Primary Structure and Functional Characteristics of a Mammalian Sodium-coupled High Affinity Dicarboxylate Transporter*

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We have cloned a Na+-dependent, high affinity dicarboxylate transporter (NaDC3) from rat placenta. NaDC3 exhibits 48% identity in amino acid sequence with rat NaDC1, a Na+-dependent, low affinity dicarboxylate transporter. NaDC3-specific mRNA is detectable in kidney, brain, liver, and placenta. When expressed in mammalian cells, NaDC3 mediates Na+-dependent transport of succinate with a $K_t$ of 2 $\mu$M. The transport function of NaDC3 shows a sigmoidal relationship with regard to Na+ concentration, with a Hill coefficient of 2.7. NaDC3 accepts a number of dicarboxylates including dimethylsuccinate as substrates and excludes monocarboxylates. Li+ inhibits NaDC3 in the presence of Na+. Transport of succinate by NaDC3 is markedly influenced by pH, the transport function gradually decreasing when pH is acidified from 8.0 to 5.5. In contrast, the influence of pH on NaDC3-mediated transport of citrate is biphasic in which a pH decrease from 8.0 to 6.5 stimulates the transport and any further acidification inhibits the transport. In addition, the potency of citrate to compete with NaDC3-mediated transport of succinate increases 25-fold when pH is changed from 7.5 to 5.5. These data show that NaDC3 interacts preferentially with the divalent anionic species of citrate. This represents the first report on the cloning and functional characterization of a mammalian Na+-dependent, high affinity dicarboxylate transporter.

Placenta plays an obligatory role in providing the developing fetus with essential nutrients and metabolic fuels (1, 2). This function is facilitated by the presence of specific transport mechanisms in the maternal facing brush border membrane and the fetal facing basal membrane of the placental syncytiotrophoblast. Several years ago, studies from our laboratory demonstrated the existence of a Na+-coupled transport system for dicarboxylates in brush border membrane vesicles prepared from human term placenta (3). This transport system accepts succinate and other Krebs cycle intermediates as substrates. The functional characteristics of the placental sodium/dicarboxylate transporter are distinct from those of the sodium/dicarboxylate transporter described in brush border membrane vesicles from mammalian kidney and intestine. The primary distinction is in substrate affinity. The transporter in the placental brush border membrane recognizes succinate with high affinity ($K_c \sim 5 \mu$M). In contrast, the transporter in the renal and intestinal brush border membranes exhibits relatively much lower affinity ($K_c \sim 100–500 \mu$M) for succinate (4–8). However, a high affinity sodium/dicarboxylate transporter, similar to the one expressed in the placental brush border membrane, is present in the renal basolateral membrane (9, 10) and in the liver canalicular membrane (11, 12).

There have been several reports on the cloning and functional characterization of the low affinity sodium/dicarboxylate transporter from mammalian kidney (13–16) and intestine (17). This transporter has been designated as either NaDC1 (Na+/dicarboxylate cotransporter 1) or SDCT1 (sodium/dicarboxylate transporter 1). We refer to this transporter as NaDC1 throughout this paper. The amino acid sequence of NaDC1 exhibits 78% identity between rabbit and human homologs (13, 14). The rat NaDC1 shows $\sim$70% identity with rabbit and human NaDC1 (15–17). Rabbit NaDC1 and human NaDC1 interact with succinate with a $K_v$ value of around 400 $\mu$M (13, 14). Interestingly, rat NaDC1 exhibits a $K_v$ value of 25–50 $\mu$M for succinate (15, 16), and the observed significant divergence in the amino acid sequence of rat NaDC1 in comparison with rabbit NaDC1 and human NaDC1 is likely to be related to the difference in the substrate affinity between rat NaDC1 and rabbit/human NaDC1. Recently a nonmammalian low affinity sodium/dicarboxylate transporter has been cloned from the intestine of Xenopus laevis (18). Cation specificity and tissue distribution of this transporter clearly differentiate it from mammalian NaDC1. Based on these differences, the X. laevis sodium/dicarboxylate transporter has been designated NaDC2. The $K_v$ value for succinate for the X. laevis transporter is in the range of 300–700 $\mu$M, depending on whether Na+ or Li+ is used as the cotransported cation. The amphibian NaDC2 may be a species-specific isoform of the mammalian NaDC1.

