J. Membrane Biol. 175, 1–8 (2000)

The Journal of
Membrane
Biology

Topical Review

Molecular Properties of Sodium/Dicarboxylate Cotransporters

A.M. Pajor
Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77555-0641, USA

Received: 20 December 1999

Introduction

The active transport of Krebs cycle intermediates, such as succinate, α-ketoglutarate and citrate, is mediated by sodium-coupled transporters found on the plasma membranes of many epithelial cells. The preferred substrates of these transporters are dicarboxylates. Tricarboxylate substrates, such as citrate, are carried in protonated form. At least two classes of Na^+/dicarboxylate cotransporters have been identified, distinguished by differences in their relative affinity for succinate. The low affinity transporters, with K_m for succinate around 0.5 mM, are found on the apical membranes of renal proximal tubule and small intestine. The high affinity transporters, with K_m for succinate around 25 μM, are found on basolateral membranes of kidney and liver, in apical membranes of placenta and in brain synaptosomes. Recent advances in the field have identified a gene family of Na^+/dicarboxylate cotransporters with functional characteristics similar to those previously identified in isolated organs or membrane vesicles. Several reviews have described the functional properties of the Na^+/dicarboxylate cotransporters in their native membranes [48, 26, 13, 31, 32]. The purpose of this review is to provide an update on new advances, particularly in the cloning and characterization of members of the NaDC/NaSi gene family.

The NaDC/NaSi Family

The Na^+/dicarboxylate cotransporters of the plasma membrane, called NaDC, belong to a distinct gene family that includes the Na^+/sulfate cotransporters: NaSi-1, from kidney and intestine and SUT-1, from endothelial cells (Table 1). The members of the NaDC/NaSi family are not related to any of the di- and tricarboxylate transporters from bacteria or from mammalian mitochondria.

The first Na^+/dicarboxylate cotransporter to be cloned was isolated from a rabbit kidney cortex cDNA library using the technique of functional expression in *Xenopus* oocytes [29]. The rbNaDC-1 corresponds to the low affinity Na^+/dicarboxylate cotransporter of the apical membrane of the renal proximal tubule. NaDC-1 orthologs have also been isolated from human and rat (Table 1). The rat NaDC-1 is represented by three cDNAs that are almost identical in sequence: rNaDC-1 and SDCT1, isolated from rat kidney [41, 8], and clone RI-19, isolated from rat intestine [18]. The sequence differences among the rNaDC-1 clones are probably due to sequencing errors because the apparent differences in predicted amino acid sequence are eliminated when frame shifts are corrected. The amino acid sequences of the NaDC-1 orthologs are at least 73% identical to one another (Table 1). The human gene coding for NaDC-1, called SLC13A2 (solute carrier family 13, member 2), has been localized to chromosome 17 p11.1–q11.1 [22, 30]. The sequence of part of chromosome 17, which includes the SLC13A2 gene, has been completed (Genbank AC005726). The human SLC13A2 gene is approximately 23.8 kB in length and contains 12 exons [1].

A cDNA coding for a Na^+/dicarboxylate cotransporter from *Xenopus laevis* intestine, called NaDC-2, was also isolated by expression cloning [4]. The amino acid sequence of NaDC-2 is approximately 62% identical to NaDC-1. It is possible that NaDC-2 is the *Xenopus* ortholog of NaDC-1 (i.e., *x*NaDC-1) because of the high sequence identity between the two transporters. However, NaDC-2 differs from the other NaDC-1 or-

Correspondence to: A.M. Pajor

Key words: Succinate — Citrate — Sodium-dependent transport — NaDC family — Kidney

Table 1. Family of transporters related to NaDC-1

Subtype	Species (prefix)	% Identity with rbNaDC-1	Organ	Genbank no.	Reference
NaDC-1	Rabbit (rb)	100	Kidney	U12186	[29]
	Human (h)	78	Kidney	U26209	[30]
	Rat (r)	73	Kidney (rNaDC-1)	AB001321	[41]
			(SDCT1)	AF058714	[8]
			Intestine (RI-19)	U51153	[18]
NaDC-2	*Xenopus laevis* (x)	62	Intestine	U87318	[4]
NaDC-3	Rat (r)	42	Placenta (rNaDC3)	AF081825	[17]
			Kidney (SDCT2)	AF080451	[7]
	Winter flounder (f)	43	Kidney	AF102261	[45]
NaSi-1	Rat (r)	43	Kidney, intestine	L19102, Q07782	[23,27]
SUT-1	Human	40	Endothelial venules	AF169301	[11]

thologs in its unusual cation selectivity, since it accepts both sodium and lithium as cotransported cations, and its localization to the intestine and not the kidney [4].

Several cDNAs coding for high-affinity Na^+/dicarboxylate cotransporters, NaDC-3, have been cloned from rat placenta and kidney (also called SDCT2), and winter flounder kidney (Table 1). In addition, the NaDC-3 ortholog from human placenta has recently been cloned (V. Ganapathy, *personal communication*). The NaDC-3 orthologs are very similar in sequence to one another, about 90%, but they are only about 42% identical to the sequences of the NaDC-1 orthologs. The gene for the human NaDC-3, SLC13A3, is found on chromosome 20 q12–13.1 based on the partial sequence of chromosome 20 (Genbank accession no. AL034424).

