Expression of slc5a8 in Kidney and Its Role in Na\(^+\)-coupled Transport of Lactate*

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We report here on the expression of slc5a8 in kidney and its relevance to Na\(^+\)-coupled reabsorption of lactate. slc5a8 is the murine ortholog of SLC5A8, a candidate tumor suppressor gene, which we recently cloned from human intestine and demonstrated its functional identity as a Na\(^+\)-coupled transporter for short-chain fatty acids and lactate. The slc5a8 cDNA, cloned from mouse kidney, codes for a protein consisting of 611 amino acids. When expressed heterologously in mammalian cells or Xenopus oocytes, slc5a8 mediates Na\(^+\)-coupled electrogenic transport of lactate/pyruvate as well as short-chain fatty acids (e.g. acetate, propionate, and butyrate). The Na\(^+\)/fatty acid stoichiometry varies depending on the fatty acid substrate (2:1 for lactate and 4:1 for propionate). This phenomenon of variable Na\(^+\)/substrate stoichiometry depending on the fatty acid substrate is also demonstrable with human SLC5A8. In situ hybridization with sagittal sections of mouse kidney demonstrates abundant expression of the transcripts in the cortex as well as the medulla. Brush border membrane vesicles prepared from rabbit kidney are able to transport lactate in a Na\(^+\)-coupled manner. The transport process exhibits the overshoot phenomenon, indicating uphill lactate transport in response to the tranmerebrane Na\(^+\) gradient. The Na\(^+\)-coupled lactate transport in these membrane vesicles is inhibitable by short-chain fatty acids. We conclude that slc5a8 is expressed abundantly in the kidney and that it plays a role in the active reabsorption of lactate. slc5a8 is the first transporter known to be expressed in mammalian kidney that has the ability to mediate the Na\(^+\)-coupled reabsorption of lactate.

L-Lactate is present in blood at a concentration of ~90 mg/liter (~1.5 mM), but the urinary excretion of L-lactate is very low (100–600 mg/days). With the normal glomerular filtration rate of 120 ml/min, the fractional reabsorption rate for L-lactate in mammalian kidney is ~95% (1). The molecular identity of the transport system that is responsible for such an effective absorption process in the kidney has not yet been established.

L-Lactate transport across mammalian cell plasma membrane is mediated by monocarboxylate transporters (MCTs) (2, 3). MCTs are H\(^+\)-coupled transporters and, therefore, the direction of lactate flux in mammalian cells depends on the net chemical gradients for H\(^+\) and lactate across the membrane. The transport process is electroneutral because of the H\(^+\)/lactate stoichiometry of 1:1. There are several members within the MCT gene family that are expressed differentially in different tissues (2, 3). Many of the MCT gene family members are expressed in the kidney (2–5), but the exact locations of these transporters in terms of cell type and the apical membrane versus the basolateral membrane of the tubular cells are not known. Because the lumen-facing brush border membrane of the renal tubular cells mediates the first step in the reabsorption of solutes present in the glomerular filtrate, studies have been carried out to determine the transport mechanisms responsible for this process (6–10). These studies have demonstrated that the renal brush border membrane possesses a Na\(^+\)-coupled transport system for L-lactate. Interestingly, there is no evidence for the presence of any H\(^+\)-coupled L-lactate transport system in this membrane (6). These findings show that MCTs do not play any role in the first step involved in the reabsorption of L-lactate in the kidney. There is no transport system expressed in mammalian tissues and known at the molecular level that has the ability to mediate Na\(^+\)-coupled transport of L-lactate. Thus, the molecular identity of the renal transport system that mediates the active reabsorption of L-lactate remains unknown.

Recently, Li et al. (11) identified a candidate tumor suppressor gene that is silenced by methylation in human colon cancer. This gene (SLC5A8) codes for a putative transporter belonging to the SLC5 gene family (also known as the Na\(^+\)/glucose cotransporter gene family). These investigators have also shown that SLC5A8 is a sodium transporter but failed to establish the identity of the cotransported substrate. The expression of this transporter in the colon and its predicted role as a tumor suppressor prompted us to test short-chain fatty acids as potential substrates for this transporter. Short-chain fatty acids such as acetate, propionate, and butyrate are produced in the colonic lumen at high concentrations by the bacterial fermentation of unabsorbed carbohydrates and dietary fiber (12–15). These fatty acids are the primary energy substrates for the colonic epithelium (12–15). In addition, they prevent colonic cell proliferation and reduce the incidence of colon cancer (16–19). Based on these findings, we recently tested the hypothesis that SLC5A8 may be a Na\(^+\)-coupled transporter for short-chain fatty acids (20). These studies showed that SLC5A8 does indeed mediate the Na\(^+\)-coupled transport of the short-chain fatty acids acetate, propionate, and butyrate. Interestingly, the
transporter also has the ability to mediate Na⁺-coupled transport of L-lactate. Because the substrate specificity of this Na⁺-coupled transporter is similar to that of the Na⁺-independent MCTs, we named this transporter the sodium-coupled monocarboxylate transporter (SMCT). The ability of SMCT to mediate Na⁺-coupled transport of L-lactate raises the question as to whether this transporter may be responsible for the active reabsorption of L-lactate in the kidney. To answer this question, we analyzed the expression of this transporter in mouse kidney by molecular cloning, in situ hybridization, functional studies of the cloned murine slc5a8, and a comparison of the functional characteristics of slc5a8 with those of L-lactate transport in purified renal brush border membrane vesicles. These studies demonstrate that SMCT is the transporter that is responsible for the active Na⁺-coupled reabsorption of L-lactate in mammalian kidney.

**EXPERIMENTAL PROCEDURES**

**Materials**—The following radiolabeled carboxylates were obtained from commercial sources: L-1⁴C-Lactate, [1⁴C]-butyrate, [1⁴C]-butyrate, and [1⁴C]-butyrate from American Radiolabeled Chemicals, St. Louis, MO; [1⁴C]-pyruvate, [1⁴C]-Cytosine, [1⁴C]-thymidine, and [1⁴C]-choline were from Moravek Biochemicals (Brea, CA); and [1⁴C]-penicillin and [1⁴C]-hexanoate were from Sigma.

