Localization of members of MCT monocarboxylate transporter family Slc16 in the kidney and regulation during metabolic acidosis

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LACTATE AND PYRUVATE ARE THE major monocarboxylates circulating in the blood (11). In the Cori cycle, the liver and kidney can utilize lactate arising from anaerobic glucose metabolism in muscle tissue to perform gluconeogenesis (1). Gluconeogenic enzymes are highly abundant in the cells of the proximal tubule (29, 43), and uptake and metabolic experiments have identified glutamine and lactate as major substrates for renal gluconeogenesis (8, 15). Conversely, lactate can serve as a substrate for further oxidation and thus, energy production in cells that are not in an anaerobic state. Lactate utilization supports renal transport activity and appears to be stimulated during alkalosis and reduced during acidosis (3). Depending on the energy and oxidative capacity of a cell, lactate can either be extruded from the cytosol or, inversely, taken up by the cell.

Lactate and other monocarboxylates are transported by transport proteins belonging to several solute carrier (SLC) families, SLC5 (26, 48), SLC16 (30), and SLC36 (19). Two members of the SLC5 family, namely, SLC5A8 (SMCT1) and SLC5A12 (SMCT2), were recently reported to be expressed in apical membranes of proximal tubule cells, suggesting to facilitate the uptake of lactate from the urinary ultrafiltrate (27). Deletion of both transporter genes in a double knockout mouse model resulted in a 29-fold increase in urinary lactate levels, indicating that the transporters are responsible for the major reabsorption of lactate (49). Similar results were obtained with Slc5a8 knockout mice, where lactate levels in urine and saliva were significantly increased due to lactate reabsorption deficiency (20). Two members of the SLC36 family of proton-dependent amino acid transporters, PAT1 and PAT2, have been shown to transport propionate and acetate (19) and are also localized to the luminal membrane of the proximal tubule. If these transporters contribute to monocarboxylate transport in vivo remains to be established.

The SLC16 family of monocarboxylate transporters comprises at least 14 members (30). However, the function, transport mode, and exact tissue distribution of several members have not been elucidated to date. MCT1 (SLC16A1), MCT2 (SLC16A7), MCT3 (SLC16A8), and MCT4 (SLC16A3) have been demonstrated to be genuine proton-driven monocarboxylate transporters. MCT1 has been shown to be present in basolateral membranes of proximal tubule cells (18), and its transport function has been investigated intensively (13, 14, 52). In addition, MCT1 and MCT2 have been shown to be present in the same tissues but in different cell types (24, 32, 40). In brain tissue, a potential lactate and pyruvate shunting mechanism between MCT1 in astrocytes and MCT2 in neurons was demonstrated (12, 13, 16, 46, 50). The MCT4 isofrom is predominantly expressed in skeletal muscle. Both MCT1 and MCT4 interact with CD147 (also known as basigin or OX-47), which may act as a chaperone enhancing membrane expression and transport activity (30). MCT8 (SLC16A2) does not transport monocarboxylates; instead, it is an efficient transporter for thyroid hormone isoforms T3 and T4 (21). Mutations of SLC16A2 cause X-linked mental retardation (17, 22). TAT1 (SLC16A10) has been identified as a transporter for aromatic amino acids, independent from proton transport and not accepting monocarboxylic acids (33). The transport substrate, mode, and tissue expression of the other SLC16 family members have not been elucidated to date. However, in a previous study investigating changes in mouse kidney transcriptome, we de-

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MCT1 Rabbit 1:200 1:2,000 Chemicon

Table 2. List of antibodies used in this study

<table>
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<th>Genes</th>
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Accession numbers and expected size of PCR products are given for each gene investigated.

Wistar rats (Janvier Elevage, Le Genest-St.-Isle, France), ~150–200 g body wt, were kept on standard chow and had free access to drinking water. Each mouse group consisted of 5 animals (total 20 animals). Four rats were used for immunohistochemistry. All experiments were performed according to Swiss Animal Welfare laws and approved by the local veterinary authority (Veterinäramt Zürich).

