Polarized Expression of Different Monocarboxylate Transporters in Rat Medullary Thick Limbs of Henle*

(Received for publication, May 27, 1999, and in revised form, July 13, 1999)

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Extracellular lactic acid is a major fuel for the mammalian medullary thick ascending limb (MTAL), whereas under anoxic conditions, this nephron segment generates a large amount of lactic acid, which needs to be excreted. We therefore evaluated, at both the functional and molecular levels, the possible presence of monocarboxylate transporters in basolateral (BLMVs) and luminal (LMVs) membrane vesicles isolated from rat MTALs. Imposing an inward H⁺ gradient induced the transient uphill accumulation of L-[^14]C]lactate in both types of vesicles. However, whereas the pH gradient-stimulated uptake of L[^14]C]lactate in BLMVs was inhibited by anion transport blockers such as α-cyano-4-hydroxycinnamate, 4,4'-disothiocyanatostilbene-2,2'-disulfonic acid (DIDS), and furosemide, it was unaffected by these agents in LMVs, indicating the presence of a L-lactate/H⁺ cotransporter in BLMVs, but not in LMVs. Under non-pH gradient conditions, however, the uptake of L[^14]C]lactate in LMVs was transstimulated 100% by L-lactate, but by only 30% by ω-lactate. Furthermore, this lactate self-exchange was markedly inhibited by α-cyano-4-hydroxycinnamate and DIDS and almost completely by 1 mM furosemide, findings consistent with the existence of a stereospecific carrier-mediated lactate transport system in LMVs. Using immunofluorescence confocal microscopy and immunoblotting, the monocarboxylate transporter (MCT)-2 isoform was shown to be specifically expressed on the basolateral domain of the rat MTAL, whereas the MCT1 isoform could not be detected in this nephron segment. This study thus demonstrates the presence of different monocarboxylate transporters in rat MTALs; the basolateral H⁺/L-lactate cotransporter (MCT2) and the luminal H⁺-independent organic anion exchanger are adapted to play distinct roles in the transport of monocarboxylates in MTALs.

The medullary thick ascending limb (MTAL) is significantly engaged in the active absorption of NaCl, NH₄⁺, and HCO₃⁻.

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1 The abbreviations used are: MTAL, medullary thick ascending limb; MCT, monocarboxylate transporter; CHC, α-cyano-4-hydroxycinnamate; DIDS, 4,4'-disothiocyanatostilbene-2,2'-disulfonic acid; BLMV, basolateral membrane vesicle; LMV, luminal membrane vesicle; TBS, Tris-buffered saline (pH 7.6); TMA, tetramethylammonium; ANOVA, analysis of variance; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; Mes, 4-morpholineethanesulfonic acid; NHE, Na⁺/H⁺ exchanger.

Extracellular lactate has been shown to be a major fuel to support this large energy demand (for review, see Ref. 1), indicating that lactate uptake is a crucial process in the regulation of energy metabolism in this nephron segment. Conversely, under anoxic conditions, the increase in lactic acid production in the MTAL greatly exceeds the increase seen in other nephron segments (2). There is thus a need for facilitating efflux of glycolytically derived lactate from the MTAL.

In most cells, transport of lactic acid occurs via monocarboxylate transporters (MCTs) exhibiting the following properties: 1) cotransport of H⁺ with a monocarboxylate (or its equivalent, O₂⁻/monocarboxylate exchange); 2) broad specificity for short chain monocarboxylates, including lactate and pyruvate; 3) inhibition by anion transport blockers such as α-cyano-4-hydroxycinnamate (CHC), DIDS, and phloretin; and 4) trans-stimulation of L-lactate uptake by pyruvate, l-lactate (but not ω-lactate), and other various substituted monocarboxylates. Seven different cDNAs have been identified that encode for MCTs in mammalian, designated MCT1 to MCT7 (3–6). MCT1 and MCT2 show a broad tissue distribution and are present in hamster (3, 4) and rat (7) kidneys. On the other hand, the expression of rat MCT3 and MCT4 is restricted to the renal pigment epithelium (5) and muscle fibers (8), respectively. Three additional MCT isoforms, MCT5, MCT6, and MCT7, have been identified in human tissues (6).