**Cloning of Mouse slc5a8 cDNA**—A cDNA library was constructed in pSPORT1 vector using poly(A) RNA isolated from mouse kidney. The full-length murine slc5a8 cDNA was isolated from this library by homology screening. The nucleotide sequence of this clone has been deposited into the GenBank™ data base under the accession number AY484422.

**Functional Expression of Mouse slc5a8 in a Mammalian Cell Line**—The vaccinia virus expression system was used to express cloned mouse slc5a8 cDNA heterologously in the human renal epithelial cell line HRPE as described previously (20). The uptake of radiolabeled lactate and other substrates was measured in these cells 18 h following transfection. The transport buffer, in most cases, was 25 mM Mes/Tris (pH 6.5) containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄, and 5 mM glucose. Transport measurements were made in parallel in vector-transfected cells and in slc5a8 cDNA-transfected cells to account for endogenous transport activity. The slc5a8-specific transport was determined by subtracting the transport rates measured in vector-transfected cells from the transport rates measured in value cDNA-transfected cells.

**Functional Expression of Mouse slc5a8 in Xenopus laevis Oocytes**—Capped cRNA from cloned mouse slc5a8 cDNA was synthesized using the mMESSAGE mMACHINE kit (Ambion Inc., Austin, TX). Mature oocytes (stage IV or V) from X. laevis were injected with 50 ng of cRNA. Whole-cell recordings were performed 4 days after injection. Transfected oocytes were used for electrophysiological studies performed by the two-microelectrode voltage-clamp method (20). Oocytes were perfused with a NaCl-containing buffer (100 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, and 10 mM Hepes/Tris, pH 7.5), followed by the same buffer containing different fatty acids. The membrane potential was clamped at −50 mV. The differences between the steady-state currents measured in the presence and absence of substrates were considered as the substrate-induced currents. To investigate the current-membrane potential (I-V) relationship, step changes in membrane potential were applied, each for a duration of 100 ms in 20-mV increments. In the analysis of the saturation kinetics of substrate-induced currents, the kinetic parameter V₅₀ (i.e., the substrate concentration necessary for the induction of half-maximal current) was calculated by fitting the values of the substrate-induced currents to a Michaelis-Menten equation. The Na⁺ activation kinetics of substrate-induced currents was analyzed by measuring the substrate-specific currents in the presence of increasing concentrations of Na⁺. From the data from these experiments, we analyzed the Hill equation to determine the Hill coefficient (h, the number of Na⁺ ions involved in the activation process). The kinetic parameters were determined using the commercially available computer program Sigma Plot, version 6.0 (SPSS, Inc., Chicago, IL). Data were analyzed by nonlinear regression and confirmed by linear regression.

**Determination of Charge/Substrate Transfer Ratio**—The charge-to-substrate transfer ratio was determined for L-lactate and propionate in five different oocytes as described previously (20–22). For the determination of the charge-to-substrate transfer ratio using the oocyte expression system, high levels of expression of heterologous cRNA are required. We found that the levels of expression of slc5a8 cRNA synthesized from the pSPORT1-slc5a8 cDNA construct were sufficient for simple electrophysiological characterization of the transport process but not for the determination of the charge-to-substrate transfer ratio. Therefore, we amplified the coding region of the murine slc5a8 cDNA by PCR and subcloned the fragment into oocyte expression vector pGHI9. The pGHI9 vector (kindly provided by Dr. Peter S. Aronson, Yale University School of Medicine) contains the 3’-untranslated region of the Xenopus β-globin gene downstream of the cloning site. cRNAs containing these elements are known to be expressed in oocytes at high levels (23, 24). The oocytes were perfused with either 50 µM L-lactate (unlabeled plus radiolabeled L-lactate) or 25 µM propionate (unlabeled plus radiolabeled propionate), and inward currents were monitored over a period of 8–10 min. At the end of the experiment, the amounts of L-lactate and propionate transported into the oocytes were calculated by using the radioactivity associated with the oocytes. The area within the curve describing the relationship between the time and inward current was integrated to calculate the charge transferred into the oocyte during incubation with L-lactate and propionate. The values for substrate transfer and charge transfer were used to determine the charge-to-substrate transfer ratio.

**Subcloning of the Mouse slc5a8 Coding Region in pGHI9 Vector**—The cDNA coding region of murine slc5a8 was amplified by PCR using the primers 5’-GATATATAGCCATGGACGCGTCGCGGGACAT-3’ (sense) and 5’-AACCTTTACAGGCGTACCCATTGATCTTGGC-3’ (antisense) and pSPORT1-slc5a8 cDNA as the template. A HindIII site (indicated by the underlining) was added to the 5’-end of the antisense primer for the purpose of subcloning. The PCR product was first subcloned in a pGEM-T vector and then the insert was released by EcoRI/HindIII double digestion. The PCR product was ligated with EcoRI/HindIII and then used for the ligation of the slc5a8 cDNA insert. The resultant product was partially sequenced to confirm the orientation of the insert.

**Data Analysis**—Experiments with HRPE cells were repeated three times with three independent transfections, and transport measurements were made in duplicate in each experiment. In most cases, electrophysiological measurements of substrate-induced currents were performed at least at least three times with three independent transfections, and transport measurements were repeated at least three times with separate oocytes. The data were repeated at least three times with separate oocytes. The data were analyzed using the Student’s t test. A p value of <0.05 was considered significant.

**Northern Blot**—A commercially (Origene, Rockville, MD) available multiple tissue blot containing mRNA from different tissues of mouse was used to analyze the tissue expression pattern of slc5a8 transcripts in the mouse. Tissue blot did not contain mRNA from human intestine or colon. We therefore prepared a separate blot with mRNA from mouse small intestine and colon. These blots were probed with a 32-P-labeled cDNA probe specific for mouse slc5a8.