For all mouse groups, during the final 2 days of the experiment, animals were placed in metabolic cages where intake (food and water) and output (urine and stool) were monitored. Metabolic acidosis was induced by supplementing drinking water with 0.28 M NH4Cl and 1% sucrose for 48 h or acid load in food for 7 days (2 g of NH4Cl/100 g of standard rodent chow). Control mice received 1% sucrose in the drinking water or standard rodent chow, respectively. Urine was collected under mineral oil during the final 24 h, and urinary pH was measured immediately using a pH microelectrode (InLab 422) connected to a pH meter (SevenEasy; Mettler Toledo, Greifensee, Switzerland). Food and water intake as well as urine and stool output were recorded. Urinary creatinine and ammonium excretion were measured by the protocols of Jaffé and Berthelot, respectively (9). Analysis of urinary and serum lactate was performed using a commercially avail-
able kit (Lactate Assay Kit; Biomedical Research Service Center, University of Buffalo, Buffalo, NY) (49).

At the end of the experiments, the mice were killed by decapitation and heparinized mixed arterial-venous blood samples were collected for immediate blood-gas analysis on a Radiometer ABL 800 blood-gas analyzer (Radiometer, Copenhagen, Denmark). Kidneys were collected, snap frozen in liquid nitrogen, and kept at -80°C until further analysis.

**Immunohistochemistry.** The fixation of mouse and rat kidneys was performed as described previously (35). Male C57BL/6j mice were anesthetized with ketamine and xylazine and subsequently fixed by vascular perfusion through the left ventricle. The fixation consisted of 3% paraformaldehyde and 0.05% picric acid. It was dissolved in a 3:2 mixture of 0.1 M cacodylate buffer (pH 7.4, adjusted to 300 mosmol/kgH2O with sucrose) and 10% hydroxyethyl starch in saline (HAES-steril; Fresenius, Stans, Switzerland). After 5 min, the fixative was washed out by perfusion with cacodylate buffer for 5 min and the kidneys were removed. Similarly, rats were anesthetized with ketamine and xylazine and subsequently perfused through the left ventricle with PBS followed by a paraformaldehyde-lysine-periodate fixative (37). Kidneys were removed and fixed overnight at 4°C by immersion in paraformaldehyde-lysine-periodate, washed three times with PBS, and cryoprotected with a PBS/30% sucrose solution.

Kidneys were cut transversely into 2-mm slices which were subsequently mounted on thin cork plates with Frozen Section medium (Richard-Allan Scientific, Kalamazoo, MI). The slices were then consequently mounted on thin cork plates with Frozen Section medium cryoprotected with a PBS/30% sucrose solution. The sections were allowed to dry at 20°C and were either directly used or further stored at -80°C.

To perform immunohistochemistry, kidney slices were rehydrated by immersion in PBS for at least 20 min. Then, depending on the antibody used, antigen retrieval was performed with 1% SDS for 3 min, followed by three steps of washing in PBS for 5 min. Then, the sections were incubated with PBS containing 1% bovine serum albumin for 15 min before application of the primary antibody. Incubation of the primary antibody was performed for 75 min at room temperature. The sections were washed two times in high-NaCl-PBS (PBS 2.7% NaCl) and once in PBS (5 min each). After the final washing step, sections were mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and subsequently stored in the dark at 4°C. Microscopic images were taken using a confocal microscope (SP1 UV CLSM; Leica, Wetzlar, Germany) and processed using Adobe Photoshop software 7.0.

**RNA extraction and real-time RT-PCR.** Total RNA from one kidney was extracted using the Qiagen RNeasy Mini Kit (Qiagen, Hombrechtikon, Switzerland). Snap-frozen kidneys were homogenized in a pestle homogenizer (Potter-Elvehjem type) together with 1 ml precooled RLT buffer supplemented with β-mercaptoethanol at a final concentration of 1%. Subsequently, 200 μl of the homogenate were used for RNA preparation, which was carried out according to the manufacturer's protocol. DNase digestion was performed using the RNase-free DNase Set (Qiagen, Hilden, Germany). Total RNA extractions were analyzed for quality, purity, and concentration using a NanoDrop ND-1000 spectrophotometer (Wilmington, DE). RNA samples were diluted to a final concentration of 100 ng/μl, and cDNA was prepared using the TaqMan Reverse Transcriptase Reagent Kit (Applied Biosystems/Roche, Foster City, CA).

