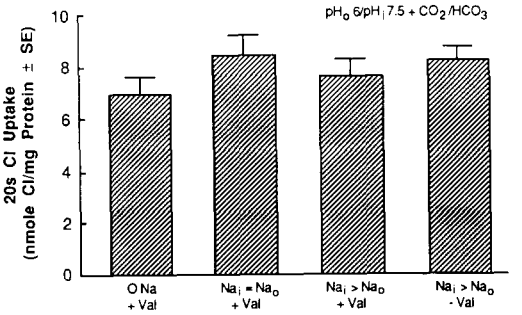

Fig. 7. Effect of Na^+ on HCO_3^- gradient-driven Cl^- influx. Basolateral membrane vesicles were pre-equilibrated under 10% CO_2/90% N_2 with: (0Na): 153 mM TMA gluconate, 57.3 mM $KHCO_3$, 45.3 mM Hepes, 23 mM TMA(OH) (pH 7.5); ($Na_i = Na_o$, $Na_i > Na_o$): 53 mM TMA gluconate, 100 mM sodium gluconate, 57.3 mM $KHCO_3$, 45.3 mM Hepes, 23 mM TMA(OH) (pH 7.5). The 20-s uptake of $^{36}Cl^-$ (5 mM) occurred from an extravesicular solution containing: (0Na): 153 mM TMA gluconate, 57.3 mM K^+, 66 mM gluconate, 37 mM Mes, 9 mM Hepes, 25 mM TMA(OH) (pH 6); ($Na_i = Na_o$): 53 mM TMA gluconate, 100 mM sodium gluconate, 57.3 mM K^+, 66 mM gluconate, 37 mM Mes, 9 mM Hepes, 25 mM TMA(OH) (pH 6); ($Na_i > Na_o$). 133 mM TMA gluconate, 20 mM sodium gluconate, 57.3 mM K^+, 66 mM gluconate, 37 mM Mes, 9 mM Hepes, 25 mM TMA(OH) (pH 6). Where indicated membranes were preincubated with valinomycin (450 μM) or an equivalent volume of ethanol (1%) for a minimum of 30 min. The data shown were compiled from three experiments.

coupling of Cl^- to Na^+/HCO_3^- cotransport consistent with a basolateral membrane Na^+-HCO_3^- for Cl^- exchange mechanism [18]. To investigate the existence of this transport pathway further the coupling of Na^+ to HCO_3^- gradient-dependent Cl^- uptake and efflux was assessed as shown in Figs. 7 and 8, respectively. As depicted in Fig. 7 the magnitude of HCO_3^- gradient-induced Cl^- uptake was not further increased when intra- and extravesicular Na^+ was equal or when an outwardly directed Na^+ gradient was imposed. Given the possibility that the apparent Na^+ insensitivity of HCO_3^- gradient-induced Cl^- uptake may have resulted from a functional asymmetry of the antiporter the effect of Na^+ on HCO_3^- gradient-induced Cl^- efflux was examined as shown in Fig. 8. With intra- and extravesicular Cl^- initially equal, the efflux of Cl^- was determined in the presence (pH_o 7.5/pH_i 6) and absence (pH_o 7.5/pH_i 7.5) of an inwardly directed HCO_3^- gradient. In the absence of Na^+ intravesicular Cl^- was significantly reduced by the imposition of an inwardly directed HCO_3^- gradient compared to level observed when intra- and extravesicular HCO_3^- was equal. However, in the continued presence of an inwardly directed HCO_3^- gradient the level of intravesicular Cl^- was not further reduced when intra- and extravesicular Na^+ was equal or an inwardly directed Na^+ gradient was imposed. Thus, similar to the previous experiment (Fig. 7) examining the effect of Na^+ on HCO_3^- gradient-induced Cl^- uptake these data also suggest a lack of coupling between Na^+ and basolateral membrane Cl^--HCO_3^- exchange.