Although the mechanisms of lactate export and import have been extensively investigated in a variety of tissues (for review, see Ref. 9), little information has been available concerning the mechanisms of lactate transport by the distal nephron, and the molecular identity of these transporters has not been established. To our knowledge, only one study has been performed on the MTAL. Vinay et al. (10) reported the presence of a lactate/OH⁻ (HCO₃⁻) antiporter in preparations of basolateral membrane vesicles isolated from the dog MTAL. That study, however, was derived from experiments in membrane preparations that were more enriched with γ-glutamyltransferase, now recognized as a luminal marker of the MTAL (11), than with Na⁺/K⁺-ATPase, a basolateral marker. As a result, the allocation of a lactate/OH⁻ (HCO₃⁻) antiporter to the basolateral or luminal membrane domains and its possible physiological role are not yet known.

This study was initiated in an attempt to characterize, at both the functional and molecular levels, the possible presence of monocarboxylate transporters at the luminal and/or basolateral cell surfaces of the MTAL. Isotopic flux studies performed on basolateral (BLMVs) and luminal (LMVs) membrane vesicles isolated from rat MTALs provide evidence for the presence of a CHC-sensitive lactate/H⁺ cotransporter restricted to BLMVs, whereas a H⁺-independent organic anion exchanger has been detected in LMVs. We also demonstrate, using immunofluorescence confocal microscopy and Western blot analysis
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of BLMV and LMV, that the recently cloned MCT2 isofrom (4) is specifically expressed on the basolateral domain of the rat MTAL, whereas the MCT1 isofrom could not be detected in this nephron segment.

**EXPERIMENTAL PROCEDURES**

**Preparation of MTAL Tubules**

Male Harlan Sprague-Dawley rats (250–300 g) were anesthetized with pentobarbital (50 mg/kg intraperitoneal), and the kidneys were removed quickly, decapsulated, and sliced sagittally. The method used for isolating MTAL tubules was the same as that previously described (11, 12). Briefly, under stereomicroscopic control, the inner stripe of the outer medulla was carefully excised. The resulting tissue was subjected to collagenase treatment. In the final suspensions, no significant activity of maltase, a marker of the proximal tubule brush-border membrane, could be detected, whereas its specific activity in homogenates from the immediately adjacent outer stripe of the outer medulla was ~150 nmol/mg of protein/min, excluding significant contamination of the starting material with tubule segments from the outer stripe of the outer medulla.

**Isolation of Plasma Membranes**

Typically, the preparation began with 30–60 mg of protein from MTAL tubules obtained from the kidneys of 20 rats. LVM and BLVM were prepared from purified rat MTAL tubules as described previously in detail (11, 12).

Apical membrane fractions were isolated from whole renal cortex using a Ca2+ aggregation method (13). Differential and Percoll gradient centrifugations were used for isolating basolateral membrane fractions from whole renal cortex (14). Transport assays were performed after overnight storage of the vesicles at ~85°C.

**Isotopic Flux Measurements**

L-14C]-lactate and 36Cl- uptake in the membrane vesicles was assayed at 20–25°C by a rapid filtration technique. For each experiment, the specific conditions are given in the figure legends. The reaction was stopped with 1.4 ml of ice-cold stop solution containing 20 mM Tris/Heps (pH 7.40) and the desired potassium gluconate concentration to neutrality with tetramethylammonium (TMA) base to produce TMA36Cl.