In brief, in a reaction volume of 40 μl, 300 ng of RNA was used as a template and mixed with the following final concentrations of RT buffer (1×): 5. 5 mM MgCl2, 2.5 μM random hexamers, 500 μM each dNTP mix, 0.4 U/μl RNase inhibitor, 1.25 U/μl multiscribe reverse transcriptase, and RNase-free water. Reverse transcription was performed with thermocycling conditions set at 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min on a thermocycler (Biometra, Goettingen, Germany).

Primers and probes of all genes of interest were designed using Primer Express Software (v.2.0.; Applied Biosystems), and primers

[Fig. 1. Specificity of antibodies against monocarboxylate transporter (MCT) and Na⁺-dependent monocarboxylate cotransporters (SMCT) isoforms in the kidney. Twenty micrograms of brush-border membrane (BBM) or 50 μg total membranes prepared from mouse or rat kidney were loaded and membranes probed with antibodies against MCT1, MCT2, MCT7, MCT8, SMCT1, or SMCT2 (on mouse kidney samples) and CD147 (on rat kidney samples). All antibodies yielded only 1 specific major band of the expected size (arrows).]
were tested by PCR with kidney cDNA and always resulted in a single product of the expected size (data not shown). Sequences of primers and probes are listed in Table 1.

Real-time PCR was performed with 22.5 ng of cDNA, 1 μM of each primer, and 100 nM 5' FAM- and 3' TAMRA- labeled probe (Microsynth, Balgach, Switzerland) and 1 × TaqMan Universal PCR Master Mix (Applied Biosystems). Reactions were run in 96-well optical reaction plates using the Prism 7500 fast Real-Time PCR cycler. Cycling conditions were set to one cycle for 2 min at 50°C, 10 min at 95°C, followed by 40 cycles at 95°C (10 s) and 60°C (1 min) with auto ramp time. All reactions were run in triplicate, and one negative control without addition of the multiscribe reverse transcription enzyme was included for each sample. The relative abundance of target mRNA was calculated to a reference mRNA (hypoxanthine-guanine phosphoribosyltransferase; HPRT). Relative expression levels were calculated as $R_2^{Ct(HPRT) - Ct(gene)}$, where Ct is the cycle number at which the fluorescence intensity is above background levels (threshold).

**Western blotting.** Frozen kidneys were used for brush-border membrane (BBM) preparation as described previously using the Mg2+-precipitation technique (10). Next, total membranes were prepared from frozen kidneys after homogenization in membrane preparation buffer (200 mM mannitol, 80 mM HEPES, 41 mM KOH, and 1 mM dissolved Complete Protease Inhibitor tablets, Roche Diagnostics, Rotkreuz, Switzerland) using a sonicator (B. Braun, Melsungen, Germany). The homogenate was centrifuged for 15 min at 4°C and 2,600 g, and the resulting supernatant was placed in an ultracentrifuge (Sorvall, Thermo Fischer Scientific) for 1 h at 100,000 g and 4°C. The pellet was resuspended in resuspension buffer, and protein concentration was quantified using a Lowry-based colorimetric assay (Bio-Rad D, Protein Assay, Bio-Rad, Hercules, CA). Fifty micrograms of total membrane protein or 20 μg of BBM protein were solubilized in Laemmli sample buffer, and SDS-PAGE was performed on 10% SDS-polyacrylamide gels. For immunoblotting, proteins were transferred electrophoretically to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA). After transfer, membranes were blocked for 1 h with 5% low-fat milk powder dissolved in Tris-buffered saline, 0.1% Tween 20 (TBS-T), at room temperature. Incubation of primary antibodies was performed overnight at 4°C or for 2 h at room temperature. Antibodies were diluted in 1% milk powder/TBS-T in dilutions as indicated in Table 2. Between incubation of primary and secondary antibodies, membranes were washed three times for 10 min with TBS-T and blocked again with 5% milk powder/TBS-T for 1 h. Primary antibody binding was detected with secondary antibodies linked to alkaline phosphatase or horseradish peroxidase, respectively (Promega, Madison, WI; GE Healthcare, Little Chalfont, Buckinghamshire, UK). Incubation of the secondary antibody was performed for 1 h at room temperature, followed by three washing steps in TBS-T for 10 min each. Finally, antibody binding was detected using chemiluminescence substrates for alkaline phosphatase or horseradish peroxidase (Millipore). The resulting signals were captured by the Diana III chemiluminescence detection system (Raytest, Straubenhardt, Germany), and the intensity of bands was quantified using Aida Image Analyzer software (Raytest). To normalize the relative protein levels detected by this method, all membranes were stripped and reprobed for β-actin, and the protein of interest/β-actin ratio was calculated and statistically analyzed.

