AN IMPORTANT FUNCTION of the mouse and rat medullary thick ascending limb (MTAL) is to reabsorb luminal 
HCO$_3^-$, and basolateral Cl$^-$/HCO$_3^-$ exchangers of rat thick limbs. Am. J. Physiol. 275 (Renal Physiol. 44): F334–F342, 1998.—Cl$^-$/HCO$_3^-$ exchange was measured in luminal (LMV) and basolateral (BLMV) membrane vesicles purified from rat medullary thick ascending limb (MTAL). Cl$^-$/HCO$_3^-$ exchange in BLMV and LMV was inhibited by DIDS, with respective IC$_{50}$ values of 3.2 ± 0.9 and 15.2 ± 5.2 µM, whereas Cl$^-$ conductances were DIDS insensitive. At constant external pH, BLMV 36Cl$^-$ influx studies, whether Cl$^-$/HCO$_3^-$ exchange not only at the basolateral membrane, but also at the luminal membrane. The BLMV and LMV exchange activities, however, could be distinguished from one another on the basis of their sensitivities to DIDS and to pH. The ∼165-kDa AE2 polypeptide was restricted to BLMV, AE1-related polypeptides were present predominantly in BLMV, as well as at variable, lower levels also in LMV.

EXPERIMENTAL PROCEDURES

Preparation of MTAL tubules. The tubule isolation procedure was similar to that described recently (4). In brief, male Sprague-Dawley rats weighing 250-300 g were anesthetized with pentobarbital sodium. Kidneys were removed quickly, decapsulated, and sliced sagittally. Slices were transferred in fresh iced (0-4°C) Hanks’ modified medium containing (in mM) 115 NaCl, 0.4 MgSO$_4$, 0.5 MgCl$_2$, 0.4 KH$_2$PO$_4$, 0.3 Na$_3$PO$_4$, 25 NaHCO$_3$, 10 HEPES, 4 KCl, 1.2 CaCl$_2$, 5 glucose, 5 l-leucine, and 1 mg/ml bovine serum albumin; pH 7.40 (bubbled with 95% O$_2$-5% CO$_2$). Under stereomicroscopic control, the inner stripe of the outer medulla, recognized by its reddish color, was carefully separated from each slice by removing completely the outer part of the outer medulla as well as the inner medulla. The resulting tissue was subjected to collagenase treatment as described (4). In the final suspensions, most of the tubules (∼95%) proved to be MTAL in origin, based on immunofluorescent staining with Tamm-Horsfall protein (4), a specific marker for the thick ascending limb. We were unable to detect significant activity of maltase, a marker of the proximal tubule brush-border membrane, in either whole homogenates or in the final apical fractions,

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Further indicating that the starting material was not significantly contaminated by tubules from the pars recta.

Isolation of plasma membranes. Typically, the preparation began with ~15 mg protein of MTAL tubules obtained from the kidneys of 10 rats. LMV and BLMV were prepared from purified rat MTAL tubules as recently described in detail (4). We have recently demonstrated that the BLMV and LM preparations have a right-side-out orientation (5). Compared with homogenate, the basolateral marker activity of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase was enriched more than 9-fold in the BLMV and only 0.5-fold in the LMV. Immunoblot analysis confirmed comparable enrichment of the α\textsubscript{1}-subunit polypeptide of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in BLMV compared with LMV (see below). In contrast, the apical marker activity of γ-glutamyltransferase was enriched >10-fold in the LMV and 2-fold in BLMV (4).

Immunoblot analysis of the 31-kDa subunit of the vacuolar H\textsuperscript{+}-ATPase (7) also confirmed its enrichment in LMV over BLMV (see below). Transport assays were performed after overnight storage of the vesicles at ~85°C.

Transport measurements. \textsuperscript{36}Cl\textsuperscript{-} uptake into the membrane vesicles was assayed at ambient temperature (20–25°C) by a rapid filtration technique. The vesicles were prequillibrated at room temperature for 2 h to load with desired constituents. For each experiment, the specific conditions are given in the legends to Figs. 1–7. In general, a 10-µl aliquot of either BLMV or LMV (10–40 µg protein) was added to 100–300 µl of appropriate reaction medium containing \textsuperscript{36}Cl\textsuperscript{-} (~1 µCi/ml). The reaction was stopped with 1.5-ml ice-cold solution containing 20 mM Tris-MES gassed with 95% N\textsubscript{2}-5% CO\textsubscript{2}, or 100 mannitol, 3 EGTA, 100 N-Methyl-D-glucamine (NMG) gluconate, and 200 Tris-MES gassed with 100% N\textsubscript{2}. Uptake in the absence of pH gradient was determined by rapid dilution of vesicles into reaction media identical to preincubation media, except for the presence of \textsuperscript{36}Cl\textsuperscript{-}.}