**In Situ Hybridization**—Sagittal sections of mouse kidneys were frozen immediately in Tissue-Tek OCT, sectioned at 60-μm thickness, and mounted on charged slides. As described previously in publications (25, 26), sections were rinsed in ice-cold PBS and treated with 1% diethylpyrocarbonate prepared in PBS to facilitate penetration of the labeled probes. Sections were permeabilized further with proteinase K (1 mg/ml) in PBS for 4 min. The proteinase K activity was stopped by rinsing the slides in glycine (2 mg/ml) in PBS. Sections were washed in PBS, equilibrated in 5% SSC, and prehybridized for 2 h at 58 °C in 50% (w/v) formamide, 5% SSC, 2% (w/v) blocking reagent (provided with the DIG nucleic acid detection kit), 0.1% (w/v) N-lauroylsarcosine, and 0.02% (w/v) SDS. Sections were hybridized with the antisense probe (1 µg/ml) and incubated overnight at 58 °C. The sections were washed twice in SSC at 25 °C, twice in 1% SSC at 55 °C, and twice in 0.1% SSC at 37 °C. For immunologic detection of the probe, sections were washed in a buffer containing 0.1% maleic acid and 0.15 M NaCl (pH 7.5) and blocked with the same buffer containing 1% blocking reagent. The anti-digoxigenin conjugated to alkaline phosphatase was diluted 1:5000, and slides were incubated with this antibody for 2 h at 25 °C. Sections were washed in the preceding wash buffer containing levamisole (200 mg/ml) twice for 10 min and equilibrated with a buffer containing 100 mM Tris-HCl (pH 8.0), 100 mM NaCl, 5 mM MgCl₂, 1 mM CaCl₂, 0.5% sodium dodecyl sulfate (SDS) developed in nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate. Slides were washed in distilled water and attached to coverslips but not counterstained so that the purplish red color precipitate, indicative of a positive reaction, could be visualized in the sections. In all cases, some cryosections were hybridized with the sense probe to determine nonspecific binding. For the preparation of antisense and sense (negative control) riboprobes, a 500-bp frag...
Results

Structural Features of Mouse slc5a8—The mouse slc5a8 cDNA is 5,346-bp-long and codes for a protein consisting of 611 amino acids. The sequence has been deposited into the GenBank™ (accession number AY484428). Hydrophobicity analysis of the amino acid sequence predicts 13 transmembrane domains in the protein with the amino terminus at the extracellular side of the membrane and the carboxyl terminus at the cytoplasmic side of the membrane. With this topology model, mouse slc5a8 has two putative N-glycosylation sites situated between transmembrane domains 12 and 13. The amino acid sequence of mouse slc5a8 is 95% identical to that of human SLC5A8. It also shows close homology to that of sodium-coupled iodide symporter (NIS) and sodium-coupled multivitamin transporter (SMVT). The sequence identity is 50% with mouse NIS and 46% with mouse SMVT. The amino acid sequence derived from the mouse clone is 100% identical to the sequence that was deposited in the GenBank™ (accession number BAC30480) as an unnamed protein product based on the mouse genome sequence.

Tissue Expression Pattern of slc5a8 in Mouse—Northern blot analysis indicates that slc5a8 transcripts are detectable only in the colon, small intestine, and kidney in mouse (Fig. 1). There is no detectable expression of the transporter in the heart, brain, spleen, lung, liver, skeletal muscle, testis, and kidney. There are three different transcripts (3, 4, and 5.4 kb). Although all three transcripts are expressed in equal amounts in the kidney, the 4- and 5.4-kb transcripts are predominant in the small intestine and colon, whereas the 3-kb transcript is not detected in these tissues.

Functional Features of Mouse slc5a8 in a Mammalian Cell Expression System—To analyze the transport function of the cloned mouse slc5a8, we first used a mammalian cell expression system. We expressed the cDNA in HEK293 cells using the vaccinia virus expression technique and compared the uptake of L-lactate in vector-transfected cells and in cDNA-transfected cells (Fig. 2A). The uptake of L-lactate was significantly higher in cDNA-transfected cells than in control cells. The increase was ~2-fold. The cDNA-induced L-lactate uptake was influenced by extracellular pH, with optimal uptake occurring at pH 6.5 (Fig. 2B).

We then evaluated the transport of eight different monocarboxylates as well as the dicarboxylate succinate and the tricarboxylate citrate using the respective radiolabeled substrates (Table I). The uptake of L-lactate, D-lactate, pyruvate, acetate, propionate, butyrate, pentanoate, and hexanoate was significantly higher in cells expressing mouse slc5a8 than in control cells transfected with empty vector (25–75% increase; p < 0.05). In contrast, the uptakes of succinate and citrate were not

Fig. 1. Northern blot for the tissue distribution pattern of SMCT transcripts in mouse. The blot obtained from commercial sources, which contains mRNA from the heart, brain, spleen, lung, liver, skeletal muscle, testis, and kidney, had 2 μg of mRNA per lane, whereas the blot prepared in our laboratory, which contain mRNA from intestinal and colon, had 5 μg of mRNA per lane. Different exposure times were used for the two blots. Therefore, the intensities of the hybridization signals are not comparable between the kidney and intestine/colon.

Fig. 2. Transport of L-lactate by mouse SMCT in HRPE cells. A, time course of L-[14C]lactate (250 μM) uptake in vector-transfected cells and cDNA-transfected cells. B, influence of extracellular pH on L-[14C]lactate (250 μM) uptake in vector-transfected cells and cDNA-transfected cells.
different between the control cells and the cDNA-transfected cells. These data show that the cloned mouse slc5a8 is capable of transporting various monocarboxylates. The Na⁺-dependent nature of mouse slc5a8 was analyzed by studying L-lactate uptake in vector-transfected cells and in cDNA-transfected cells in the presence or absence of Na⁺. The uptake of L-lactate was ~90% higher in cells expressing mouse slc5a8 than in control cells when measured in the presence of Na⁺. But, the cDNA-induced L-lactate uptake was not detectable when measured in the absence of Na⁺. K⁺ and Li⁺ were unable to substitute for Na⁺ in the uptake process mediated by mouse slc5a8. We also tested the Na⁺-dependent nature of the transporter with four other substrates (pyruvate, acetate, propionate, and butyrate), and the data showed that the uptakes of these substrates mediated by mouse slc5a8 were also absolutely dependent on the presence of Na⁺ (data not shown).