**Statistics.** All data are presented as means ± SE and were tested for significance using the unpaired Student’s t-test or one-way ANOVA followed by Bonferroni’s multiple comparisons test. The level of statistical significance was set at P < 0.05.
ANOVA. Results with a $P$ value <0.05 were considered statistically significant.

RESULTS

Antibody testing. Specificity of antibodies was assessed by testing total membrane and BBM preparations of untreated mouse kidney. Each antibody revealed one major band at the expected molecular weight of the respective transport protein (Fig. 1). Of note, enrichment of BBM proteins in the BBM preparations resulted in more intense signals only for SMCT1 and SMCT2, in agreement with their previous localization in the BBM of the proximal tubule (27).

Localization of MCTs and SMCTs in mouse kidney. Indirect immunohistochemical staining for MCT1 in mouse kidneys localized the protein to the basolateral membranes of the proximal tubule cells. Here, costaining with 4F2hc served to confirm that both proteins localized to basolateral membranes and that MCT1 was restricted to S1 and S2 segments (Fig. 2). In contrast, S3 segments of the proximal tubule were negative for MCT1 and 4F2hc.

![Fig. 3. MCT2 is present in thick ascending limbs and distal convoluted tubules of mouse kidney. Mouse kidney sections were stained with 2 different antibodies against MCT2. A: the commercial antibody against MCT2 stained only the basolateral side of cortical and medullary thick ascending limbs of the loop of Henle (TAL) as previously reported (18). Original magnification ×400. B: the second antibody against MCT2 (red) stained also the basolateral side of the cortical and medullary TAL. C–E: MCT2 staining was also detected in distal convoluted tubules (DCT). Costaining with antibodies against calbindin D28K (green) was used to identify distal convoluted tubules. cTAL cortical TAL; G, glomerulum. Original magnification ×400.](http://ajprenal.physiology.org/)
Using a commercial antibody, positive immunoreactivity for MCT2 was detected in the thick ascending limb of the loop of Henle with the highest intensity in outer medullary regions (Fig. 3A), confirming previously published data demonstrating that MCT2 was confined to basolateral membranes of the thick ascending limbs and is absent from other regions of the mouse kidney (18). However, using a second antibody against MCT2 (53), we found MCT2-related staining not only in the thick ascending limb of the loop of Henle but also in the subsequent distal convoluted tubule. Costaining experiments together with calbindin D28k confirmed identification of the distal convoluted tubule (Fig. 3, B and C). The staining for MCT2 in the thick ascending limb of the loop of Henle and the distal convoluted tubule is in agreement with recently published in situ hybridizations for MCT2 (SLC16A7) in mouse kidney (see The Kidney Atlas at www.euregene.org).

MCT7 was detected in the outer medulla, and at the basolateral membranes of the thick ascending limbs (Fig. 4). In the cortex, MCT7 was localized in medullary rays. Interestingly, a distinct staining pattern in regions close to the glomerulum could be observed. Here, the macula densa showed positive immunoreactivity for MCT7. A structural hallmark of macula densa cells is their large and closely packed nuclei. Furthermore, MCT7 was detected in distal convoluted tubules. To identify distal convoluted tubules, costaining with the Na\(^+/\)Cl\(^-\) cotransporter was performed. This transporter was previously shown to be present only in apical membranes of the distal convoluted tubule (41). Double labeling of Na\(^+/\)Cl\(^-\) cotransporter and MCT7 showed colocalization in the same cells but at different sites, indicating that MCT7 is indeed confined to the basolateral membrane.