To ascertain the presence of MCT2 in medullary thick ascending limbs, sections were dual labeled with anti-MCT2 antibody and an antibody to NHE3, which specifically stains apical membranes of thick ascending limbs within the inner stripe of the outer medulla (15). Microwaved sections were first stained for MCT2 as described above. Sections were then incubated with a 1:100 dilution of monoclonal anti-NHE3 antibody (Chemicon International, Inc.), followed by Cy3-conjugated anti-mouse immunoglobulin G (Amersham Pharmacia Biotech) diluted 1:200 in TBS, each for 30 min with three TBS washes in between. Sections were then washed in TBS (3 × 5 min). Sections were mounted and observed using the confocal microscope. To avoid cross-talk (unwanted overlap range) of emission spectra of Cy2 and Cy3, sequential scanning was used. Cy2 was excited at 488 nm and detected at 502–601 nm. Control experiments using anti-MCT2 antibody preadsorbed with a 100 μg/ml concentration of the immunizing peptide revealed no labeling.

**Materials**

From Amersham Pharmacia Biotech, we obtained L-[U-14C]lactic acid. H36Cl obtained from NEN Life Science Products was titrated to neutrality with tetrathymelammonium (TMA) base to produce TMA36Cl. Rabbit polyclonal antibody to NHE3 was a gift of Dr. R. Alpern (University of Texas Southwestern Medical Center, Dallas, TX). Mouse monoclonal antibody to NHE3 and chicken anti-rat MCT2 peptide, anti-rat MCT1 peptide, and anti-Na+/K+/ ATPase polypeptide antibodies were from Chemicon International Inc.

**Statistical Methods**

All data are represented as the means ± S.E., and error bars in the figures also represent S.E. Comparison between groups was generally carried out by ANOVA. For all analyses, statistical significance was accepted as p < 0.05.

**RESULTS**

Since outwardly directed OH- gradients have been reported to stimulate 1-lactate uptake in partially purified basolateral membrane vesicles isolated from the dog MTAL (10), we investigated for the presence of this possible mode of lactate transport in both BLMV and LMV. These experiments were conducted in the presence of a valinomycin voltage clamp. As illustrated in Fig. 1, an inside alkaline pH gradient (intravesicular pH (pHi) = 7.8, extravesicular pH (pHe) = 5.5) stimulated the rate of 1-[14C]lactate uptake compared with no pH gradient (pH = pHi = 7.8) and induced transient accumulation of 1-[14C]lactate to levels ~8- and 3-fold greater than equilibrium values in BLMV and LMV, respectively. The pH gradient-stimulated uptake of lactate could be explained by the transport of this anion via non-ionic diffusion of lactate acid or via carrier-mediated H+/lactate cotransport.

To distinguish between these possibilities, we evaluated the effect of CHC, a relatively specific inhibitor of H+-coupled monocarboxylate transporters (16), on the initial rates of 1-[14C]lactate uptake under the same conditions as in the Fig. 1 experiments. Fig. 2 shows that pH gradient-stimulated 1-[14C]lactate uptake in BLMV was sensitive to CHC inhibition with IC50 of 63 ± 16 μM. In contrast, CHC, even at a concentration of 5 mM, had no effect on the pH gradient-stimulated 1-[14C]lactate uptake in LMV. These results are consistent with carrier-mediated lactate/H+ cotransport (or lactate/OH- exchange) in BLMV and with transport of lactate via non-ionic diffusion of lactate acid in LMV.

We next compared the effect of several less specific anion...
transport blockers such as furosemide, DIDS, and phloretin (9) on the pH gradient-stimulated transport of L-[14C]lactate in BLMVs and LMVs. As shown in Fig. 3, with the exception of phloretin, DIDS and furosemide significantly inhibited the BLMV transporter. In contrast, the pH gradient-stimulated uptake of L-[14C]lactate in LMVs was not significantly inhibited by all of the tested agents. These findings confirm the view that lactate/H+ cotransport is present only in BLMVs.