**Insensitivity of slc5a8 Transport Function to α-Cyano-4-hydroxycinnamic Acid**—Because the substrate specificity of slc5a8 was similar to that of MCTs, we examined the influence of α-cyano-4-hydroxycinnamic acid, a specific inhibitor of MCTs (2, 3), on the transport function of slc5a8. We measured the uptake of L-lactate and pyruvate in vector-transfected cells and cDNA-transfected cells in parallel in the absence and presence of 5 mM α-cyano-4-hydroxycinnamic acid (Fig. 3). In the absence of the MCT inhibitor, the expression of slc5a8 increased the uptake of L-lactate by 55 ± 5%. Interestingly, the MCT-specific inhibitor did not inhibit L-lactate uptake mediated by slc5a8. Instead, it stimulated the transport function of slc5a8 to a significant extent. In the presence of the inhibitor, the uptake of L-lactate increased 170 ± 7% in cDNA-transfected cells compared with control cells. A similar phenomenon was observed with pyruvate as the substrate. In the absence of α-cyano-4-hydroxycinnamic acid, the uptake of pyruvate was only 30 ± 3% higher in slc5a8-expressing cells than in control cells. However, when the inhibitor was present, the uptake of pyruvate in slc5a8-expressing cells was 165 ± 15% higher than in control cells. We then monitored the ability of short-chain fatty acids to inhibit slc5a8-mediated L-lactate uptake in the presence of the MCT inhibitor. All three short-chain fatty acids tested (acetate, propionate, and butyrate) inhibited the uptake of L-lactate via slc5a8 (Table I). At a concentration of 5 mM, the inhibition was almost complete with propionate and butyrate. Acetate was comparatively less potent, the inhibition being ~70% under similar conditions.

**Characteristics of Endogenous Monocarboxylate Transport System in HRPE Cells**—HRPE cells transfected with vector alone show considerable transport activity for various monocarboxylates (Table I). We investigated the characteristics of this endogenous activity to determine whether this activity is mediated by MCTs or SLC5A8. The uptake of acetate and L-lactate in HRPE cells transfected with vector alone was completely Na⁺-independent. For acetate (50 μM), the uptake was 0.45 ± 0.02 nmol/10⁶ cells/20 min in the presence of Na⁺ and 0.44 ± 0.01 nmol/10⁶ cells/20 min in the absence of Na⁺. For L-lactate (50 μM), the uptake was 0.75 ± 0.06 nmol/10⁶ cells/20 min in the presence of Na⁺ and 0.78 ± 0.04 nmol/10⁶ cells/20 min in the absence of Na⁺. The uptake process was dependent on extracellular pH, with maximal uptake occurring at pH 6.5 (Fig. 2). Furthermore, the uptake of acetate (20 μM) was inhibitable by L-lactate (54 ± 7% inhibition at 10 mM) and pyruvate (61 ± 3% inhibition at 10 mM). These data show that endogenous monocarboxylate transport in HRPE cells is mediated by

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**Table I**

<table>
<thead>
<tr>
<th>Carboxylate substrate</th>
<th>pSPORT1</th>
<th>slc5a8</th>
<th>Increase</th>
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<tbody>
<tr>
<td>L-lactate (50 μM)</td>
<td>0.77 ± 0.05</td>
<td>1.18 ± 0.04</td>
<td>53</td>
</tr>
<tr>
<td>d-Lactate (20 μM)</td>
<td>0.19 ± 0.01</td>
<td>0.33 ± 0.02</td>
<td>74</td>
</tr>
<tr>
<td>Pyruvate (50 μM)</td>
<td>0.65 ± 0.02</td>
<td>0.89 ± 0.05</td>
<td>37</td>
</tr>
<tr>
<td>Acetate (50 μM)</td>
<td>0.45 ± 0.02</td>
<td>0.76 ± 0.06</td>
<td>69</td>
</tr>
<tr>
<td>Propionate (50 μM)</td>
<td>0.57 ± 0.03</td>
<td>0.79 ± 0.04</td>
<td>39</td>
</tr>
<tr>
<td>Butyrate (50 μM)</td>
<td>1.66 ± 0.09</td>
<td>1.99 ± 0.04</td>
<td>20</td>
</tr>
<tr>
<td>Pentanoate (500 μM)</td>
<td>3.93 ± 0.12</td>
<td>4.93 ± 0.22</td>
<td>26</td>
</tr>
<tr>
<td>Hexanoate (400 μM)</td>
<td>2.53 ± 0.05</td>
<td>3.11 ± 0.11</td>
<td>23</td>
</tr>
<tr>
<td>Succinate (50 μM)</td>
<td>0.11 ± 0.01</td>
<td>0.12 ± 0.02</td>
<td>9</td>
</tr>
<tr>
<td>Citrate (50 μM)</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0</td>
</tr>
</tbody>
</table>

* p < 0.05.
studies showed that the uptake of L-[14C]lactate in oocytes was not detectable only when the perfusion medium contained NaCl. When NaCl was replaced with choline, the L-lactate-induced currents were not detectable, indicating the absolute requirement for Na⁺ in the slc5a8-mediated transport process. There was no involvement of external Cl⁻, because the L-lactate-induced currents were similar whether the perfusion medium contained NaCl or sodium gluconate.