MCT8 expression was found throughout the entire proximal tubule on the basolateral side of the epithelial cells (Fig. 5). Costaining with 4F2hc, a specific marker of basolateral membranes of S1 and S2 segments, revealed that MCT8 overlapped in these segments. However, the expression of MCT8 extended beyond the S1 and S2 segments; the S3 segment was also positive for MCT8 expression. Interestingly, proximal tubule lateral membranes of epithelial cells (Fig. 5D) were intensely stained, in contrast to earlier segments where a more basal staining was observed.

SMCT1 and SMCT2, members of the SLC5 transporter family, were of interest as they are also known to transport lactate and pyruvate. In contrast to MCTs, they were found in apical membranes of epithelial cells in the nephron. In agreement with a previous publication (27), SMCT1 and SMCT2 localized to BBM of the proximal tubule (data not shown). In addition, we could detect both transport proteins also in the collecting duct. Here, expression was restricted exclusively to type A and B intercalated cells, as confirmed by costaining with aquaporin-2 and AE1 (see Fig. 6, A–D).

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**Fig. 4.** Localization of the MCT7 orphan transporter in mouse kidney. A: overview of medullary regions with positive staining of TAL in the outer stripe of the outer medulla (OSOM). In contrast, nephron segments of the inner stripe of the outer medulla (ISOM) were negative for MCT7. B: cortical TAL were also stained for MCT7 whereas adjacent S3 proximal tubule segments were negative. C: MCT7-related staining was also detected in the cells of the macula densa (arrow). D: colocalization of MCT7 (red) and the thiazide-sensitive NaCl cotransporter NCC (green) in the same segment, indicating expression of MCT7 in the distal convoluted tubule. Original magnification ×400–600.
Staining for CD147 (basigin) was performed on rat kidney slices since the antibody was generated in mouse and gave very high background staining in mouse tissues. The scaffolding protein CD147 has been reported to interact with MCT1 and MCT4 by affecting their expression at the cell surface (30). We found positive immunoreactivity for CD147 in proximal tubule cells (S1 segment) and type A intercalated cells of the collecting duct (Fig. 7). In particular, a distinct basolateral expression of CD147 was observed confirming previous data favoring the interaction of MCT1 with this scaffolding protein. Furthermore, costaining of AE1 and CD147 revealed overlapping expression of both proteins in the collecting duct (Fig. 7D). The potential partner(s) of CD147 in these cells is unknown.

Metabolic acidosis induces transient loss of lactate into urine. During analysis of transcriptome data, we had previously observed alterations in several mRNAs of SLC5 and SLC16 family members during 2 or 7 days of metabolic acidosis in mouse kidneys (39). Thus metabolic acidosis was induced for 2 and 7 days by administration of NH₄Cl in the drinking water. Blood-gas analysis measured standard parameters: pH, PCO₂, PO₂, bicarbonate, lactate, and glucose. A summary of relevant parameters is given in Table 3. Metabolic acidosis was confirmed by a significant decrease in blood pH and bicarbonate concentration in acidotic animals. Furthermore, urine analysis revealed more acidic urine and increased ammonia excretion in animals treated with NH₄Cl.

Blood glucose levels were not altered, and total blood and serum lactate concentrations were slightly lower in acidotic mice. Measurement of lactate concentrations in 24-h urine revealed a significant increase in acidotic animals after 2 days but not after 7 days. Correspondingly, the calculated total urinary lactate excretion was significantly higher in animals treated with 2 days of NH₄Cl treatment but not after NH₄Cl treatment for 7 days (Fig. 8).

Regulation of monocarboxylate transporters during metabolic acidosis. After 2 days of NH₄Cl loading, the mRNA abundance of SMCT2 was significantly decreased, whereas MCT7 was strongly increased (Fig. 9). In contrast, after 7 days, MCT1 and MCT8 mRNAs were strongly diminished and all other changes were nonsignificant.

Immunoblotting was employed to assess the impact of metabolic acidosis on protein abundance of these transporters. After 2 days of NH₄Cl addition, no significant changes in the protein abundance could be detected (Fig. 10). However, after 7 days, the expression of MCT7 was significantly reduced. All other changes detected were not significant.