Fig. 1. Effect of H\textsuperscript{+} and HCO\textsubscript{3}\textsuperscript{-} gradient on Cl\textsuperscript{-} uptake by BLMV (A) and LMV (B) isolated simultaneously. Vesicles were preincubated for 2 h with a pH 7.8 medium in the presence of HCO\textsubscript{3}\textsuperscript{-} (in mM: 100 mannitol, 3 EGTA, 100 N-methyl-D-glucamine (NMG) gluconate, 55 NMG HCO\textsubscript{3}, and 100 Tris-MES gassed with 95% N\textsubscript{2}-5% CO\textsubscript{2} or in the absence of HCO\textsubscript{3}\textsuperscript{-} (110 mannitol, 3 EGTA, 100 NMG gluconate, and 200 Tris-MES gassed with 100% N\textsubscript{2}). Chloride (2 mM \textsuperscript{36}Cl\textsuperscript{-}) uptake was then assayed by diluting vesicles 1:21 into pH 5.5 buffer containing either (in mM) 100 mannitol, 3 EGTA, 155 NMG gluconate, and 100 Tris-MES gassed with 95% N\textsubscript{2}-5% CO\textsubscript{2}, or 100 mannitol, 3 EGTA, 100 NMG gluconate, and 200 Tris-MES gassed with 100% N\textsubscript{2}. Uptake in the absence of pH or HCO\textsubscript{3}\textsuperscript{-} gradient was determined by rapid dilution of vesicles into reaction media identical to preincubation media, except for the presence of \textsuperscript{36}Cl\textsuperscript{-}. Values are means of 6 determinations from 2 different BLMV and LMV preparations. When SE bar is not shown, it was smaller than the symbols. pH\textsubscript{i}, intracellular pH; pH\textsubscript{o}, external pH.
accumulation achieved within ~2 and 6 min, respectively, in BLMV and LMV. The peak Cl\(^{-}\) uptakes (overshoots), were 3- and 1.5-fold greater than the respective equilibrium values for BLMV (measured at 3 h) and LMV (5 h). In both preparations, however, even the equilibrium levels of intravesicular Cl\(^{-}\) remained two to five times greater than in the absence of either H\(^{+}\) or HCO\(_3\)\(^{-}\) gradients. These differences in the 3-h and 5-h levels of Cl\(^{-}\) may suggest that the HCO\(_3\)\(^{-}\) and H\(^{+}\) gradients across the vesicular membranes dissipated very slowly, thus retarding efflux of Cl\(^{-}\) that had been concentrically transported into the plasma membrane vesicles.

To test this possibility, 36Cl\(^{-}\) uptake was estimated under the different conditions described in Fig. 1. After 6 min, the BLMV and the LMV were incubated for an additional period of 5 h in the presence or absence of the protonophore FCCP (100 µM). The results are presented in Table 1. In the presence of a gradient of pH and/or HCO\(_3\)\(^{-}\), FCCP markedly decreased the 5-h levels of 36Cl\(^{-}\) in the BLMV. In the presence of this ionophore, values for equilibrium 36Cl\(^{-}\) uptakes were not different, regardless of whether these were measured in the absence or presence of HCO\(_3\)\(^{-}\) and/or pH gradients. Comparable results were also obtained with the luminal preparations, in which FCCP markedly reduced the 5-h levels of 36Cl\(^{-}\) in the membrane vesicles incubated in the presence of gradients of pH and/or HCO\(_3\)\(^{-}\). These findings suggested the presence in both BLMV and LMV of H\(^{+}\) and/or HCO\(_3\)\(^{-}\) conductive pathways of very low magnitude. Recent stopped-flow fluorometry experiments have demonstrated directly very low H\(^{+}\) permeabilities of the basolateral and apical membrane vesicles isolated from rat MTAL (29). Taken together, these data strongly support the presence of Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchangers in both basolateral and apical plasma membrane fractions isolated from the rat MTAL.

Effect of an electrical potential across the membrane vesicles on 36Cl\(^{-}\) uptake. We next investigated whether significant Cl\(^{-}\)-conducting pathways exist in these membrane vesicles and whether DIDS, an established inhibitor of Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchangers, would inhibit Cl\(^{-}\) uptake. These experiments were performed in the absence or presence of transmembrane potentials generated by preincubation of membrane vesicles with valinomycin. Generation of an inside-positive membrane potential by imposition of an inwardly directed K\(^{+}\) gradient markedly stimulated 36Cl\(^{-}\) uptake in BLMV (Fig. 2, left) and in LMV (Fig. 2, right), indicating significant Cl\(^{-}\)-conductive pathways in both preparations. Figure 2 shows, however, that 2 mM DIDS had no significant effect on 36Cl\(^{-}\) uptake in BLMV and LMV under both unclamped and voltage-clamped conditions.