There is no information available in the literature on the molecular nature of the high affinity sodium/dicarboxylate transporter. We report here on the cloning and functional characterization of a high affinity sodium/dicarboxylate transporter NaDC3 from rat placenta. This transporter, when heterologously expressed in mammalian cells, mediates the transport of succinate with a $K_v$ value of 2 $\mu$M. The rat NaDC3 is 48% identical to rat NaDC1 in amino acid sequence. Northern blot analysis indicates that NaDC3 transcript is also present in the brain, in addition to placenta, kidney, and liver, the tissues in which the existence of a high affinity sodium/dicarboxylate transporter has been demonstrated by functional studies. This represents the first report on the molecular nature of a mammalian high affinity sodium/dicarboxylate transporter.

* This work was supported by National Institutes of Health Grant HD 33347 and DA 10045. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF081825.

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**EXPERIMENTAL PROCEDURES**

**Materials**—SuperScript Plasmid System for cDNA cloning and Lipo- 
fectin were purchased from Life Technologies, Inc. Restriction enzymes 
were obtained from New England Biolabs. NitroType transfer membranes 
were purchased from Micron Separations, Inc. The human reti- 
nal pigment epithelial (HRPE) cell line was kindly provided by M. A. 
Del Monte (Department of Ophthalmology, W. K. Kellogg Eye Center, 
Ann Arbor, MI). The cells were routinely cultured in Dulbecco’s mod- 
fied Eagle’s medium/F-12 medium supplemented with 10% fetal bovine 
serum and 100 μg/ml of penicillin and 100 units/ml of streptomycin. 

| 2,3-3H]Succinic acid (specific radioactivity, 37.5 Ci/mmol) was pur- 
| chased from Moravek Biochemicals (Brea, CA), and [14C]Citrate (specific- 
| ic radioactivity, 65 Ci/mmol) was purchased from American Radiola- 
| beled Chemicals (St. Louis, MO). [3H]Succinate (specific radioactivity, 37.5 
| Ci/mmol) was purchased from American Radiolabeled Chemicals. 

**Northern Blot Analysis**—Tissue distribution of the NaDC3-specific 
transcripts was determined by Northern analysis. A commercially 
available membrane blot containing size-fractionated mRNA (2 μg 
| each) from brain, heart, kidney, stomach, small intestine, and skeletal 
| muscle of rat was used for this purpose. A second blot containing mRNA 
| from rat placenta and liver was prepared in our laboratory. A BamHI– 
| EcoR1 fragment (~2.0 kilobase pairs) consisting of most of the open 
| reading frame of the NaDC3 cDNA was used as the probe.

**Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and Rec- 
striction Analysis**—To detect the presence of NaDC3 transcripts in rat 
placenta and in rat liver, RT-PCR was done with poly(A) RNA isolated 
from these tissues. The primers used were 5′-GGCTTCCACCGCAAT- 
GAT-3′ (upstream primer) and 5′-TGAAAGCGCTAGGAAACACG-3′ 
(downstream primer). These primers corresponded to nucleotide positions 465–482 and 1571–1588, respectively, in the rat NaDC3 cDNA. The 
expression of the RT-PCR product in HRPE cells was ascertained by 
Southern blot analysis. The PCR procedure consisted of 35 cycles: 94°C for 
1 min, 60°C for 1 min, and 72°C for 1 min. The PCR products were 
separated on a 2% agarose gel, transferred to nylon membranes, and 
exposed to X-ray films. The lengths of the PCR products were assessed 
by comparison with the molecular size markers.

**Functional Expression of the cDNA**—The cDNA was functionally 
expressed in HRPE cells by vaccinia virus expression system (23) as 
described previously (20, 21). HRPE cells were used instead of HeLa 
cells because they had less endogenous succinate transport activity 
(data not shown). Transport measurements were made at room temperature using [3H]succinate or [14C]citrate. In most experiments, the 
influence of KCl and calcium gluconate for CaCl2. When the influence of Na+ on succinate transport was being investigated, the buffers containing 140 
mm NaCl or 140 mm N-methyl-D-glucamine (NMDG) chloride were mixed to give uptake buffers of desired Na+ composition. When the influence of pH on transport was investigated, transport buffers of different pH values were prepared by varying the concentration of Tris, 
Hepes, and Mes.