DISCUSSION

Localization of monocarboxylate transporters along the nephron. Lactate and other monocarboxylates are filtered in the kidney and reabsorbed at least in part in the proximal tubule by sodium-dependent transport processes. It was previously dem-

Fig. 5. MCT8 is localized in the proximal tubule in mouse kidney. A and B: kidneys were stained with antibodies against the MCT8 (red) and the 4F2hc subunit of heteromeric amino acid transporters (green) that is mainly expressed in the early proximal tubule and with low abundance in the S2 segment. C and D: overlays of MCT8- and 4F2hc-related signals show colocalization of both in the early proximal tubule (S1) but distinct staining for MCT8 also in the late proximal tubule (S3) negative for 4F2hc. Of note, distinct and strong lateral MCT8 staining were observed in the late proximal tubule (arrow). Original magnification ×650.
onstrated that SMCT1 and SMCT2 are the major transporters facilitating this uptake (20, 27, 49). We have extended these observations by demonstrating here that both SMCT1 and SMCT2 are also localized to the luminal membrane of type A and type B intercalated cells along the collecting duct. The role of intercalated cells in lactate reabsorption is unclear. However, it has been shown that bicarbonate secretion by type B intercalated cells in the cortical collecting duct as well as bicarbonate reabsorption by type A intercalated cells in the outer medulla can be supported by monocarboxylates such as acetate and is completely independent from glycolysis (31).

The source and uptake route for monocarboxylates in these cells has not been characterized in detail. Moreover, we detected expression of CD147 on the basolateral side of the same intercalated cells, pointing to the possibility that CD147 may interact with another MCT isoform there and may be involved in mediating uptake of monocarboxylates from blood as fuel for intercalated cell metabolism. The identity of this putative interacting (MCT) transport protein is elusive to date. We did not detect MCT1 staining in intercalated cells, but the localization of MCT4, the other known interactor of CD147, was not tested here.
The MCT1 transporter was detected on the basolateral side of the proximal tubule (Table 4). Previously, MCT1 localization was reported in the hamster kidney (24), and our data confirm the localization of this transporter in the early proximal tubule. This localization is further supported by the detection of CD147, a necessary interactor of MCT1, at the same site as MCT1 along the early proximal tubule. Similar data were reported from the rabbit kidney, where CD147 staining was detected in the proximal tubule, the thick ascending limb, along the collecting duct, and the papillary surface epithelium (47).

Immunohistochemistry confirmed the localization of MCT2 to the medullary thick ascending limbs, as reported previously by Eladari et al. (18). In addition, we detected strong staining for MCT2 also along the distal convoluted tubule, which is in agreement with mRNA data from in situ hybridization of mouse kidneys (see www.euregene.org, The Kidney Atlas). However, we were unable to detect MCT2-related staining in principal cells as previously suggested (54). Nevertheless, the role of MCT1 and MCT2 in these nephron segments is unknown. MCT1 and MCT2 can import or export lactate and ketone bodies depending on the cellular environment and metabolism (30). Thereby, MCT1 and MCT2 are closely linked to gluconeogenesis, glycolysis, and β-oxidation (30). The major difference between MCT1 and MCT2 is that the latter has a higher affinity for lactate. In the kidney, the proximal tubule is engaged in gluconeogenesis and β-oxidation, and MCT1 may be involved in taking up lactate or pyruvate for these processes. Conversely, MCT1 may act as an efflux pathway for lactate from glycolysis or from the reabsorption of filtered lactate that has been taken up from urine via SMCT1 and SMCT2.

MCT7 is a transporter of an as yet unknown substrate and was previously described to be present in the brain, pancreas, and muscle. In mouse kidneys, we detected MCT7 on the basolateral side of cortical and medullary thick ascending limbs and distal convoluted tubules. Of note, MCT7 was also present in macula densa cells, highly specialized cells critical for sensing urine composition and regulation of glomerular filtration rate via the tubuloglomerular feedback mechanism.

The MCT8 transporter mediates thyroid hormone transport (51), and its localization to the proximal tubule is in agreement with the presence of type I iodothyronine 5'-deiodinase, the enzyme responsible for the conversion of T4 to T3 (34). The maturation and function of the proximal tubule is highly
regulated by thyroid hormones (2, 5, 6, 25, 38, 42), but whether MCT8 is involved in the regulation of the proximal tubule by thyroid hormones remains to be investigated.