Comparison of the effects of DIDS on Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchange in BLMV and LMV. Figure 3 shows that pH- and HCO\(_3\)\(^{-}\) gradient-stimulated 36Cl\(^{-}\) uptakes in BLMV and LMV were sensitive to DIDS inhibition in the micromolar range, suggesting that the stimulation of 36Cl\(^{-}\) uptake by HCO\(_3\)\(^{-}\) gradients in BLMV and LMV is direct rather than secondary to generation of an inside-positive membrane potential. Figure 3 also shows that the BLMV and LMV inhibition curves differed significantly (P < 0.05), with IC\(_{50}\) values for DIDS of 3.2 ± 0.9 and 15.2 ± 5.2 µM.

pH\(_{\text{d}}\) dependence of the BLMV and LMV anion exchangers. Figure 4 compares the effect of pH\(_{\text{d}}\) on 36Cl\(^{-}\) uptake by basolateral and LMV, measured in the presence and absence of 2 mM DIDS. pH\(_{\text{d}}\) was varied from 6.1 to 8.0 by equilibrating the vesicles in media of appropriate HCO\(_3\)\(^{-}\) concentration gassed with 95% N\(_2\)-5% CO\(_2\) while keeping the extravesicular pH constant during the transport asays. In the BLMV (Fig. 4A), 36Cl\(^{-}\) uptake displayed a sigmoidal pattern of activation as pH\(_{\text{d}}\) increased from 6.1 to 8.0, consistent with the presence of an internal H\(^{+}\) and/or OH\(^{-}\) modifier site on this exchanger. In contrast (Fig. 4B), uptake of 36Cl\(^{-}\) by the luminal exchanger exhibited a concave velocity curve as pH\(_{\text{d}}\) increased from 6.1 to 8.0. At the acidic pH\(_{\text{d}}\) (6.7) prevailing in MTAL cells (14), the DIDS-sensitive component of 36Cl\(^{-}\) uptake expressed relative to maximal uptake measured at pH 8.0 was threefold higher in LMV than in BLMV (30% vs. 10%).

In the experiments in Fig. 4, increasing pH\(_{\text{d}}\) from 6.1 to 8.0 at constant PCO\(_2\) was accompanied by increasing intravesicular concentrations of HCO\(_3\)\(^{-}\) (from 1.1 to 87 mM). Thus the pH\(_{\text{d}}\) dependence of the Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchangers could have been due to intravesicular HCO\(_3\)\(^{-}\) and not to H\(^{+}\). To distinguish between these possibilities, we evaluated the pH\(_{\text{d}}\) dependence of the BLMV and LMV exchangers operating in the 36Cl\(^{-}\)/Cl\(^{-}\) exchange mode in the nominal absence of HCO\(_3\)\(^{-}\). In the BLMV (Fig. 5A), total and DIDS-sensitive 36Cl\(^{-}\) uptakes displayed a sigmoidal pattern of activation over the pH\(_{\text{d}}\) range 6.1 to 8.0, suggesting that this exchanger is regulated by the intravesicular H\(^{+}\) (OH\(^{-}\)) concentration. In contrast, DIDS-sensitive 36Cl\(^{-}\) uptake by LMV was completely unresponsive to changes in pH\(_{\text{d}}\) from 6.7 to 7.8.

Cl\(^{-}\)/HCO\(_3\)\(^{-}\) antiporter kinetics in BLMV and LMV. Figure 6 compares the effect of external Cl\(^{-}\) concentra-