It is well established (3, 9, 17, 18) that MCTs have affinity for several substituted monocarboxylates in addition to L-lactate (or OH-/lactate cotransporter (or its equivalent, a OH- or lactate exchanger), we measured L-[14C]lactate uptake in BLMVs in the presence of outwardly directed gradients of various substituted and unsubstituted monocarboxylates (transstimulation). These experiments were conducted at pH 7.8 under non-pH gradient conditions using vesicles equilibrated with a highly buffered medium. To further minimize possible alkalization of the intravesicular space, a valinomycin/FCCP pH clamp was used. As shown in Fig. 4, the uptake of L-[14C]lactate in BLMVs was stimulated 183 ± 14% by prior incubation with unlabeled L-lactate (lactate self-exchange) compared with the gluconate non-gradient control, whereas D-lactate-stimulated L-[14C]lactate uptake by only 53 ± 8%. Because D-lactate is not available as a sodium salt, these experiments were performed using either L-lactate as a lithium or sodium salt. The results were identical; thus, the two sets of data were averaged. Prior loading of BLMVs with other α-substituted anions (pyruvate and α-hydroxybutyrate) and with β-hydroxybutyrate also significantly stimulated the uptake of L-[14C]lactate. In contrast, we detected only marginal transstimulation with formate. The other unsubstituted anions tested (acetate, propionate, and butyrate) stimulated the uptake of L-[14C]lactate, but the stimulation decreased progressively as the carbon chain length increased from C2 to C4.

Because of the absence of lactate/H+ cotransport in LMVs, we next considered whether an anion-exchange pathway for lactate may represent an alternative transport system in these membranes. We tested this hypothesis in two ways. In the first approach, we compared the effects of outwardly directed L- and D-lactate gradients on the initial rates of L-[14C]lactate uptake (transstimulation). As shown in Fig. 5, the uptake of L-[14C]lactate was stimulated nearly 100% by prior incubation of LMVs with unlabeled L-lactate (L-lactate self-exchange) compared with the gluconate non-gradient control, but by only 30% with the D-isomer. Because these experiments were conducted under non-pH gradient conditions using a valinomycin/FCCP pH clamp and because L- and D-lactate are stereoisomers with similar physical properties, stimulation of L-[14C]lactate uptake by an outward L-lactate gradient must be due either to secondary alterations in pH, or as the result of transmembrane potential. In the second approach, we examined the possible effects of some anion transport blockers on L-lactate self-exchange. As shown in Fig. 6, L-[14C]lactate uptake was markedly inhibited by DIDS and CHC and almost completely by furo-
We next investigated whether additional mechanisms of lactate transport were present in BLMVs and LMVs. Because the rat MTAL possesses both luminal and basolateral Na\(^+\)-independent Cl\(^-\)/HCO\(_3\)\(^-\) exchangers (19), we examined the effect of outwardly directed lactate gradients on \(^{36}\)Cl\(^-\) uptake under conditions in which the vesicles were voltage- and pH-clamped. As indicated in Fig. 7, imposing an outward Cl\(^-\) gradient stimulated by \(-800\%\) the rates of \(^{36}\)Cl\(^-\) influx in both BLMVs and LMVs compared with their respective gluconate non-gradient controls, confirming the presence Cl\(^-\)/HCO\(_3\)\(^-\) exchangers operating on the Cl\(^-\)/HCO\(_3\)\(^-\) exchange mode in both preparations. In contrast, imposing an outward lactate gradient had no significant effect on \(^{36}\)Cl\(^-\) uptake in BLMVs and LMVs. Taken together, these results demonstrate that lactate is not a substrate for the BLMV and LMV Cl\(^-\)/HCO\(_3\)\(^-\) exchangers.

We also examined whether lactate could be cotransported with Na\(^+\), slowly approached equilibrium. Imposing an inward Na\(^+\) gradient caused no significant additional uptake of L-[\(^{14}\)C]lactate at the \(p < 0.05\) level for incubation periods of 0.5, 1, and 3 min and failed to induce an overshoot of this anion. Thus, these data argue against the existence of direct coupling between the flux of Na\(^+\) and the flux of L-[\(^{14}\)C]lactate (i.e. no Na\(^+\)/lactate cotransport) in both BLMVs and LMVs.