**Regulation of monocarboxylate transporters during metabolic acidosis.** We had previously observed alterations of thyroid hormones remains to be investigated. MCT8 is involved in the regulation of the proximal tubule by thyroid hormones (2, 5, 6, 25, 38, 42), but whether regulatory mechanisms.

Using real-time PCR, we confirmed the previous microarray data and found significant changes in mRNA abundances for MCT1, MCT8, and SMCT2 (lower) and MCT7 and SMCT1 (higher). However, immunoblotting found reduced protein abundances only for MCT7. The reason(s) for the discrepancy in mRNA and protein expression data are not clear to date. Differences in the turnover and/or stability of mRNA and protein under conditions of metabolic acidosis might contribute to the apparent paradox. Clearly, this point requires further investigation.

Thus downregulation of protein abundance of known lactate transporters cannot explain the increased excretion of lactate with urine. However, the more acidic urine during metabolic acidosis may affect transport activity of luminal and/or basolateral lactate transporters. The transport of lactate by MCT transporters is energized by the cotransport of protons; thus acidification of the extracellular medium may reduce transport of lactate by these transporters (30). On the other hand, metabolic acidosis increases the expression and activity of the proximal tubular basolateral electrogenic sodium/bicarbonate cotransporter NBCe1 (SLC4A4), exporting bicarbonate into the interstitium and blood space. MCT1 and NBCe1 colocalize in the same membrane (44), and coexpression experiments in *Xenopus laevis* oocytes indicate that both transporters may functionally couple to enhance lactate fluxes (7). Whether this is the case in vivo and in the proximal tubule remains to be established.

In summary, we describe the localization of two sodium-dependent luminal monocarboxylate transporters, SMCT1 and SMCT2, in the proximal tubule and surprisingly also in intercalated cells along the collecting duct. Moreover, we observed segment-specific distribution of the MCT1, MCT2, MCT7, and MCT8, members of the SLC16 family of monocarboxylate transporters, as well as the chaperone CD147 in nephrons. These data collectively suggest that the kidney actively reabsorbs monocarboxylates from urine and that MCT transporters are involved in specific metabolic pathways in the kidney. Moreover, during metabolic acidosis a

**Table 3. Metabolic parameters**

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<td>147.4 ± 0.5</td>
<td>148.5 ± 0.7</td>
<td>148.3 ± 0.6</td>
</tr>
<tr>
<td>K⁺, mM</td>
<td>6.8 ± 0.2</td>
<td>5.6 ± 0.3*</td>
<td>6.4 ± 0.2</td>
<td>6.5 ± 0.2</td>
</tr>
<tr>
<td>Cl⁻, mM</td>
<td>111.4 ± 0.8</td>
<td>118.0 ± 0.7</td>
<td>113.3 ± 0.6</td>
<td>118.1 ± 0.7*</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>10.1 ± 0.5</td>
<td>10.2 ± 0.5</td>
<td>11.1 ± 0.8</td>
<td>10.4 ± 0.4</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>9.1 ± 0.4</td>
<td>10.6 ± 1.3</td>
<td>7.0 ± 0.7</td>
<td>8.1 ± 1.3</td>
</tr>
<tr>
<td>Lactate excretion, mg/24 h</td>
<td>0.49 ± 0.16</td>
<td>1.81 ± 0.40*</td>
<td>0.93 ± 0.25</td>
<td>0.83 ± 0.17</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 animals/group. A summary of blood and urinary data from mice on a control diet (2% sucrose) and 2 or 7 days of NH₄Cl plus 2% sucrose in drinking water is shown. Mice were kept in metabolic cages, and urine was collected for further analysis. *P < 0.05, †P < 0.01, ‡P < 0.001.
transient loss of lactate into urine was found; however, we did not observe dysregulation on the protein level of any of the known lactate transporters that could explain this loss. Thus acidosis may affect the activity of lactate transporters rather than their expression.