Table 1. Effect of FCCP on the 5-h level of 36Cl\(^{-}\) uptake in BLMV and LMV

<table>
<thead>
<tr>
<th>Conditions</th>
<th>BLMV</th>
<th>LMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH(<em>{\text{d}})=5.5/pH(</em>{\text{c}})=7.8</td>
<td>Control</td>
<td>3.98±0.16</td>
</tr>
<tr>
<td>HCO(_3)(^{-})=3.95±0.25</td>
<td>2.28±0.26*</td>
<td></td>
</tr>
<tr>
<td>pH(<em>{\text{d}})=5.5/pH(</em>{\text{c}})=7.8</td>
<td>FCCP</td>
<td>1.99±0.29</td>
</tr>
<tr>
<td>no HCO(_3)(^{-})</td>
<td>2.70±0.42</td>
<td>1.99±0.12</td>
</tr>
<tr>
<td>pH(<em>{\text{d}})=pH(</em>{\text{c}})=7.8</td>
<td>Control</td>
<td>1.30±0.12</td>
</tr>
<tr>
<td>HCO(_3)(^{-})</td>
<td>1.52±0.09</td>
<td>1.67±0.45</td>
</tr>
<tr>
<td>pH(<em>{\text{d}})=pH(</em>{\text{c}})=7.8</td>
<td>FCCP</td>
<td>1.72±0.12</td>
</tr>
<tr>
<td>no HCO(_3)(^{-})</td>
<td>2.11±0.38</td>
<td>1.93±0.43</td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 determinations on 2 separate preparations of basolateral membrane vesicles (BLMV) and luminal membrane vesicles (LMV), expressed as nmol/mg protein. pH\(_{\text{d}}\), extracellular pH; pH\(_{\text{c}}\), intracellular pH. Both types of vesicles were incubated for 6 min with 2 mM 36Cl\(^{-}\) in presence or absence of a pH and HCO\(_3\)\(^{-}\) gradient, as described in the legend to Fig. 1. Then, ethanol (control) or FCCP in ethanol was added to final concentrations of 1% and 100 µM, respectively, and incubation continued for 5 h. *P < 0.01 and †P < 0.05 vs. respective controls by ANOVA.
tion ([Cl\(^-\)]\(_o\)) on pH and HCO\(_3^-\) gradient-stimulated \(^{36}\)Cl\(^-\) uptake by BLMV and LMV measured in the presence and absence of 2 mM DIDS. In the presence of DIDS, best fits were obtained with a linear function (r = 0.99) for both BLMV (Fig. 6A) and LMV (Fig. 6B). When uptakes observed in the presence of DIDS were subtraced from total uptakes, curves were obtained in BLMV and LMV that described saturable Cl\(^-\) dependant transport processes with Michaelis-Menten kinetics. Eadie-Scatchard plots of these data were consistent with the participation of a single Cl\(^-\)/HCO\(_3^-\) antiport system in BLMV with an apparent K\(_m\) of 3.0 ± 0.5 mM and a V\(_{max}\) of 9.2 ± 2.2 nmol·mg\(^{-1}\)·s\(^{-1}\) (Fig. 6A, inset). The corresponding values in LMV were an apparent K\(_m\) of 4.63 ± 0.61 mM and a V\(_{max}\) of 4.9 ± 0.6 nmol·mg\(^{-1}\)·s\(^{-1}\) (Fig. 6B, inset). These values of apparent K\(_m\) and V\(_{max}\) did not significantly differ between BLMV and LMV, as determined by unpaired two-tailed t-tests.

AE2 anion exchanger polypeptides in BLMV and LMV. Five independent preparations of BLMV and LMV were assessed by immunoblot for the presence of AE2 and AE1 polypeptides. As shown in Fig. 7A, for two representative preparations, the 165-kDa AE2 polypeptide detected by antibody to mouse AE2 COOH-terminal amino acids 1224–1237 (top bracket) was present only in BLMV (lanes 2 and 4) and was undetectable in LMV (lanes 1 and 3). This distribution coincided with immunofluorescent studies of this AE2 epitope in MTAL of rat (2) and mouse (32). The faint 145-kDa AE2 polypeptide (Fig. 7A, top bracket on left), although likely a degradation product, could represent AE2c, whose transcript is also present in rodent kidney (2, 32). The AE2 bands were not detected when antibody incubations were carried out in the presence of peptide antigen (lanes 5 and 6). Treatment of BLMV with peptidyl-N-glycosidase F reduced the intensity of AE2 polypeptides by ∼20 kDa (not shown), as observed previously in gastric membranes (37).

In all five membrane preparations, the antibody to mouse AE2 amino acids 1224–1237 also detected in BLMV poorly resolved doublet bands at M\(_r\) ∼115 and 95 kDa (Fig. 7A, bottom, lanes 2 and 4). Interestingly, lower levels of a doublet band of ∼95 kDa were also present at variable levels in LMV (Fig. 7A, lanes 1 and 3). Since no currently known AE2 transcript encodes polypeptides of these smaller masses, since this anti-AE2 antibody cross reacts with AE1 (2, 6, 32), and since a preliminary report has noted the presence of AE1 mRNA in microdissected rat MTAL (31), we stripped these blots and reprobed them with antibodies that recognize only AE1.