The inward currents induced by substrates were saturable. With L-lactate as the substrate, the currents saturated with a $K_{0.5}$ value of 0.08 ± 0.02 μM at −50 mV (Fig. 5A). The L-lactate-induced currents showed a sigmoidal relationship with Na⁺ concentration (Fig. 5B). At −50 mV, the Hill coefficient for the activation of the transport process by Na⁺ was 1.7 ± 0.1. We also performed the electrophysiological studies with propionate as the substrate. The currents induced by propionate saturated with a $K_{0.5}$ value of 0.23 ± 0.03 μM at −50 mV (Fig. 6A). The propionate-induced currents showed a sigmoidal relationship with Na⁺ concentration (Fig. 6B). At −50 mV, the Hill coefficient for the activation of the transport process by Na⁺ was 1.8 ± 0.2. Because L-lactate and propionate exist primarily as monovalent anions under the experimental conditions, the involvement of more than one Na⁺ ion in the transport process provides the molecular basis for the electrogenic nature of the transport process. We calculated the $K_{0.5}$ values for pyruvate, acetate, and butyrate at −50 mV by analyzing the saturation kinetics of induced currents with each of these substrates. Acetate showed the lowest affinity for the transporter with a $K_{0.5}$ of 1.51 ± 0.15 mM. The corresponding values were 0.13 ± 0.02 mM for pyruvate and 0.08 ± 0.03 mM for butyrate. The magnitude of the currents induced by the substrates of slc5a8 was dependent on the membrane potential as evidenced from the I-V relationship (Fig. 7). The substrate-induced currents increased as the oocyte membrane was hyperpolarized. With propionate as the substrate, we analyzed the influence of membrane potential on various kinetic parameters (data not shown). The maximal inward current induced by propionate increased with hyperpolarization of the membrane. The value for $I_{\text{max}}$ was 23 ± 5 nA at −50 mV, and the value increased to 41 ± 8 nA at −150 mV. This suggests that the membrane potential serves as an additional driving force for the transport process. The value for $K_{0.5}$ for propionate was also influenced by membrane potential. The value for $K_{0.5}$ was 0.23 ± 0.03 mM at −50 mV, and the value decreased as the membrane potential became hyperpolarized and increased as the membrane potential became depolarized. This shows that the substrate affinity of the transporter is influenced by membrane potential to a significant extent. The value for the Hill coefficient (n) was, however, not affected by membrane potential. The value remained at 1.7–1.8 over the membrane potential range of −30 mV to −150 mV. We also tested the influence of pH of the perfusion medium on substrate-induced currents under voltage-clamp conditions. There were no differences between pH 7.5 and 6.5 in terms of substrate-induced currents (data not shown).

### Analysis of Charge-to-Substrate Transfer Ratio

With cDNA prepared from a pSPORT1-slc5a8 cDNA construct, the currents induced in oocytes by saturating concentrations of various substrates (5 mM) never exceeded 25 nA. In our experience, we have found these currents to be too low to allow us to determine the charge-to-substrate transfer ratio. Because the analysis involves perfusion of oocytes with radiolabeled substrates for simultaneous measurements of substrate uptake and substrate-induced currents, we had to use much lower concentrations (25–50 μM) of substrates to avoid a dilution of radioactivity in order to obtain a measurable uptake of radiolabeled substrate into oocytes within the 8–10 min period used to monitor the substrate-induced currents. With these low concentrations of substrates, the currents were even lower than 25 nA. Therefore, we decided to subclone the coding region of slc5a8 in a different vector in an attempt to increase the expression levels of the transporter. For this purpose, we chose pGH19 (23, 24). We first compared the uptake of L-[14C]lactate (50 μM) in oocytes injected with cRNAs prepared from pSPORT1-slc5a8 cDNA (full-length) and pGH19-slc5a8 cDNA (coding region) (Fig. 8A). The uptake in uninjected oocytes was 19.6 ± 1.6 pmol/oocyte/h. The uptake increased ~3-fold in

### Table II

<table>
<thead>
<tr>
<th>Short-chain fatty acid</th>
<th>L-lactate uptake</th>
<th>cDNA-specific uptake</th>
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<tbody>
<tr>
<td></td>
<td>pmol/10⁶ cells/20 min</td>
<td>pmol/10⁶ cells/20 min</td>
</tr>
<tr>
<td>Control</td>
<td>45.2 ± 2.9</td>
<td>83.7 ± 8.3</td>
</tr>
<tr>
<td>Acetate</td>
<td>27.1 ± 2.0</td>
<td>70.9 ± 4.3</td>
</tr>
<tr>
<td>Propionate</td>
<td>20.8 ± 0.9</td>
<td>34.0 ± 1.3</td>
</tr>
<tr>
<td>Butyrate</td>
<td>19.0 ± 0.5</td>
<td>23.6 ± 0.6</td>
</tr>
</tbody>
</table>

MCTs rather than by SLC5A8. We performed reverse transcription PCR to determine whether these cells express SLC5A8 (data not shown). The data showed that these cells do express SLC5A8 mRNA but at barely detectable levels. Human colon tissue was used as a positive control in these reverse transcription PCR experiments. Functional data, however, show that there is no detectable contribution of SLC5A8 to endogenous monocarboxylate transport activity in HRPE cells.

### Functional Features of Mouse slc5a8 in the X. laevis Oocyte System

Even though the mammalian cell expression system clearly indicated that the cloned mouse slc5a8 is able to transport L-lactate and other monocarboxylates in a Na⁺-dependent manner, the cDNA-induced uptake activity was small. This low activity made it difficult to carry out detailed kinetic analysis of the transport function. Therefore, we employed the X. laevis oocyte expression system. Initial studies showed that the uptake of L-[14C]lactate in oocytes injected with mouse slc5a8 cDNA was 3-fold higher than in water-injected oocytes (data not shown). We then used the electrophysiological approach to characterize the transport function of mouse slc5a8. Under voltage-clamp conditions, exposure of slc5a8-expressing oocytes to L-lactate and other monocarboxylates (5 mM) induced detectable inward currents when the perfusion buffer contained NaCl (Fig. 4A). Among the substrates tested, inward currents were detectable with monocarboxylates containing 2–7 carbon atoms. Octanoate, which contains eight carbon atoms, failed to induce any detectable currents. However, the magnitude of induced currents varied markedly depending on the substrate when examined at a fixed concentration (5 mM) of each substrate (Fig. 4B). L-Lactate and pyruvate produced the maximal currents, followed by acetate and propionate. Starting with butyrate, the currents decreased as the carbon chain length of the fatty acid increased. The transporter does not interact with monocarboxylates whose carbon chain length is greater than seven. The dicarboxylate succinate and the tricarboxylate citrate were not recognized as substrates (data not shown). Thus, the cloned mouse slc5a8 is specific for L-lactate and other short-chain monocarboxylates. The currents induced by L-lactate were detectable only when the perfusion medium contained Na⁺ (Fig. 4C). When Na⁺ was replaced with choline, the L-lactate-induced currents were not detectable, indicating the absolute requirement for Na⁺ in the slc5a8-mediated transport process. There was no involvement of external Cl⁻, because the L-lactate-induced currents were similar whether the perfusion medium contained NaCl or sodium gluconate.
oocytes injected with cRNA from pSPORT1-slc5a8 cDNA (53.0 ± 3.6 pmol/oocyte/h). Under similar conditions, the uptake increased 70-fold in oocytes injected with cRNA from pGH19-slc5a8 cDNA (1396 ± 91 pmol/oocyte/h). We then compared the currents induced by saturating concentrations (5 mM) of L-lactate in these oocytes (Fig. 8B). There were no detectable currents in uninjected oocytes when perfused with L-lactate. In oocytes injected with cRNA from pSPORT1-slc5a8 cDNA, the magnitude of L-lactate-induced currents was 25 ± 1 nA. Under similar conditions, the currents induced by L-lactate in oocytes injected with cRNA from pGH19-slc5a8 cDNA were severalfold higher (555 ± 36 nA).