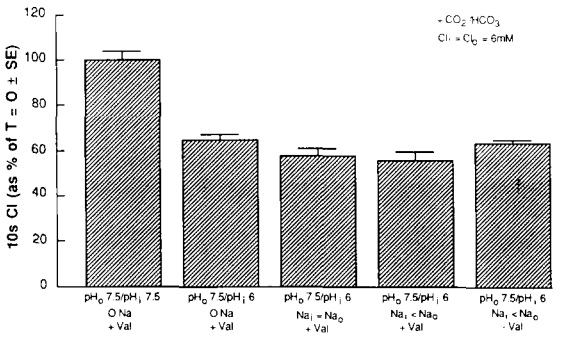

Fig. 8. Effect of Na^+ on HCO_3^- gradient-driven Cl^- efflux. Basolateral membrane vesicles were pre-equilibrated under 10% CO_2/90% N_2 with: (pH_o 7.5/pH_i 7.5, 0Na): 101 mM TMA gluconate, 41 mM Hepes, 26 mM TMA(OH), 57.3 mM $KHCO_3$, 14.2 mM potassium gluconate, 52 mM mannitol, 6 mM $^{36}Cl^-$; (pH_o 7.5/pH_i 6, 0Na, $Na_i < Na_o$): 101 mM TMA gluconate, 41 mM Hepes, 26 mM TMA(OH), 71.5 mM potassium gluconate, 52 mM Mes, 6 mM $^{36}Cl^-$; (pH_o 7.5/pH_i 6, $Na_i = Na_o$): 51 mM TMA gluconate, 50 mM sodium gluconate, 41 mM Hepes, 26 mM TMA(OH), 71.5 mM potassium gluconate, 52 mM Mes, 6 mM $^{36}Cl^-$. The extravesicular solutions were: (pH_o 7.5/pH_i 7.5, 0Na): same as intravesicular solution described above; (pH_o 7.5/pH_i 6, 0Na): 101 mM TMA gluconate, 41 mM Hepes, 10 mM Mes, 37 mM TMA(OH), 57.2 mM $KHCO_3$, 14.3 mM potassium gluconate, 31 mM mannitol, 6 mM ^{36}Cl; (pH_o 7.5/pH_i 6, $Na_i = Na_o$): 51 mM TMA gluconate, 50 mM sodium gluconate, 41 mM Hepes, 10 mM Mes, 37 mM TMA(OH), 57.2 mM $KHCO_3$, 14.3 mM potassium gluconate, 31 mM mannitol, 6 mM $^{36}Cl^-$; (pH_o 7.5/pH_i 6, $Na_i < Na_o$): 61 mM TMA gluconate, 40 mM sodium gluconate, 41 mM Hepes, 10 mM Mes, 37 mM TMA(OH), 57.2 mM $KHCO_3$, 14.3 mM potassium gluconate, 31 mM mannitol, 6 mM $^{36}Cl^-$. Where indicated membranes were preincubated with valinomycin (450 μM) or an equivalent volume of ethanol (1%) for a minimum of 30 min. The data from three experiments are shown as the % of initial ($T = 0$) Cl^- content remaining after 10 s.

Discussion

The present investigation was conducted to gain further insight as to the mechanism(s) of HCO_3^- permeation across the basolateral membrane of mammalian proximal tubule cells. Specifically, the possible presence of an anion antiporter mediating the exchange of Cl^- for HCO_3^- was assessed directly by tracer flux and indirectly by acridine orange absorbance measurements using preparations of purified basolateral membrane vesicles. Evidence was obtained suggesting the existence of a basolateral membrane anion antiporter operative as a Cl^--HCO_3^- exchanger.

The presence of a basolateral membrane Cl^--HCO_3^- exchanger was first assessed by examining the ability of HCO_3^- to serve as a driving force for the accumulation of intravesicular Cl^-. In appreciation of the reported presence of basolateral membrane-associated carbonic anhydrase [36] experiments were conducted in the presence or nominal absence of CO_2 so as to distinguish between OH^- and HCO_3^- as the preferred driver ion for Cl^- uptake. The observed pH gradient-induced stimulation of Cl^- uptake in the absence of CO_2/HCO_3^- suggests the coupled exchange of Cl^- for OH^- (or H^+/Cl^- cotransport) and implies the presence of a Cl^--HCO_3^- exchanger. This implication was

strengthened by the further stimulation of Cl^- uptake noted in the presence of the same pH gradient and an outwardly directed HCO_3^- gradient. That the concentrative uptake of Cl^- (overshoot) was observed in the presence but not the absence of CO_2/HCO_3^- also argues favorably for the mediated exchange of Cl^- for HCO_3^-.

Apart from the coupled exchange of Cl^- for HCO_3^- an alternate mechanism may be invoked to explain the observed HCO_3^- gradient-induced stimulation of Cl^- uptake. As the experiment described above was performed in the absence of maneuvers designed to minimize the development of membrane potential differences, the noted stimulation of Cl^- uptake may have resulted from ion-gradient (H^+, OH^-, HCO_3^-) induced diffusion potentials. This possibility was evaluated by determining HCO_3^- gradient-induced Cl^- uptake in the presence and absence of valinomycin when $K_o = K_i$. Whereas HCO_3^- gradient-induced Cl^- uptake was slightly reduced in the presence of the ionophore, concentrative Cl^- uptake persisted which suggests a negligible contribution of ion gradient-induced diffusion potentials to Cl^- uptake. Thus, the HCO_3^- gradient-induced stimulation of basolateral membrane Cl^- uptake would appear to be the result of direct coupling i.e. anion exchange, rather than indirect electrostatic interactions. However, as we did not verify the adequacy with which valinomycin in the presence of symmetrical K^+ concentrations offset the development of ion-gradient induced diffusion potentials the issue remained open and was investigated further. To the extent that HCO_3^- gradient-induced Cl^- uptake occurred indirectly via electrostatic interactions then a voltage sensitive pathway for Cl^- uptake should be present as has been previously described for this membrane preparation [37]. As defined by a valinomycin inducible stimulation of Cl^- uptake in the presence of an inwardly directed K^+-gradient, a basolateral membrane Cl^--conductive pathway was detected in the present study as well. In an attempt to distinguish the Cl^--conductive pathway from Cl^--HCO_3^- exchange each was tested for sensitivity to DIDS inhibition. Whereas the conductive uptake of Cl^- was essentially DIDS insensitive, HCO_3^- gradient-driven Cl^- uptake was markedly inhibited by DIDS ($I_{50} = 20\ \mu M$). The observed difference in sensitivity to DIDS inhibition further suggests HCO_3^- gradient-induced Cl^- uptake does not occur via a Cl^- conductive pathway and most likely represents coupled exchange of Cl^- for HCO_3^-.