**Data Analysis**—Uptake measurements were made in duplicate and 
each experiment was repeated two or three times with separate 
transfections. Results are given as means ± S.D. of these replicate values. 
Kinetic analyses were carried out by nonlinear as well as linear regres- 
sion methods using the commercially available computer programs 
Fig.P, version 6.0 (Cambridge, UK) or Sigma Plot (Chicago, IL).
present at positions 584 and 594. When rat NaDC3 is modeled to accommodate these N-glycosylation sites toward the extracytoplasmic side, both the N-terminal and the C-terminal ends of the protein are directed toward the extracellular side of the cell membrane. This is, however, only a hypothetical model, and further experimentation is needed to confirm the exact number of transmembrane domains and the orientation of the N and C termini and the loops between the transmembrane domains. A comparison of the amino acid sequence of rat NaDC3 with the protein sequences in the SwissProt sequence database revealed that NaDC3 bears significant homology to the known sodium-dependent dicarboxylate and sodium-dependent sulfate transporters (13–17, 26). The closest relative, rat NaDC1, has a sequence similarity of 71% and identity of 48% at the level of amino acid sequence (Fig. 1).

**Tissue Distribution of NaDC3 Transcripts—**Poly(A)⁺ RNA isolated from several tissues of rat was analyzed by Northern blot hybridization for the presence of mRNA transcripts of NaDC3 (Fig. 3). In the Northern blot obtained commercially, transcripts were detected in brain and kidney (Fig. 3A). mRNA from heart, stomach, small intestine, and skeletal muscle did not show any hybridization signal. The size of the primary transcript was 3.3 kilobases. An additional minor hybridizing transcript of 5.4 kilobases was also seen in both the positive tissues. In the second blot with mRNA from placenta and liver, although NaDC3-specific transcripts of 3.3 kilobases were detected in both placenta and liver, the hybridization signal obtained from placental mRNA was very weak. The hybridization signal in the mRNA from liver was comparable with that obtained in the mRNA from brain (data not shown). Since the cDNA was isolated from a rat placental cDNA library, RT-PCR was performed on mRNA isolated from rat placental tissue to conclusively prove the presence of NaDC3-specific mRNA in the placenta. Poly(A)⁺ RNA isolated from liver was used as a positive control, and reaction in which reverse transcriptase was omitted was used as negative control. As seen in Fig. 3B, an RT-PCR product of the expected size (1124 bp) was obtained with mRNA isolated from the rat placenta as well as liver. The identity of the RT-PCR product was confirmed by restriction analysis using the enzymes DraIII and NcoI as detailed under “Experimental Procedures.” The restriction fragments obtained from the RT-PCR products are of the expected size (Fig. 3C), confirming that NaDC3 is expressed in rat placenta.

**Functional Characterization—**The functional expression of the NaDC3 cDNA was done in HRPE cells by transient transfection followed by vaccinia virus-induced expression of the cDNA. The function was monitored by the transport of radio-labeled succinate. Fig. 4 shows the time course of the uptake of succinate by HRPE cells transfected with vector alone or NaDC3 cDNA. Na⁺-dependent uptake of succinate in control cells transfected with empty vector showed negligible transport, indicating that HRPE cells themselves do not have significant levels of NaDC3-like transport activity. Under these conditions, the uptake of succinate in NaDC3 cDNA-transfected cells was 75–100-fold higher. The uptake of succinate in the absence of extracellular Na⁺ in HRPE cells expressing NaDC3 was similar to the levels of Na⁺-dependent uptake measured in cells transfected with empty vector. These results clearly demonstrate that NaDC3 is a sodium-dependent succinate transporter. Since the NaDC3-mediated Na⁺-dependent uptake of succinate linearly increased at least up to 3 min, subsequent initial transport rate measurements were made using a 1-min incubation period.