Therefore, we performed an analysis of the charge-to-substrate transfer ratio for slc5a8 in oocytes injected with cRNA prepared from pGH19-slc5a8 cDNA. With five different oocytes, the ratio was 1.1 ± 0.1 for L-lactate (Fig. 9A). This value agreed well with the results from the Na⁺ activation kinetics, which yielded a value of 1.7 ± 0.1 for the Hill coefficient. The Hill coefficient suggests that the Na⁺/L-lactate stoichiometry is most likely 2:1. With this stoichiometry, the transport process is expected to be associated with the transfer of one positive charge per L-lactate molecule, giving a charge-to-substrate transfer ratio of 1. Because the Hill coefficient for the activation of propionate-induced currents was also 1.8 ± 0.2, we analyzed the charge-to-substrate transfer ratio for this substrate, expecting to see a value similar to that obtained with L-lactate. Surprisingly, the charge-to-substrate transfer ratio for propionate was not 1. The value was much higher (3.0 ± 0.2) (Fig. 9B), suggesting a Na⁺/propionate stoichiometry of 4:1. This was an intriguing finding not only because the Hill coefficient for the Na⁺-dependent activation of propionate-induced currents was 1.8 ± 0.2 (a value similar to that for L-lactate), but also because the Na⁺/substrate coupling ratio varied depending on the substrate. Interestingly, the value for propionate agreed with the results we recently reported for human SLC5A8 (20). However, we did not analyze the charge-to-substrate transfer ratio for human SLC5A8 with L-lactate as the substrate in our previous study. Therefore, we were interested to find out if the charge-to-substrate transfer ratio varies depending on the substrate even in the case of human SLC5A8. This prompted us to repeat the analysis of the charge-to-substrate transfer ratio for human SLC5A8 for L-lactate and propionate under identical conditions. The ratio was 1.3 ± 0.1 for L-lactate and 2.7 ± 0.2 for propionate (Fig. 9, A and B). These data show that the substrate-specific variation in the coupling ratio is not unique for mouse slc5a8. A similar phenomenon is also seen with human SLC5A8.

Expression Pattern of slc5a8 mRNA in Mouse Kidney—As evidenced from the Northern blot analysis, slc5a8 mRNA transcripts are expressed primarily in the kidney and the intestinal tract. To determine the regional distribution pattern of slc5a8 expression in the kidney, we performed in situ hybridization...
using sagittal sections of mouse kidney (Fig. 10). Positive signals with the antisense riboprobe were observed throughout the kidney, with expression being clearly evident in the cortex as well as in the medulla (Fig. 10 A). The hybridization signals with the antisense probe were specific, because the sense probe did not yield any detectable signals (Fig. 10, B, D, and F). At higher magnifications of the cortical (Fig. 10C) and medullary (Fig. 10E) sections, it was evident that the expression was restricted to tubular epithelial cells. The hybridization signals were more predominant in the cortex than in the medulla. The distribution pattern of the signals in the cortex indicates that the transporter is expressed solely in the proximal tubule. The presence of the signals in the cortex as well as in the medulla shows that the transporter is expressed not only in the convoluted portions of the proximal tubule (pars convoluta) but also in the straight portions of the proximal tubule (pars recta) and in the loop of Henle.

Characteristics of L-Lactate Uptake in Rabbit Renal Brush Border Membrane Vesicles—The uptake of L-lactate has been studied in purified renal brush border membrane vesicles in several laboratories (6–10). These studies have shown that renal reabsorption of L-lactate across the tubular epithelial apical membrane occurs via a Na⁺/H⁺-coupled process. However, there is very little information available in the literature regarding the substrate specificity of this transport process. d-

Lactate and pyruvate have been shown to share the L-lactate transport system in the kidney brush border membrane (6–10). Because slc5a8 is a Na⁺/H⁺-coupled transporter not only for L-
lactate but also for short-chain fatty acids such as acetate, propionate, and butyrate, it would be of interest to know if the Na\(^+\)-coupled l-lactate transporter expressed in mammalian kidney. Therefore, we studied l-lactate uptake in rabbit renal brush border membrane vesicles. These vesicles were prepared from kidney cortex. The uptake of l-lactate in these membrane vesicles was Na\(^+\)-dependent (Fig. 11A). The presence of an inwardly directed Na\(^+\) gradient stimulated the initial uptake rates by 12-fold. In the presence of a Na\(^+\) gradient, l-lactate uptake exhibited the overshoot phenomenon, the uptake values at a 0.5-min incubation being nine times higher than the equilibrium values measured with a 60-min incubation. When Na\(^+\) in the extracellular medium was replaced with K\(^+\), the uptake was very low and barely exceeded the equilibrium value. The Na\(^+\)-coupled l-lactate uptake was inhibited markedly by pyruvate as well as by various short-chain fatty acids (Fig. 11B). At a concentration of 5 mM, acetate, propionate, butyrate, pentanoate, and hexanoate inhibited the uptake of l-lactate (10 mM) by >70%. Dose-response studies showed that unlabeled l-lactate inhibited the uptake of radiolabeled l-lactate with an IC\(_{50}\) value of 0.27 ± 0.06 mM (Fig. 11C). The corresponding values for inhibition by propionate and butyrate were 0.08 ± 0.02 and 0.12 ± 0.02 mM. These data clearly show that the Na\(^+\)-coupled transport system for l-lactate in renal brush border membrane vesicles recognizes short-chain fatty acids as substrates. Thus, the characteristics (Na\(^+\) dependence and substrate specificity) of the Na\(^+\)/l-lactate cotransport system in the kidney brush border membrane are similar to those of the cloned slc5a8.