Mouse monoclonal antibody to rat AE1 (2) detected the same bands near 100 kDa but did not detect the 165-kDa AE2 polypeptide (Fig. 7B). Moreover, rabbit anti-mouse AE1 917–929 (2) detected the same bands in the presence of irrelevant peptide (Fig. 7C, lanes 1–4) but not in the presence of excess peptide antigen (lanes 5 and 6). Thus these bands were defined as AE1 or AE1-related polypeptides by virtue of immunospecific reactivity with three different antibodies recogniz-
ing AE1. Comparison of the AE1-related bands of LMV and those of BLMV was hindered by the presence in BLMV of abundant comigrating Na\(^+\)-K\(^+\)-ATPase \(\alpha\)-subunit polypeptide (white bands in Fig. 7C and dark bands in Fig. 7D). However, experiments in which increasing quantities of partially purified pig kidney Na\(^+\)-K\(^+\)-ATPase was doped in parallel into LMV and red blood cell membrane samples (not shown) confirmed that the slightly different mobilities of the \(\sim 95\)-kDa bands in LMV and BLMV was secondary to distortion of the basolateral membrane lanes by very large amounts of Na\(^+\)-K\(^+\)-ATPase, and thus the \(\sim 95\)-kDa proteins in BLMV and LMV are likely the same polypeptide(s). Moreover, addition of increasing amounts of kidney ATPase to red blood cell ghost membranes suppressed the immunoreactivity of red blood cell AE1 on immunoblot, suggesting that the abundance of AE1-related polypeptides in BLMV might be greater (and the relative abundance in LMV lower) than apparent from Fig. 7.

**DISCUSSION**

The objective of this study was to detect and characterize the Cl\(^-\)/HCO\(_3\)^- exchangers on rat MTAL of Henle. To accomplish this, a new fractionation procedure was developed for the simultaneous isolation of LMV and BLMV from purified suspensions of rat MTAL, thereby permitting direct and separate characterization of the Cl\(^-\)/HCO\(_3\)^- exchangers present at the luminal and basolateral cell surfaces of this nephron segment. The present study revealed that the rat MTAL possesses both luminal and basolateral Na\(^+\)-independent Cl\(^-\)/HCO\(_3\)^- exchangers (Fig. 1). Unlike the LMV exchanger, Cl\(^-\)/HCO\(_3\)^- exchange by the BLMV transporter was markedly accelerated by increasing pHi from 6.7 to 7.8, consistent with the presence of a proton-sensitive modifier site on this exchanger (Fig. 4). Both exchangers were DIDS-inhibitable; however, the BLMV exchanger was approximately fivefold more sensitive to DIDS (Fig. 3). The present study also established that a \(\sim 165\)-kDa AE2 polypeptide was detected only in BLMV, whereas AE1-related polypeptides were detected predominantly in BLMV and in lower abundance in LMV (Fig. 7). Our studies demonstrate for the first time the polarized distribution of distinct isoforms of anion exchanger polypeptides in the cells of the MTAL of Henle.

Earlier studies have failed to detect significant Cl\(^-\)/HCO\(_3\)^- exchange in the absence of AVP and under isotonic conditions in mouse (23) and rat (24) MTAL.
suspensions by measuring pH1. Leviel et al. (24) and Kikeri et al. (23) concluded that Cl−/HCO3− exchange was absent, because pH1 recovery in tubules incubated in the presence of an outwardly directed CO2/HCO3− gradient was insensitive to removal of external Cl−. In both studies, however, substitution of chloride with gluconate was incomplete, resulting in final [Cl−]o levels ranging from 2.5 to 7.5 mM. These values of [Cl−]o likely supported significant Cl−/HCO3− exchange activity in MTAL, since in the current study the apparent Km for [Cl−]o of the BLMV and LMV Cl−/HCO3− exchangers averaged 3 and 4.6 mM, respectively (Fig. 6). Previous studies of mouse AE2 in Xenopus oocytes have yielded low apparent Km values of AE2 for [Cl−]o in Cl−/Cl− exchange mode of 5.6 mM, as determined from influx measurements (18), and 3.7 mM from efflux measurements (Y. Zhang and S. L. Alper, unpublished observations). Moreover, the Na+–containing solutions used in the experiments of Leviel et al. (24) and Kikeri et al. (23) may have enhanced alkali-loading processes (e.g., the BLMV and LMV Na+/H+ exchangers), thus counteracting acid-loading processes such as the Cl−/HCO3− exchangers of BLMV and LMV. These possibilities are supported by the subsequent

![Fig. 6. Cl−/HCO3− antiporter kinetics in BLMV (A) and LMV (B). Effect of increasing extravascular concentrations of Cl− (from 1 to 10 mM) on pH gradient- and HCO3− gradient-stimulated 9s 36Cl− uptake in BLMV and LMV in presence or absence of 2 mM DIDS (see Fig. 3 for composition of media). Total 36Cl− uptakes measured in absence (△) and presence of DIDS (●) were used to calculate DIDS-sensitive uptake (○). TMA salt concentration was maintained at 155 mM using appropriate concentrations of chloride and gluconate. Values are means ± SE of 6 determinations on 3 separate preparations of BLMV and LMV. Insets: Eadie-Scatchard plots of DIDS-sensitive 36Cl− uptake in BLMV (A) and LMV (B).](http://ajprenal.physiology.org/)