The ionic dependence of the cDNA-stimulated succinate uptake was investigated by measuring succinate transport in the presence of various inorganic salts (Table I). Control uptake was measured in the presence of NaCl. Replacement of Na⁺ with equimolar concentrations of cations such as K⁺, choline, or NMDG almost completely (~99%) inhibited the succinate uptake, indicating that Na⁺ is essential for the transport func-
Replacement of Na\(^+\) with Li\(^+\) decreased the uptake markedly, but there was, however, significant residual uptake of succinate, indicating that Li\(^+\) can substitute for Na\(^+\) to a small extent. When Cl\(^-\) in the uptake buffer was replaced with F\(^-\), I\(^-\), SCN\(^-\), or NO\(_3\)^-, the initial uptake rate of [\(^3\)H]succinate was inhibited only marginally, suggesting the noninvolvement of the anion in the transport process. This anion independence is typical of transporters belonging to the sodium-dependent glucose transporter family (27).

Substrate saturation kinetics of the cloned NaDC3 were analyzed in cDNA-transfected HRPE cells (Fig. 5). The initial uptake rate of succinate was measured at varying concentrations of succinate in the uptake buffer (0.5–15 pmol/10^6 cells/min) (Fig. 5) and confirmed by linear regression (Fig. 5, inset). The cDNA-induced transport of succinate was saturable. The experimental values were found to fit best to a transport model consisting of a single transport system. The values for the kinetic constants, K\(_m\) (Michaelis-Menten constant) and V\(_{max}\) (maximal velocity), for the uptake of succinate in cDNA-transfected cells are 2.0 ± 0.1 pmol/10^6 cells/min, respectively.

The effect of Na\(^+\) on the uptake of succinate was investigated by measuring the uptake of succinate in HRPE cells transfected with NaDC3 cDNA in the presence of varying concentrations of extracellular Na\(^+\). The concentration of NaCl in the extracellular medium was varied from 0 to 75 mM. The relationship between the uptake rate and the Na\(^+\) concentration was sigmoidal (Fig. 6), suggesting the involvement of more than one Na\(^+\) per succinate molecule transported. The data were fit to the Hill equation, and the Hill co-efficient, which is the number of Na\(^+\) ions interacting with the carrier, was calculated. The value, determined from the slope of the Hill plot (Fig. 6, inset) was 2.7 ± 0.2. This indicates that for every succinate molecule transported, three Na\(^+\) ions are cotransported. Since succinate is a divalent anion at physiological pH, one extra positive charge enters the cell with every succinate molecule, thereby rendering the transport process rheogenic. The rheogenic nature of the transport process is supported by the findings that K\(^+\)-induced depolarization of the cells inhibit the transport function of NaDC3. Transport of succinate (20 nm) in cells exposed to a physiological concentration of K\(^+\) (5.6 mM) was 6.5 ± 0.4 pmol/10^6 cells/min, whereas this transport was reduced 32% to 4.4 ± 0.2 pmol/10^6 cells/min in cells exposed to 56 mM K\(^+\). Thus, both the Na\(^+\) gradient as well as the difference in the membrane potential across the cell membrane energize the transport process.

The substrate specificity of the transporter was evaluated by assessing the ability of various unlabeled mono-, di-, and tri-carboxylic compounds to inhibit the transport of labeled succinate in cDNA-transfected HRPE cells (Table II). Monocarboxylates lactate and pyruvate showed very little inhibition of [\(^3\)H]succinate uptake, indicating that monocarboxylates are not substrates. The two- and three-carbon chain dicarboxylates oxalate and malonate also were poor inhibitors, suggesting that the length of the carbon chain plays a crucial role in substrate recognition.Dicarboxylates of four- and five-carbon chain length (e.g., succinate, fumarate, and glutarate) markedly inhibited the uptake of radiolabeled succinate, indicating that these are the most ideal substrates recognized by the trans-
porter. The transporter is also able to distinguish between trans and cis isomers of unsaturated dicarboxylates. Fumarate (a trans isomer) was able to inhibit totally (99%) the uptake of [3H]succinate, whereas the corresponding cis isomer, male- 
ate, caused significantly much lesser inhibition (25%). Malate and α-ketoglutarate also inhibited the uptake of radiolabeled succinate very potently, indicating that the presence of a hy- 
droxyl group or a keto group on the α-carbon atom does not 
interfere with the ability to interact with the transporter. Sim-
ilarly, dimethylsuccinate and dimercaptosuccinate were also 
potent inhibitors of succinate uptake, indicating that substitu-
ition at the second and third carbon atoms in the succinate 
molecule with methyl or thiol groups does not abolish the 
interaction with the transporter. Dipicolinate, on the other 
hand exhibited a very small but significant ability to inhibit 
succinate uptake. Citrate, which is predominantly trivalent at 
PH 7.5, also inhibited succinate uptake by 60%. While the 
amino acid aspartate inhibited succinate uptake significantly 
(60%), glutamate had no effect.