Five independent preparations of BLMVs and LMVs were assessed by immunoblotting for the presence of MCT1 and MCT2 polypeptides. As shown in Fig. 9A, the affinity-purified anti-MCT2 peptide antibody recognized a 37-kDa protein that was enriched in basolateral membranes (B) compared with luminal membranes (L). In contrast, MCT1 could not be detected neither in BLMV nor in LMV preparations isolated from rat MTALs (Fig. 9A). In immunoblotting performed with apical and basolateral membrane-enriched preparations from rat renal cortex, anti-MCT2 and anti-MCT1 antibodies recognized proteins with molecular masses of 37 and 41 kDa, respectively.
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In basolateral, but not in apical, membrane preparations. Labeling was blocked by the specific immunizing peptides (data not shown). Blots were also probed with an antibody to the Na\(^+\)/H\(^+\) exchanger NHE3 (Fig. 9B), which serve as markers for basolateral and apical membranes, respectively. Anti-NHE3 antibody reacted with a 55-kDa protein that was enriched in luminal membranes compared with basolateral membranes. Conversely, the Na\(^+\)/K\(^+\)-ATPase \(\beta\)-subunit identified as a 50-kDa protein was enriched in basolateral membranes and completely absent from luminal membranes. These results confirm the view (11, 19) that basolateral and luminal membranes isolated from rat MTALs are largely separated from each other.

Fig. 10 shows the localization of MCT2 in the inner stripe of the outer medulla of the rat kidney using indirect immunofluorescence on sections of paraformaldehyde-fixed kidney embedded in paraffin. Anti-MCT2 antibody binds intensely to thick ascending limbs and, to a lesser extent, to collecting ducts. When anti-MCT2 peptide antibody was incubated with the specific peptide antigen before application to a section, no binding of anti-MCT2 antibody was observed (data not shown).

To confirm the polarized expression of MCT2 in thick ascending limbs, we performed dual labeling indirect immunofluorescence for MCT2 and the apical NHE3 protein. In the inner stripe of the outer medulla, only the apical membrane of the MTAL showed intense staining for NHE3. Anti-MCT2 antibody (Fig. 10A) labeled the same tubules more intensely than did anti-NHE3 antibody (Fig. 10B). However, all labeling of MCT2 did not spatially colocalize with that of NHE3 (Fig. 10C), indicating that MCT2 expression is restricted to the basolateral domain.

DISCUSSION

This study provides the first description, at both the functional and molecular levels, of distinct monocarboxylate transporters at the basolateral and luminal surfaces of rat MTALs. In this report, we found evidence compatible with the presence of a member of the MCT family in the basolateral membranes isolated from rat MTALs. First, an inside alkaline pH gradient induced uptake of L-[\(^{14}\)C]lactate accumulation (overshoot). Second, the pH gradient-stimulated transport of L-[\(^{14}\)C]lactate was inhibited by CHC as well as by less specific anion transport blockers such as furosemide and DIDS. Third, L-[\(^{14}\)C]lactate uptake was markedly transstimulated by prior equilibration of the vesicles with l-lactate, but only marginally with d-lactate. Fourth, the MCT2 isoform was shown by immunochromical methods to be expressed exclusively on the basolateral membranes of the MTAL, whereas MCT1 could not be detected in this nephron segment.