![Fig. 7. Immunoblot analysis of AE2 and AE1 in two independent preparations of BLMV (B) and LMV (L). Each lane is loaded with 50 µg membrane protein. A: polyclonal rabbit antibody to mouse AE2 amino acids 1224–1237 detects predominant 165-kDa and less abundant 145-kDa AE2 polypeptides in BLMV but not in LMV. This antibody also detects additional polypeptides of lower Mr in BLMV and, at lower levels, in LMV [lane 1; longer exposure (not shown) confirms presence of these bands in lane 3, also]. All bands detected in the presence of irrelevant peptide (lanes 1–4) are competed by peptide antigen (lanes 5 and 6). B: monoclonal mouse antibody to rat AE1 fails to detect AE2 bands but detects all bands of lower Mr, confirming their identity as AE1 related. C: polyclonal antibody to mouse AE1 amino acids 917–929 fails to detect AE2 bands but detects all AE1-related bands of lower Mr. Asterisk in C indicates nonspecific reactivities not competed by peptide antigen (compare lanes 1–4 with lanes 5 and 6). Asterisk in B indicates band comigrating with this nonspecific band. AE2 and AE1 polypeptides were detected in 5 of 5 preparations examined. D: Na+–K+–ATPase α-subunit is appropriately enriched in BLMV (the signal is oversaturated) but is present in nonnegligible amount in LMV, consistent with previously reported enzymatic assays (4). E: 31-kDa subunit of vacuolar H+–ATPase is appropriately enriched in LMV but also present in nonnegligible amount in BLMV.](http://ajprenal.physiology.org/)
preliminary studies of Sun and Dworkin (33), who detected basolateral DIDS-sensitive, Na\(^{+}\)-independent and Cl\(^{-}\)-dependent HCO\(_3\)\(^{-}\) transport in mouse MTAL segments perfused in vitro in the absence of AVP and under isotonic conditions. The possible presence of luminal Cl\(^{-}\)/HCO\(_3\) exchange was not reported by these authors.

The major functional difference between the basolateral and apical anion exchanger activities was observed in the effects of pH, (Fig. 4). The steep activation of BLMV 36Cl\(^{-}\)/Cl\(^{-}\} exchange by increasing pH, in normally CO\(_2\)-free media (Fig. 5) suggested that internal H\(^{+}\) exerts a modifier effect on the BLMV Cl\(^{-}\)/HCO\(_3\) exchanger. The presence of a strong internal modifier site for Cl\(^{-}\)/HCO\(_3\) exchange reflected in steep pH\(_{i}\) dependence has previously been observed in rabbit parietal cell BLMV (27). A weaker internal modifier site, reflected in a broader pH\(_{i}\) dependence and decreased magnitude of activation, has also been noted in rabbit ileal brush-border membrane vesicles (26, 27). In contrast, 36Cl\(^{-}\}/Cl\(^{-}\} exchange in rat MTAL LMV was unresponsive to pH\(_{i}\) values between 6.7 and 7.8 (Fig. 5). Thus the apparent pH\(_{i}\) dependence of Cl\(^{-}\)/HCO\(_3\) exchange in LMV (Fig. 4) presumably reflects its dependence on the intravesicular HCO\(_3\)\(^{-}\) concentrations that varied in parallel with pH\(_{j}\), as opposed to a modifier effect of OH\(^{-}\)/H\(^{+}\). Our observations that anion exchange in the BLMV isolated from rat MTAL displays a pH\(_{i}\) sensitivity consistent with regulation by an internal pH modifier site, whereas the apical AE is insensitive to pH\(_{j}\), along with the uniquely basolateral distribution of AE2 (Fig. 7 and Ref. 2), are consistent with the known steep pH\(_{i}\) dependence of AE2 (18, 36). The latter studies found that AE2 and AE1 function in Xenopus oocytes differs markedly in their regulation by pH, with AE2 having a steeper pH\(_{i}\) dependence than AE1. However, unlike in the present study, combined variation of pH\(_{j}\) and pH\(_{o}\) were used, such that the “allosteric” control of AE2 may have been exerted by both pH\(_{j}\) and pH\(_{o}\) (36).