When we examined the effect of Li⁺ on [3H]succinate uptake in the absence of Na⁺, we observed significant residual uptake of succinate, which indicated that in the absence of Na⁺, Li⁺ was able to substitute for Na⁺ to a small extent. But several studies with native renal brush border membrane vesicles as well as with cloned NaDC1 have shown that Li⁺ can inhibit the activity of the low affinity sodium/dicarboxylate transporter in the presence of Na⁺. Therefore, we studied the effect of Li⁺ on the activity of NaDC3 in the presence of Na⁺ to determine if the high affinity sodium/dicarboxylate transporter also interacts with Li⁺ in a similar manner. Potent inhibition was seen at very low concentrations of Li⁺, which seemed to plateau off at around 2.5 mM (data not shown). At this concentration, Li⁺ was able to inhibit the transport activity of NaDC3 by 60%. Further increase in the concentration of Li⁺ up to 40 mM did not
increase the inhibition significantly. Thus, Li⁺ is a potent inhibitor of NaDC3-mediated Na⁺-dependent succinate uptake. Interestingly, the inhibition is only partial.

Citrate exists predominantly as a tricarboxylate anion at pH 7.5, but the concentration of the dicarboxylate anion species increases considerably when the pH is changed to 5.5. In contrast, succinate exists predominantly as a dicarboxylate anion at pH 7.5 as well as at pH 5.5. Therefore, we investigated the influence of pH on the interaction of succinate and citrate with NaDC3 by assessing the ability of these compounds in unlabeled form to inhibit NaDC3-mediated succinate uptake at pH 7.5 and pH 5.5 (Fig. 7). These experiments revealed that the uptake of NaDC3-mediated [3H]succinate uptake was markedly influenced by pH, the uptake rate at pH 7.5 being about 3–4-fold higher than at pH 5.5. However, when compared with the corresponding control uptake, unlabeled succinate was able to inhibit [3H]succinate uptake at both pH values. There was a 2-fold difference in the inhibitory potency between pH 5.5 and 7.5. The IC₅₀ value (concentration of unlabeled compound to inhibit the uptake of radiolabeled succinate by 50%) for succinate was 1.5 ± 0.1 μM at pH 7.5. This value decreased to 0.7 ± 0.2 μM at pH 5.5. The maximal velocities for rNaDC3-mediated succinate uptake, calculated from these experiments, were 75 ± 2 pmol/10⁶ cells/min at pH 7.5 and 19 ± 4 pmol/10⁶ cells/min at pH 5.5, respectively. When citrate was used as the inhibitor, pH was found to have a much greater effect on the inhibitory potency. The IC₅₀ value for the inhibition of [3H]succinate uptake by citrate was 2.1 ± 0.2 mM at pH 7.5. This value decreased 25-fold to 0.08 ± 0.01 at pH 5.5. These data show that the transport function of NaDC3 is significantly inhibited by acid pH and that citrate is preferentially recognized by NaDC3 as a substrate in the divalent form.