**DISCUSSION**

This is the first report of the molecular identity of the Na\(^+\)-coupled l-lactate transporter expressed in mammalian kidney. This is the same transporter that is present in the mammalian colon, where it participates in the Na\(^+\)-coupled absorption of short-chain fatty acids (11, 20). We have named this transporter SMCT for sodium-coupled monocarboxylate transporter because its substrate specificity is similar to that of MCTs. The substrates of MCTs include l-lactate, d-lactate, acetate, propionate, and butyrate (2, 3). All of these monocarboxylates are also substrates for the cloned mouse SMCT. The primary difference between MCTs and SMCT is in the nature of the driving force. Although MCTs are coupled to a transmembrane H\(^+\) gradient, SMCT is coupled to a transmembrane Na\(^+\) gradient. The stoichiometry for the coupled ion is, however, different for MCTs and SMCT. MCTs mediate the H\(^+\)/monocarboxylate stoichiometry of 1:1 (2, 3), whereas SMCT mediates the Na\(^+\)-coupled transport of monocarboxylates via an energetic mechanism with a Na\(^+\)/monocarboxylate stoichiometry depending on the monocarboxylate substrate. Interestingly, despite the similarity in substrate specificity between SMCT and MCTs, SMCT has no sequence homology to MCTs. SMCT and MCTs belong to two different gene families, the former being a member of the SLC5 gene family (also called the Na\(^+\)/glucose cotransporter gene family) (29), whereas the latter are members of the SLC16 gene family (2, 3). Another major difference between MCTs and SMCT is their tissue distribution pattern. MCTs are expressed widely in mammalian cells (2, 3), whereas the expression of SMCT is limited to the kidney and the intestinal tract. The abundant expression of SMCT in the kidney signifies its physiological function as a transporter responsible for the Na\(^+\)-coupled active reabsorption of l-lactate. In the intestinal tract, the primary substrates for the transporter are most likely the short-chain fatty acids acetate, propionate, and butyrate produced by bacterial fermentation of dietary fiber. There is evidence for the expression of SMCT in the apical membrane of thyroid epithelial cells (30, 31). But functional studies have shown that the transporter plays a role in the transport of iodide in these cells, a finding which led to the naming of the transporter as AIT (for apical iodide transporter) (30, 31). However, we could not demonstrate iodide transport via human SMCT (20) or mouse SMCT (data not shown) cloned in our laboratory. The reasons for the discrepancy are not known.

Previous studies on the electrogenic nature of the Na\(^+\)-coupled l-lactate transport process in mammalian kidney have produced conflicting data, with some studies providing evidence for the electrogenicity of the transport process (6, 8, 9), whereas others provide evidence for the electroneutral nature of the transport process (7). Our studies with the cloned SMCT show unequivocally that the transport process is electrogenic. Interestingly, the Na\(^+\)/monocarboxylate stoichiometry varies depending on the substrate. The value for this stoichiometry is 2:1 for l-lactate and 4:1 for propionate. This variable stoichiometry is not evident from the analysis of Na\(^+\) activation kinetics of substrate-induced inward currents. The value for the Hill coefficient obtained from these studies was 2 for l-lactate as well as propionate. The Hill coefficient does not always reflect the actual number of Na\(^+\) ions involved in the
transport process. For example, the Hill coefficient for the transport of succinate, a divalent anion, via the Na\(^+/\)H\(^+\)-coupled dicarboxylate transporters NaDC1 and NaDC3 is 2, but the transport process is definitively associated with substrate-induced inward currents, suggesting the involvement of at least three Na\(^+/\)H\(^+\) ions in the transport process (21, 32). However, the variable stoichiometry of mouse and human SMCT, depending on the substrate, is interesting. The only structural difference between L-lactate and propionate is the hydroxyl group. The presence (L-lactate) or absence (propionate) of the hydroxyl group appears to influence the conformation of the transporter protein upon binding of the substrate, and such substrate-dependent changes in the conformation may underlie the difference in Na\(^+/\)H\(^+\) coupling. It has to be mentioned here, however, that the exact order of binding of the substrates (Na\(^+/\) and L-lactate/propionate) is not known. Furthermore, the values for Na\(^+/\)/substrate stoichiometry for SMCT were obtained by simultaneous measurements of the transfer of the monocarboxylate substrates and the transfer of charge. Additional studies that involve direct measurements of Na\(^+/\) transfer would be needed to confirm these findings.