The polarized expression of different isoforms of the Cl\(^{-}\)/HCO\(_3\) exchanger gene family in a nephron segment that resorbs NaCl and HCO\(_3\) is consistent with the possibility that each isoform exerts distinct roles in the regulation of pH\(_{j}\) and cell volume as well as in vectorial ion transport. Recent studies found that the rat MTAL is the nephron segment with the highest concentration of AE2 transcript (6) and protein (2). Our current membrane fractionation studies (Fig. 7A) agree with previous immunocytochemical studies (2) that AE2 is located exclusively on the basolateral membrane of the rat MTAL, whereas the Na\(^{+}\)/H\(^{+}\) exchanger NHE-1 is also expressed (4). This NHE isoform has been shown, in stable transfection studies, to be stimulated by hypertonicity (22), suggesting that AE2 and NHE-1 are the most likely candidates for cell volume regulation in the rat MTAL. Stimulation of NHE-1 by hypertonicity would alkalinize the cell and thereby activate the BLMV Cl\(^{-}\)/HCO\(_3\) exchanger. Such a mechanism has already been proposed by Mason et al. (25) in lymphocytes and has also been described in Xenopus oocytes expressing heterologous AE2 (19). As was recently shown in oocytes (21), coupled operation of NHE-1 and AE2 produces a net NaCl gain that, together with osmotically obliged water, restores cell volume toward its original value.

In addition to its possible role in volume regulation, the presence of a pH-sensitive internal modifier site for BLMV Cl\(^{-}\)/HCO\(_3\) exchange suggests that AE2 could have other functions in the rat MTAL. Although as assayed in the current experiments, the BLMV AE2 exchanger is maximally active only at alkaline pH\(_{i}\) values that are not reached physiologically in the cells of this nephron segment, it might in vivo undergo additional forms of regulation. This possibility is supported by recent evidence demonstrating that, at acidic pH\(_{i}\) values, NH\(_{4}\)\(^{+}\) at concentrations physiologically achieved in the MTAL activated AE2 expressed in Xenopus oocytes (17). Whether NH\(_{4}\)\(^{+}\) can also overcome the inhibitory effect of intracellular H\(^{+}\) similarly to activate AE2 of the rat MTAL at acidic pH\(_{i}\) remains to be determined.

At least two studies using isolated, perfused rat MTAL are consistent with the possibility that the BLMV anion exchanger of this nephron segment could play a role in HCO\(_3\)\(^{-}\) transport and/or pH\(_{i}\) regulation. Watts and Good (35) found a background acid loading process, whose activity resembled that of the BLMV anion exchanger, with negligible activity at pH\(_{j}\) values below 6.7, which markedly increased to a maximal value at pH\(_{j}\) 7.5. Although these experiments were performed in the nominal absence of HCO\(_3\), the pH\(_{i}\)-sensitive background acid loading process could have been due to Cl\(^{-}\)/HCO\(_3\) exchange supported by low levels of ambient HCO\(_3\) and/or Cl\(^{-}\)/OH\(^{-}\} exchange. These processes presumably account for H\(^{+}\) gradient-stimulated Cl\(^{-}\} uptake in the nominal absence of HCO\(_3\) (Fig. 1) and are potentiated in AE2 by hypertonicity (21).

More recently, Good et al. (14) found that 1 µM bath ethylisopropylamiloride (EIPA), a potent inhibitor of the Na\(^{+}\)/H\(^{+}\) exchangers, inhibited HCO\(_3\)\(^{-}\) absorption by 35%. They also found that 50 µM bath EIPA secondarily could inhibit the luminal membrane Na\(^{+}\)/Cl\(^{-}\} exchanger which, at pH\(_{j}\) of ~7.1, is exquisitely sensitive to small pH\(_{j}\) variations (Fig. 4). This would in turn inhibit basolateral NaCl entry via the parallel operation of the basolateral membrane Na\(^{+}\)/H\(^{+}\) and Cl\(^{-}\)/HCO\(_3\) exchangers, leading to cell shrinkage that secondarily could inhibit the luminal membrane Na\(^{+}\)/H\(^{+}\) exchanger (35).

AE1-related polypeptides were also found in BLMV, in agreement with the preliminary report of the AE1 mRNA in rat MTAL (31), but in contrast to immunocytochemical studies (2). Since the pH\(_{i}\) dependence of
known isoforms of AE1 (20, 36) is not consistent with that displayed by BLMV anion exchange (Figs. 4 and 5), the relationship between basolateral AE1 and H+\textsuperscript{-}inhibited BLMV anion exchange remains unclear. It is possible, however, that the small DIDS-sensitive component of Cl\textsuperscript{-} uptake (0.80 ± 0.093 nmol·mg\textsuperscript{-1}·s\textsuperscript{-1}) observed in BLMV at pH 6.1 (Fig. 5A) represented operation of the basolateral AE1, since AE2-mediated \textsuperscript{36}Cl\textsuperscript{-} uptake into Xenopus oocytes is absent at or below pH\textsubscript{a} 6.2 (18), corresponding to a pH\textsubscript{i} value of ~7.3 (36).