Consistent with the operation of a basolateral membrane anion exchange mechanism coupling the flows of HCO_3^- and Cl^- we have demonstrated the ability of HCO_3^- to serve as a driving force for intravesicular Cl^- accumulation. In keeping with the properties of this anion exchanger to couple the flows of HCO_3^- and Cl^- then gradients of Cl^- should serve as a driving force for intravesicular HCO_3^- accumulation. This was shown indirectly by monitoring changes in acridine orange absorbance in response to the collapse of an imposed inside acid pH gradient. In the nominal absence of CO_2/HCO_3^- the imposition of an outwardly directed Cl^- gradient was observed to have no effect on the rate of intravesicular alkalinization. However, in the presence of CO_2/HCO_3^- the same outwardly directly Cl^- gradient caused a significant increase in the rate of intravesicular alkalinization suggesting a more rapid influx of HCO_3^- consistent with Cl^--HCO_3^- exchange. Furthermore, the Cl^- gradient-induced increase in the rate of intravesicular alkalinization was abolished by anion exchange inhibitor furosemide. The observation of an inhibitor-sensitive, HCO_3^--dependent, Cl^- gradient-induced increase in the rate of intravesicular alkalinization supports the findings obtained from the tracer flux measurements and further suggests the existence of mediated Cl^--HCO_3^- exchange in rat basolateral membrane vesicles.

Owing to the possible existence of a brush border membrane Cl^--HCO_3^- exchange mechanism [37–43] and the small amount of luminal membrane contamination detected in the basolateral membrane preparation used in this study the membrane origin of the anion exchange activity was assessed. Although a significant stimulation of brush-border membrane vesicle Cl^- uptake was observed in the presence of an outwardly directed HCO_3^- gradient, the magnitude of stimulation was reduced by more than 60% when membrane potential development was blunted. This finding suggests that much of the Cl^- uptake occurring in the absence of maneuvers controlling for membrane potential development resulted from

ion-gradient-induced diffusion potentials. However, it should be noted that a small but significant stimulation of Cl^- uptake persisted in the presence of valinomycin and symmetrical K^+ concentrations which may indicate luminal membrane Cl^--HCO_3^- exchange or incomplete shunting of membrane potential. When considering the low level of brush-border membrane contamination present in the basolateral membrane preparation, we conclude that the magnitude of this putative luminal membrane Cl^--HCO_3^- exchange activity is not sufficient to account for the anion exchange observed in basolateral membranes.

Finally, prompted by our previous observations of the effects of Cl^- on HCO_3^- gradient-induced basolateral membrane Na^+ uptake [18] consistent with the operation of a Na^+-HCO_3^- for Cl^- exchange mechanism, we investigated the effects of Na^+ on HCO_3^- gradient-driven Cl^- uptake and efflux. Compared to the absence of Na^+, the level of HCO_3^- gradient-induced Cl^- uptake remained constant when intra- and extravesicular Na^+ was equal or when an outwardly directed Na^+ gradient was imposed which suggests a lack of Na^+ coupling to Cl^--HCO_3^- exchange. In an effort to compensate for a possible functional asymmetry of the Na^+-HCO_3^- for Cl^- exchange mechanism the coupling of Na^+ to Cl^--HCO_3^- exchange was further investigated by examining the effect of Na^+ on HCO_3^- gradient-driven Cl^- efflux. Again, as with HCO_3^- gradient-induced Cl^- uptake, Na^+ was without effect on HCO_3^- gradient-driven Cl^- efflux. The apparent Na^+ insensitivity of basolateral membrane Cl^--HCO_3^- exchange noted in this membrane vesicle study contrasts the recent report of Na^+ dependent Cl^--HCO_3^- exchange located at the basolateral membrane of microperfused rat proximal convoluted tubules [24]. While it is possible that the properties of the anion exchange mechanism (Na^+ dependency) may have been altered during the routine preparation of basolateral membrane vesicles, this is inconsistent with the effects of Cl^- on HCO_3^- gradient-induced Na^+ uptake we previously described [18]. Perhaps a more likely explanation may be the existence of two basolateral membrane Cl^--HCO_3^- exchange mechanisms distinguished by the property of coupling to Na^+. Our inability to detect an effect of Na^+ on HCO_3^- gradient-driven Cl uptake may reflect an additional difference between these two mechanisms in their respective affinities for Cl^-. This is suggested by the apparent Na^+-independent anion exchange observed in the present study using a low Cl concentration (5 mM) and the Na^+ dependence demonstrated when much higher Cl^- concentrations were used [24].

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