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GRANTS

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Fig. 9. Altered mRNA abundance of MCT and SMCT transporters in kidneys from acidic mice. Mice received either NH4Cl in food for 2 days or NH4Cl/sucrose in drinking water for 7 days or only sucrose (control groups). Total RNA preparations of kidneys were reverse transcribed and used for real-time PCR analysis. Hypoxanthine-guanine phosphoribosyltransferase was used as a housekeeping gene, and relative gene expression levels were calculated as described in MATERIAL AND METHODS. Data were normalized to the respective control groups. A: MCT1 mRNA abundance is decreased in kidneys of acidic animals. A significant decrease in MCT1 mRNA levels was observed only after 7 days of NH4Cl loading. B: MCT2 mRNA showed no significant changes in expression levels during 2- or 7-day NH4Cl treatment. C: MCT7 mRNA expression is upregulated during 2-day NH4Cl, but this effect returned to normal levels after 7-day NH4Cl and no difference in expression of MCT7 is observed. D: mRNA expression of MCT8 is decreased after both 2- and 7-day NH4Cl loading, becoming significant only after 7 days. E: MCT9 mRNA abundance did not change significantly. F: SMCT1 mRNA was significantly increased after 2-day acidosis, whereas this effect was not seen after 7 days. G: mRNA expression levels of SMCT2 were significantly downregulated after 2-day NH4Cl; the same trend was observed after 7 days. *P < 0.05, **P < 0.01, ***P < 0.001.
Effect of NH4Cl loading on protein expression of MCT and SMCT isoforms in mouse kidney. Total protein preparations of kidneys were subjected to SDS-PAGE, followed by immunoblotting against specific MCT and SMCT isoforms. All membranes were stripped and reprobed with β-actin to control for loading. Band intensities were determined by densitometric analysis, and expression of transporters was subsequently normalized against the corresponding β-actin bands. Protein abundance of MCT1, MCT2, MCT8, SMCT1 and SMCT2 remained unaltered. In contrast, protein expression of MCT7 in kidneys of acidicotic mice was significantly decreased after 7-day NH4Cl loading. The resulting ratio of target protein to actin was used for statistical analysis, and means ± SD are shown as bar graphs (2-day control and 2-day NH4Cl, n = 4), (7-day control and NH4Cl, n = 5). *P < 0.05, **P < 0.01, ***P < 0.001.

Table 4. Summary of renal localization studies and known substrates for transporter subunits belonging to SLC5 and SLC16 families

<table>
<thead>
<tr>
<th>Transporter Subunit</th>
<th>Nephron Localization</th>
<th>Subcellular Localization</th>
<th>Known Substrates</th>
<th>Ref. No(s.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCT1</td>
<td>Proximal tubule S1.2</td>
<td>Basolateral</td>
<td>Lactate, pyruvate, ketone bodies</td>
<td>Present study, 30</td>
</tr>
<tr>
<td>MCT2</td>
<td>Thick ascending limb, distal convoluted tubule</td>
<td>Basolateral</td>
<td>Pyruvate, lactate, ketone bodies</td>
<td>Present study, 18, 30</td>
</tr>
<tr>
<td>MCT7</td>
<td>Thick ascending limb, distal convoluted tubule</td>
<td>Basolateral</td>
<td>Unknown</td>
<td>Present study, 30</td>
</tr>
<tr>
<td>MCT8</td>
<td>Proximal tubule S1–S3</td>
<td>Basolateral</td>
<td>T4, T3 thyroid hormone</td>
<td>Present study, 30, 51</td>
</tr>
<tr>
<td>SMCT1</td>
<td>Proximal tubule S3 type A and B intercalated cells</td>
<td>Luminal</td>
<td>Lactate, ketone bodies</td>
<td>Present study, 27</td>
</tr>
<tr>
<td>SMCT2</td>
<td>Proximal tubule S1–S3 type A intercalated cells</td>
<td>Luminal</td>
<td>Lactate, pyruvate, nicotinate</td>
<td>Present study, 27</td>
</tr>
<tr>
<td>CD147</td>
<td>Proximal tubule A intercalated cells</td>
<td>Basolateral</td>
<td>Interacts with MCT1</td>
<td>Present study, 30</td>
</tr>
</tbody>
</table>

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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