The data showing that extracellular pH influences SMCT function in the mammalian cell expression system but not in the X. laevis oocyte expression system are interesting. The influence of pH on the function of SMCT was assessed in the mammalian cell expression system without clamping the membrane potential, whereas the oocyte studies were done under voltage-clamp conditions. It is not clear whether these differences in the experimental conditions contributed to the observed differences in the influence of pH between the two expression systems. Alternatively, the apparent influence of extracellular pH on SMCT function in mammalian cells may actually be due to changes in intracellular pH induced by variations in extracellular pH. In contrast to mammalian cells, oocytes are more resistant to changes in intracellular pH in response to changes in extracellular pH. It is also possible that pH may have differential effect on the function of SMCT, depending on the cell type used for the heterologous expression. For example, the reduced folate transporter is influenced by extracellular pH when monitored in a mammalian cell expression system using an intestinal cell line, but pH has no effect on the function of the same transporter when monitored in the Xenopus oocyte expression system (33). If it is indeed true that the SMCT transport function is stimulated by extracellular
acidic pH, these findings may have a physiological significance. This stimulation cannot be due to the role of a transmembrane H⁺/H11001 gradient as an additional driving force for the transport process because, if that were the case, extracellular pH should have influenced the transport function in *Xenopus* oocytes as monitored by the substrate-induced inward currents. Lactic acid has a pKₐ of 3.8 and, therefore, exists predominantly (>99%) in the ionized form in the pH range 6.5–7.5. Because an extracellular acidic pH is expected to decrease rather than increase the concentration of the ionized form, these findings cannot be explained by pH-dependent changes in the concentration of lactate, the transportable form for SMCT. One possibility is that SMCT possesses a regulatory site for H⁺/H11001 on its exoplasmic surface. Such a possibility can be investigated in the future in renal tubular cell lines and in isolated renal brush border membrane vesicles by studying the influence of pH on the transport function of SMCT. One possibility is that SMCT possesses a regulatory site for H⁺ on its exoplasmic surface. Such a possibility can be investigated in the future in renal tubular cell lines and in isolated renal brush border membrane vesicles by studying the influence of pH on the transport function of SMCT. It is interesting to note that the pH of the fluid in the proximal tubule of the nephron is slightly acidic (~6.5–6.8) due to the secretion of H⁺ via a Na⁺-H⁺ exchanger. Therefore, irrespective of the mechanism of the stimulatory effect of extracellular acidic pH on SMCT function, these findings may have physiological relevance. The observed stimulation of SMCT by extracellular acidic pH in mammalian cells following heterologous expression of the cloned transporter may suggest that reabsorption of lactate in the renal tubule is optimized under physiological conditions by the stimulatory effect of H⁺. This means that there may be a functional link between SMCT and the Na⁺-H⁺ exchanger in the kidney. Additional studies are needed to investigate these interesting possibilities. Electrophysiological measurements of SMCT transport activity show that butyrate, pentanoate, and hexanoate induce less current compared with L-lactate, pyruvate, propionate, and acetate when monitored at a fixed concentration (5 mM) of each substrate (Fig. 4). However, kinetic analyses show that these differences are not due to differences in the substrate affinities, because the Kₐₐ values for butyrate and L-lactate are similar. Therefore, the differences in currents induced by 5 mM sub-


*FIG. 11. Characteristics of L-lactate uptake in rabbit kidney brush border membrane vesicles.* A, time course of uptake of L-[¹⁴C]lactate (10 µM) in the presence of either an inwardly directed Na⁺ gradient (●) or an inwardly directed K⁺ gradient (∇). B, inhibition of Na⁺-dependent uptake of 10 µM L-[¹⁴C]lactate (30-s incubation) by various unlabeled short-chain fatty acids (5 mM). Data are given as percent of control uptake (100%) measured in the absence of inhibitors. C, dose-response relationship for the inhibition of Na⁺-dependent uptake of 10 µM L-[¹⁴C]lactate (30-s incubation) by unlabeled L-lactate (●), propionate (○), and butyrate (□). Data are given as percent of control uptake (100%) measured in the absence of inhibitors.
strates most likely represent differences in the maximal current inducible by these substrates. This probability is corroborated by the data from membrane vesicle studies (Fig. 11). When examined at a fixed concentration (5 mM) for the ability of various short-chain fatty acids to inhibit the uptake of L-[14C]lactate, butyrate, pentanoate, and hexanoate are as effective as L-lactate and propionate.

In situ hybridization shows that SMCT mRNA is detectable, at least in the mouse kidney, in the cortex as well as the medulla. Even though the hybridization signals are more predominant in the cortex than in the medulla, detectable signals are present throughout the kidney, indicating that the transporter is expressed in most parts of the nephron. These findings are interesting, because micropuncture studies in rat kidney have shown that the absorption of lactate occurs predominantly in the proximal tubule (34). The most likely explanation for the differences between the in situ data and the micropuncture study is that the expression pattern of SMCT is heterogeneous among superficial nephrons, which are accessible for micropuncture studies and the nephrons present at deeper locations. A similar phenomenon has been observed in the absorption of certain amino acids (35).

The present studies suggesting that SMCT may be involved in the active reabsorption of not only L-lactate but also other short-chain fatty acids such as acetate, propionate, and butyrate in the kidney are of physiological significance. Specific cell surface receptors for short-chain fatty acids have been identified recently in mammals (36–38). Acetate, propionate, and butyrate are specific ligands for the G-protein-coupled receptors GPR41 and GPR43, and these receptors are expressed predominantly in immune cells (36, 37) and adipocytes (38). The presence of an efficient reabsorption mechanism for these important ligands in the kidney may effectively prevent their loss in the urine. Because SMCT is also expressed in the colon where the concentrations of short-chain fatty acids may reach as high as 100 mM in the lumen (12–15), the ability of the transporter to transport these fatty acids may be of more importance in the colon than in the kidney. The effective concentrations of these fatty acids to activate the receptors on immune cells is in the range of 0.01–1 mM. Only acetate is present in peripheral blood at concentrations relevant to receptor activation (39). Because the luminal concentrations of these fatty acids are in the millimolar range in the colon, the local concentrations of these fatty acids are likely to be much higher on the serosal side of the colonic epithelial layer than in the peripheral blood because of effective absorption of these fatty acids via SMCT. The intestinal tract constitutes one of the largest immune systems in the body, with immune cells residing predominantly in the lamina propria just below the mucosal cell layer. Therefore, the resident immune cells in the intestinal tract are likely to be exposed to relatively higher levels of short-chain fatty acids than the immune cells elsewhere in the body. The localized interaction of GPR41 and GPR43 in immune cells with short-chain fatty acids in the intestinal tract may have relevance to various physiological and pathophysiological conditions related to intestinal inflammation and anti-tumor immunity. Short-chain fatty acids in the intestinal lumen have been shown to be beneficial in reducing the risk for inflammatory bowel disease and colon cancer (16–19). The modulation of immune function in the intestinal tract by these fatty acids via the short-chain fatty acid receptors may play a role in this phenomenon.

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Expression of *slc5a8* in Kidney and Its Role in Na\(^+\)-coupled Transport of Lactate
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