The influence of pH on the NaDC3-mediated uptake of succinate and citrate is compared in Fig. 8. The uptake of succinate was markedly inhibited when the pH was changed from 8.0 to 5.5 (Fig. 8A). The uptake at pH 8.0 was almost 5-fold greater than the uptake at pH 5.5. The influence of pH on citrate uptake was very different from the influence on succinate uptake (Fig. 8B). The process of citrate uptake displayed a distinct pH optimum at about pH 7.0. Decreasing the pH from 8.0 to 6.5–7.0 stimulated citrate uptake, but further decrease below pH 6.5 inhibited citrate uptake. The uptake of succinate and citrate in cells transfected with vector alone was much less compared with uptake in cells transfected with NaDC3 cDNA, and this endogenous uptake was not influenced by pH. These data show that the function of NaDC3 is inhibited by acidic pH, NaDC3 accepts preferentially dicarboxylates as substrates, and the biphasic pH influence on citrate uptake is due to the opposing effects of acidic pH on the function of NaDC3 and on the concentration of the diionic species of citrate.

**DISCUSSION**

This report describes the cloning and functional characterization of a mammalian high affinity Na⁺-dependent dicarboxylate transporter (NaDC3). The NaDC3 was cloned from a rat placental cDNA library. This is the first high affinity dicarboxylate transporter to be characterized at the molecular level. The functional characteristics of rat NaDC3 were investigated by heterologously expressing the NaDC3 cDNA in HRPE cells. Rat NaDC3 transports succinate, and its function is obligatorily dependent on the presence of Na⁺. Li⁺ is able to substitute for Na⁺ to a very small but significant extent. The Na⁺:succinate stoichiometry is 3:1. The Kₛ for succinate is 2 μM. The transporter recognizes various dicarboxylates consisting of four- or five-carbon chain length. Dimethylsuccinate and dimercaptosuccinate exhibit greater effect on the inhibitory potency. The IC₅₀ value for the citrate was used as the inhibitor, pH was found to have a much higher influence of pH on the interaction of succinate and citrate with NaDC3. At pH 7.5, but the concentration of the dicarboxylate anion species markedly influenced by pH, the uptake rate at pH 7.5 being about 3–4-fold higher than at pH 5.5. Therefore, we investigated the influence of pH on the interaction of succinate and citrate with NaDC3 by assessing the ability of these compounds in unlabeled form to inhibit NaDC3-mediated succinate uptake at pH 7.5 and pH 5.5 (Fig. 7). These experiments revealed that the uptake of NaDC3-mediated [3H]succinate uptake was markedly influenced by pH, the uptake rate at pH 7.5 being about 3–4-fold higher than at pH 5.5. However, when compared with the corresponding control uptake, unlabeled succinate was able to inhibit [3H]succinate uptake at both pH values. There was a 2-fold difference in the inhibitory potency between pH 5.5 and 7.5. The IC₅₀ value (concentration of unlabeled compound to inhibit the uptake of radiolabeled succinate by 50%) for succinate was 1.5 ± 0.1 μM at pH 7.5. This value decreased to 0.7 ± 0.2 μM at pH 5.5. The maximal velocities for rNaDC3-mediated succinate uptake, calculated from these experiments, were 75 ± 2 pmol/10⁶ cells/min at pH 7.5 and 19 ± 4 pmol/10⁶ cells/min at pH 5.5, respectively. When citrate was used as the inhibitor, pH was found to have a much greater effect on the inhibitory potency. The IC₅₀ value for the inhibition of [3H]succinate uptake by citrate was 2.1 ± 0.2 mM at pH 7.5. This value decreased 25-fold to 0.08 ± 0.01 at pH 5.5. These data show that the transport function of NaDC3 is significantly inhibited by acid pH and that citrate is preferentially recognized by NaDC3 as a substrate in the divalent form.

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Cloning of High Affinity Dicarboxylate Transporter NaDC3

sucinate are also recognized as substrates by NaDC3.