In marked contrast, we found that l-lactate cannot be transported across the luminal membrane in exchange for OH\(^-\) (i.e. no lactate/OH\(^-\) exchange or H\(^+\)/lactate cotransport), but have detected the presence of a carrier-mediated system for lactate transport in these membranes. This conclusion is based on the following findings. First, the pH gradient-stimulated transport of L-[\(^{14}\)C]lactate is completely resistant to inhibition by anion transport blockers, suggesting that nonionic diffusion of lactic acid is the mechanism underlying pH gradient-stimulated lactate uptake. Second, under non-pH gradient conditions, L-[\(^{14}\)C]lactate uptake is markedly transstimulated by prior equilibration of the vesicles with l-lactate, but only marginally with d-lactate. Third, l-lactate self-exchange is sensitive to inhibition by anion transport blockers such as DIDS, CHC, and furosemide.

Immunofluorescence confocal microscopy using antibodies to MCT1 and MCT2 revealed the presence of MCT2 at the basolateral surfaces of the MTAL, whereas MCT1 could not be detected in this nephron segment (Fig. 10). We also found that the antibody to MCT2 detected a band of 37 kDa on immunoblots of BLVs isolated from rat MTALs, whereas this band was barely detectable on LMVs (Fig. 9). This apparent molecular mass of MCT2 on SDS-polyacrylamide gels is close to the value of 43 kDa reported by others on immunoblots from membranes of various hamster tissues (4). In agreement with our immunofluorescence microscopy experiments, Western blotting using the antibody to MCT1 showed no evidence for the presence of this isoform in both BLVs and LMVs (Fig. 9). As a positive control, we found that the antibody to MCT1 detected a band of 41 kDa on immunoblots of basolateral membranes isolated from rat kidney cortex (Fig. 9). In apparent contrast with our data, previous immunofluorescent studies (4) have shown expression of MCT2 restricted to the basolateral surfaces of the inner medullary collecting ducts of the hamster.
kidney. Using immunofluorescence confocal microscopy, we have detected MCT2 not only at the basolateral surfaces of the MTAL (Fig. 10), but also at the basolateral domains of the inner medullary collecting ducts, cortical medullary thick ascending limbs, and distal convoluting tubules. These differences between the results of Garcia et al. (4) and those reported here for the localization of the MCT2 isoform in rat kidneys are not surprising. Indeed, as recently pointed out in detail by Jackson et al. (7), there are markedly different patterns of expression of MCT1 and MCT2 in hamster and rat tissues. For example, Garcia et al. (4) found MCT2 to be abundant in hamster heart, but not brain, whereas the opposite was found by Jackson et al. (7) in membrane fractions prepared from rat heart and brain.

The intense expression of the MCT2 polypeptide in the basolateral membranes of rat MTALs, as demonstrated here by immunochemical methods (Figs. 9 and 10), completely matches the robust functional expression of the H⁺/monocarboxylate cotransporter (Figs. 1–3). Conversely, the absence of MCT1 and MCT2 immunoreactivities in the luminal membranes of rat MTALs (Figs. 9 and 10) is consistent with our functional data demonstrating that carrier-mediated lactate transport in these membranes (Figs. 5 and 6) does not occur via operation of a H⁺/monocarboxylate cotransporter (Fig. 3).

Transstimulation experiments were used to investigate the substrate specificity of the BLMV H⁺/lactate cotransporter (Fig. 4). These studies were performed using vesicles equilibrated in a highly buffered medium and in the presence of FCCP and valinomycin. It is therefore highly unlikely that the organic acids tested caused stimulation indirectly, i.e. either by alkalinizing the intravesicular space via non-ionic diffusion and/or by generating an interior positive. Consistent with this possibility is the observation that L-lactate was more than three times as potent as the D-isomer in stimulating L-[14C]lactate uptake (Fig. 4). The BLMV H⁺/lactate cotransporter (MCT2) is stereospecific and exhibits a broad specificity for monocarboxylates, as expected for an MCT isoform (9). L-Lactate and other α-substituted anions (pyruvate and α-hydroxybutyrate) are the best substrates. The possible importance of the 2-OH group in substrate recognition is suggested by the observations that butyrate has no affinity for the transporter and that β-hydroxybutyrate is significantly less effective than α-hydroxybutyrate in stimulating L-[14C]lactate uptake.