Although luminal AE1 has been proposed to mediate bicarbonate secretion by type B intercalated cells of the collecting duct (1), the identity of this apical anion exchanger remains controversial (2, 11, 30). Similarly, interpretation of putatively apical AE1-related polypeptides in LMV of MTAL is complicated by several issues. First of these is the purity of the LMV fraction, since LMV are largely (but not completely) purified away from BLMV (Fig. 7D; and Ref. 4). Other possible sources of contamination in both LMV and BLMV fractions include eAE1 from residual red blood cell membrane and kAE1 from both plasmalemma and intracellular membranes of type A intercalated cells of medullary collecting duct.\textsuperscript{1} Another problem is the comparison of the AE1-related polypeptides in LMV to the more abundant AE1-related polypeptides in BLMV, whose immunoblot signal is likely attenuated by very abundant, comigrating, α-subunit of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (Fig. 7, C and D).

Nonetheless, Fig. 7 is consistent with the presence of AE1-related Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchanger (AE1) in rat MTAL luminal membrane, where two NHE isoforms, NHE-2 (8, 34) and NHE-3 (3, 4), are also present. Since AE1 can operate at the acidic pH of MTAL cells, the Na\textsuperscript{+}/H\textsuperscript{+} exchangers and the Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchanger may “counteract” one another under physiological circumstances, providing negative feedback regulation of HCO\textsubscript{3} absorption. AE1 might also contribute to NaCl reabsorption via functional coupling with either NHE-2 and/or NHE-3, allowing to the MTAL cell the option to regulate independently NaCl absorption via coupled ion exchangers and NH\textsubscript{4}\textsuperscript{+} absorption via the Na\textsuperscript{+}K\textsuperscript{+} (NH\textsubscript{4}\textsuperscript{+})-2Cl\textsuperscript{-} cotransporter. Of note, in the isolated, perfused mouse (12) cortical thick ascending limb, but not in mouse MTAL (16), it has been shown that 50% of luminal NaCl absorption requires the presence of HCO\textsubscript{3}. This HCO\textsubscript{3}-dependent NaCl absorption is abolished by inhibition of carbonic anhydrase and inhibited by luminal SITS, suggesting that it is due to parallel action of Na\textsuperscript{+}/H\textsuperscript{+} and Cl\textsuperscript{-}/HCO\textsubscript{3} exchange. Further studies are needed to examine the physiological import of these exchange modes in the apical membrane of the rat MTAL, a nephron segment containing high levels of carbonic anhydrase (10).

In conclusion, the present study directly demonstrates the presence of distinct Cl\textsuperscript{-}/HCO\textsubscript{3} exchangers in basolateral and apical plasma membranes isolated from rat MTALs. AE2 polypeptide is detected only in BLMV, whereas AE1-related polypeptides are detected predominantly in BLMV, and in lower, variable abundance in LMV. The most prominent functional difference between the anion exchangers in the two membrane fractions is the effect of pH\textsubscript{i}. In contrast to the rather pH\textsubscript{i}-insensitive LMV exchanger, the H\textsuperscript{+} concentration dependence of the BLMV exchanger suggests the existence of an intracellular H\textsuperscript{+} (OH\textsuperscript{-}) modifier site. Proton sensitivity of the BLMV Cl\textsuperscript{-}/HCO\textsubscript{3} antipor might play a role in pH\textsubscript{i} homeostasis and volume regulation. We further propose that Cl\textsuperscript{-}/HCO\textsubscript{3} exchange in the MTAL basolateral membrane may contribute to HCO\textsubscript{3} absorption, whereas the luminal membrane exchanger may play a role in NaCl absorption via functional coupling with the luminal Na\textsuperscript{+}/H\textsuperscript{+} antipor and/or may provide negative feedback regulation of HCO\textsubscript{3} absorption.

We thank F. Pezy for excellent technical assistance.

S. L. Alper is an established investigator of the American Heart Association and was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-43495 and DK-51059 and by the Harvard Digestive Diseases Center Grant DK-34854. R.-A. Podevin is an Established Investigator of the Institut National de la Santé et de la Recherche Médicale.

Portions of this work were presented at the 30th Annual Meeting of the American Society of Nephrology, San Antonio, TX, and have been published in abstract form (J. Am. Soc. Nephrol. 8: 5, 1997).

Address for reprint requests: R.-A. Podevin, Institut Biomédical des Cordeliers, 15 rue de l’Ecole de Médecine, 75270 Paris Cedex 06, France (E-mail: podevin@crcc.jussieu.fr).

Received 7J anuary 1998; accepted in final form 7 May 1998.

REFERENCES