There are marked differences in substrate affinity and substrate specificity between NaDC3 and NaDC1. The $K_t$ for succinate is 500–800 μM in the case of rabbit and human NaDC1 when evaluated by tracer uptake in X. laevis oocytes (13, 14). The rat NaDC1 exhibits a much lower $K_t$ for succinate, in the range of 25–30 μM, when analyzed by electrophysiological approaches in X. laevis oocytes (15, 16). These differences in $K_t$ values among NaDC1s from various animal species may not be entirely due to the differences in the experimental approaches employed in the determination of the $K_t$ values. A recent study (28) has shown that rabbit NaDC1 has a $K_t$ of 180 μM in X. laevis oocytes when analyzed electrophysiologically, in contrast to the $K_t$ value of 25–30 μM for rat NaDC1 obtained with similar experimental approaches (15, 16). Therefore, the possibility of actual differences in the affinity of NaDC1 among various animal species cannot be ruled out. The $K_t$ for the transport of succinate by rat NaDC3 is 2 μM, a value 10- to 15-fold less than the corresponding value for rat NaDC1. The difference is even greater when compared with $K_t$ values reported for rabbit and human NaDC1. Another significant difference between NaDC3 and NaDC1 is in substrate specificity. NaDC1s from various animal species exhibit similar substrate specificity. This includes preferential recognition of dicarboxylates with four- or five-carbon chain length, trans isomer selectivity for unsaturated dicarboxylates, and lack of interaction with monocarboxylates (13–16). NaDC3 also exhibits all of these characteristics with respect to substrate specificity. However, NaDC3 and NaDC1 markedly differ in the case of interaction with dimethylsuccinate. Rabbit NaDC1 does not recognize dimethylsuccinate as a substrate (13). In contrast, rat NaDC3 interacts very well with this substituted succinate derivative. These differences in the affinity for succinate and in the interaction with dimethylsuccinate between NaDC1 and NaDC3 constitute essential criteria for the identification of NaDC3 as the high affinity dicarboxylate transporter. Mammalian kidney expresses a low affinity dicarboxylate transporter as well as a high affinity dicarboxylate transporter, the former in the brush border membrane and the latter in the basolateral membrane (4–6, 9, 10). Only the high affinity transporter in the basolateral membrane interacts with dimethylsuccinate, and therefore this succinate derivative is considered as the test substrate for the high affinity transporter (29). Since NaDC3 has a $K_t$ of 2 μM for succinate and also interacts with dimethylsuccinate, it is clearly evident that NaDC3 represents the high affinity dicarboxylate transporter.

The high affinity dicarboxylate transporter has so far been shown to be expressed by functional studies in three tissues, namely kidney, liver, and placenta (3, 9–12). The present study provides evidence for the expression of NaDC3 in these three tissues. A surprising finding, however, is the evidence for the presence of NaDC3 mRNA transcripts in brain. Dicarboxylate transport function has not been described in this tissue.

With respect to interaction with Li$^+$, NaDC3 behaves similar to NaDC1. In the presence of Na$^+$, the transport function of NaDC3 is inhibited by Li$^+$. The inhibition is maximal (60%) at 1 mM Li$^+$, Interestingly, the inhibition does not go beyond 60% even when the concentration of Li$^+$ is increased to as high as 40 mM. In the case of NaDC1, there appears to be a species-dependent variation in sensitivity to Li$^+$ (30). The rabbit NaDC1 is inhibited 60% at 2.5 mM Li$^+$, whereas the human NaDC1 is inhibited only 20% even at 10 mM Li$^+$. These results show that rat NaDC3 and rabbit NaDC1 interact with Li$^+$ with high affinity. Furthermore, the present study shows that Li$^+$ can substitute for Na$^+$, although to a small extent, in supporting succinate transport by NaDC3. Similar results have been obtained for rabbit NaDC1 (28). Thus, Li$^+$ is a stimulator as well as an inhibitor for NaDC3 and NaDC1. This cation stimulates the activity of these transporters in the absence of Na$^+$ but inhibits the activity for these transporters in the presence of Na$^+$, (30). Pajor et al. (28) have recently analyzed the interaction of Na$^+$ and Li$^+$ with rabbit NaDC1 in great detail. This analysis has led to the following conclusions: (a) although Na$^+$ is the preferred cation for NaDC1, Li$^+$ can support transport; (b) one of the three cation binding sites in NaDC1 exhibits a higher affinity for Li$^+$ than for Na$^+$, and (c) the binding of Li$^+$ to this site results in inhibition of Na$^+$-dependent transport function of NaDC1. Our current data with NaDC3 suggest that NaDC3 behaves essentially in a similar manner with regard to interaction with Na$^+$ and Li$^+$.