Since it is generally accepted that the proximal tubule depletes the tubular fluid in organic solutes and since MCT2 is restricted to the basolateral membrane of the MTAL (Figs. 9 and 10), it is likely that this isoform may play a major role in the uptake of lactate and other monocarboxylates. Consistent with this view, microperfusion studies indicated that lactate was effective to support Na⁺ transport only on the basolateral side of the cortical thick ascending limb (20), a nephron segment that exhibits a substrate preference comparable to that of the MTAL. The relatively high lactate concentration (10 mM) observed in rat medulla (21) probably maintains an inward lactate gradient sufficient to override the outward H⁺ gradient. The possibility exists, however, that MCT2 can mediate net lactate transport in either direction, depending on the physiological status and thus the actual driving forces. Under anoxic conditions, there is an increase in lactic acid production in all distal segments, and the maximum increase was observed in

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**Fig. 10. Immunohistochemical localization of MCT2 and NHE3 proteins in sections of paraformaldehyde-fixed rat kidney embedded in paraffin.** Sections were dually labeled with green fluorescence for MCT2 (A) and red fluorescence for NHE3 (B). Superimposition of the separately recorded images revealed no spatial colocalization of green and red fluorescence (yellow), demonstrating that there is no colocalization of the MCT2 protein with the apical NHE3 protein (C).
the MTAL (2), a situation that may be associated with marked intracellular acidification, which may damage the MTAL (2). Thus, a large thermodynamic driving force favoring net efflux of glycolytically derived lactic acid via MCT2 and via non-ionic diffusion is expected. The MTAL is particularly sensitive to anoxic damage because of the low medullary PO$_2$ in the range of 10–20 mm Hg and the high rate of active NaCl transport (22). Thus, under anoxic conditions, stimulation of MCT2 activity may contribute to limit intracellular acidification by increasing efflux of glycolytically derived lactic acid. The resultant lactic acid accumulation in the medullary interstitium probably acidifies the interstitial fluid, which may stimulate H$^+$ secretion in the adjacent collecting duct. Moreover, under normal conditions, lactic acid secretion in the tubular fluid across the luminal membrane via non-ionic diffusion is expected because there is no lactate in the lumen, and so, at least theoretically, there is a large gradient for lactate exit, and because pHi (23) is markedly lower than the pH of the tubular fluid (6.6 versus 7.4) (24).

The transport studies reported here on the luminal membrane vesicles are preliminary and were designed to detect the presence in these vesicles of an organic anion transporter, distinct from the monocarboxylate transporters of the MCT family. Clearly, evaluation of the physiological role of this new H$^+$-independent organic anion exchanger, demonstrated here, requires additional studies. With the exception of HCO$_3^-$, which is known to be present at a non-negligible concentration (~20 mM) in the tubular fluid of the rat MTAL (24), little information has been available concerning the concentrations of other anions that might be substrates for the newly described LMV organic anion exchanger (i.e. no lactate/HCO$_3^-$ exchange). Recently, we have also demonstrated that the BLMV H$^+$/monocarboxylate cotransporter has no affinity for HCO$_3^-$ (25).

In conclusion, we have demonstrated the existence of distinct monocarboxylate transporters in basolateral and luminal plasma membranes isolated from rat MTALs. The results of our immunolocalization studies demonstrate that the basolateral transport system is the H$^+$/monocarboxylate cotransporter MCT2 isoform. Under normal conditions, MCT2 may be of special importance in mediating the active uptake of l-lactate and of other substituted monocarboxylates of physiological interest. Under hypoxic conditions, MCT2 may limit the degree of intracellular acidification, potentially damaging for the MTAL, by extruding glycolytically derived lactic acid into the medullary interstitium. On the other hand, the luminal anion exchanger identified in this report has not been characterized molecularly, and its physiological function remains to be evaluated.

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