NaDC3 and NaDC1 differ markedly in pH sensitivity. With succinate as the substrate, rat NaDC1 shows no change in transport function between pH 7.5 and 5.5 (15). Rabbit NaDC1 also behaves similarly in the pH range of 7.5–5.5 with glutarate as the substrate (30). Transport of glutarate by human NaDC1, on the other hand, is stimulated when pH is changed from 7.5 to 5.5 (30). The influence of pH on the transport of succinate by rat NaDC3 is dramatically different. The transport function of NaDC3 is markedly inhibited by acidic pH. The pH sensitivity profile of NaDC3 in the present study is similar to that of succinate transport via the high affinity dicarboxylate transporter in rat renal basolateral membrane vesicles (9).

The results of the influence of pH on the interaction of
Cloning of High Affinity Dicarboxylate Transporter NaDC3

NaDC3 with citrate are interesting. Citrate exists predominantly as a trivalent anion at pH 7.5, but the concentration of the divalent anionic species of citrate increases to significant levels when the pH is changed from 7.5 to 5.5. Our results with NaDC3 show that citrate is 25-fold more potent in competing with the transport of succinate at pH 5.5 than 7.5. This suggests that the divalent anionic species of citrate rather than the trivalent anionic species is preferred as the substrate by NaDC3. This suggestion is supported by the differential influence of pH on the NaDC3-mediated transport of succinate and citrate. While the transport of succinate is inhibited steadily by a decrease in pH from 8.0 to 5.5, the transport of citrate actually increases when the pH is changed from 8.0 to 6.5. However, when the pH is made more acidic by decreasing the pH below 6.5, the transport of citrate is inhibited. These observations can be explained based on the opposing effects of acidic pH on the transport activity of NaDC3 and the concentration of the transportable ionic species of citrate. A gradual decrease in pH from 8.0 to 5.5 steadily decreases the transport function of NaDC3 but steadily increases the concentration of the transportable divalent anionic species of citrate. These two opposing effects of pH on the transport function of NaDC3 and on the concentration of the transportable citrate species are responsible for the biphasic influence of pH on NaDC3-mediated citrate transport. These results are significant because Chen et al. (15) have recently suggested that the high affinity dicarboxylate transporter present in the renal basolateral membrane is capable of transporting the divalent as well as trivalent forms of citrate. This suggestion was primarily based on the findings by Wright and Wunz (10) that citrate transport in renal basolateral membrane vesicles is not affected markedly by pH. However, if the inhibitory influence of acidic pH on the carrier function is taken into account, it is obvious that the actual stimulatory effect of acidic pH on citrate transport is significantly underestimated under these conditions. We therefore conclude that there is no difference between the low affinity and high affinity dicarboxylate transporters in terms of preferential recognition of the divalent citrate over trivalent citrate as the substrate.

Functional studies have demonstrated that the low affinity and high affinity dicarboxylate transporters are differentially localized in the kidney tubular cells (4–6, 9, 10). The low affinity transporter is expressed in the brush border membrane, whereas the high affinity transporter is expressed in the basolateral membrane. Immunohistochemical studies using antibodies raised against the cloned rat NaDC1 has recently established the brush border membrane localization of the low affinity dicarboxylate transporter in kidney (16). Functional studies have also demonstrated that the low affinity transporter is expressed in the brush border membrane of intestinal epithelial cells (7, 8) and the high affinity transporter is expressed in the canalicular membrane of liver cells (11, 12). In the placenta, the high affinity dicarboxylate transporter is present in the brush border membrane (3). This membrane is in direct contact with maternal blood, and therefore the transporter may mediate the entry of succinate and other Krebs cycle intermediates from maternal blood into the placental syncytiotrophoblast. We speculate that monocarboxylates such as lactate and pyruvate and dicarboxylates such as succinate, α-ketoglutarate, malate, and fumarate may be actively transported from mother to fetus across the placenta to serve as metabolic fuels for fetal utilization. Our earlier studies have demonstrated the existence of a H+-dependent monocarboxylate transporter in the placental brush border membrane (31). The exit mechanisms for these monocarboxylates and dicarboxylates in the placental basal membrane remain to be investigated.

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