Structure of the Na^+/Dicarboxylate Cotransporters

The transporters of the NaDC/NaSi family contain 590–600 amino acids and are likely to have similar protein structures. Hydropathy analysis of the primary sequences suggests anywhere from 8 to 12 hydrophobic regions long enough to cross the membrane as alpha-helices. The current model of rbNaDC-1 contains 11 transmembrane domains (Fig. 1). The carboxy terminus contains the N-glycosylation site at Asn-578 [35], conserved in all members of this family, which places the carboxy terminus on the outside of the cell (Fig. 1). The rbNaDC-1 also contains a consensus sequence for N-glycosylation at Asn-160 that is not glycosylated, which supports the proposed location of Asn-160 in the intracellular loop between transmembrane domains 4 and 5 [35]. The rest of the secondary structure model remains to be tested.

Tissue Distribution of Na^+/ Dicarboxylate Cotransporters

Low affinity Na^+/dicarboxylate cotransporters, with K_ms for succinate around 0.5 mM, have been identified functionally in brush border membranes of renal cortex and small intestine from rabbit and rat [19, 47, 50]. The primary function of the renal transporter is to reabsorb filtered Krebs cycle intermediates, particularly citrate. The regulation of urinary citrate concentrations is of considerable importance because of the role of citrate as a calcium chelator. Hypocitraturia is associated with a tendency to form kidney stones. In the small intestine, the Na^+/dicarboxylate cotransporter absorbs di- and tricarboxylates from the diet and from gastrointestinal secretions [32].

As predicted by the functional studies, the mRNA coding for the low affinity Na^+/dicarboxylate cotransporter, NaDC-1, is found in kidney cortex and small intestine [8, 29, 30, 40]. The NaDC-1 protein is located on the apical membranes in kidney, predominantly in the S2 segment of the renal proximal tubule, and in the small intestine [35, 40]. In addition, there is evidence suggesting that the mRNA for NaDC-1, or a closely related message, is found in organs such as lung, adrenal, liver, and epididymis, that had not been known previously to have low affinity Na^+/dicarboxylate transporters [8, 29]. NaDC-1 mRNA is not found in testis, brain, eye, heart, or skeletal muscle [29, 40]. Interestingly, the rat NaDC-1 cDNA also hybridizes with mRNA from large intestine [40]. Since the rat large intestine contains a low affinity Na^+/dicarboxylate cotransporter that is functionally distinct from the low affinity transporter of the small intestine [47] it is possible that the large intestine transporter is closely related in sequence to NaDC-1, and could possibly be a splice variant.

High affinity Na^+/dicarboxylate cotransporters have been identified functionally in basolateral membranes of renal cortex and liver, in brush border membranes of placenta, and in brain synaptosomes [5, 9, 10, 25, 28, 42, 43, 50, 52]. The high-affinity NaDC-3 transporters cloned from placenta and kidney have identical amino acid sequences [7, 17]. Northern blots have identified mRNA coding for NaDC-3 (or a closely-related message) in kidney, placenta, liver and brain, but not in

Table 2. Functional properties of Na^+/dicarboxylate cotransporters measured by radiotracer uptakes (Tracer) or substrate-dependent currents in two-electrode voltage clamp experiments (TEVC) at Vh −50 mV

Clone	K_m succinate (μM)		K_m citrate (μM)		Lithium		Reference
	Tracer	TEVC	Tracer	TEVC	Inhibition	Transport	
rbNaDC-1	450	180	900	*n.d.*	Yes	Yes	[29, 33]
hNaDC-1	800	1100	7000	6800	No	No	[30, 51]
rNaDC-1	*n.d.*	24–29	*n.d.*	320–640	Yes	No	[8, 40]
xNaDC-2	278	*n.d.*	*n.d.*	*n.d.*	No	Yes	[4]
rNaDC-3	2	15	*n.d.*	220	Yes	No	[7, 17]
fNaDC-3	30	*n.d.*	*n.d.*	*n.d.*	Yes	*n.d.*	[45]

In some of the Na^+ dicarboxylate cotransporters, lithium acts as a competitive inhibitor of sodium. Lithium can also substitute for sodium to drive uphill transport of dicarboxylates. (*n.d.*, not determined).

heart, spleen, lung, skeletal muscle, or testis, which resembles the distribution based on functional studies [7, 17].

Functional Properties of Na^+/ Dicarboxylate Cotransporters

All of the members of the NaDC/NaSi family are sodium-coupled, electrogenic transporters that share similar transport mechanisms. The transporters couple three sodium ions and one divalent anion substrate, with the net transfer of one positive charge across the membrane. The transport mechanism appears to be ordered, with sodium binding first followed by substrate.

Substrates

The NaDC-1 orthologs correspond to the low affinity Na^+/dicarboxylate cotransporters found on the apical membrane in the renal proximal tubule and small intestine [19, 50]. The rabbit and human NaDC-1 have similar K_ms for succinate, 0.5 and 0.8 mM, respectively (Table 2). In contrast, the rat NaDC-1 (SDCT1) has a K_m for succinate of 24–29 μM [8, 40], similar to the K_m for methylsuccinate of 130 μM in rat renal brush border membranes [44]. The high affinity Na^+/dicarboxylate cotransporters are represented by the NaDC-3 orthologs, with K_ms for succinate between 2 and 30 μM [7, 17, 45], similar to previous studies with isolated membrane vesicles.

All of the cloned Na^+/dicarboxylate cotransporters have broad, overlapping substrate specifies for a wide range of di- and tricarboxylates, including succinate, citrate and α-ketoglutarate. There are some species and isoform differences, probably related to different affinities for the substrates. Although previous studies with rat renal vesicles suggested that dimethylsuccinate (DMS) is a specific substrate for the high affinity transporter, the cloned transporters show different interactions with DMS [5, 46]. Both the high and low affinity transporters from rat, rNaDC-1 (SDCT1) and rNaDC-3 (SDCT2), exhibit currents in the presence of dimethylsuccinate, but the rabbit NaDC-1 does not [7, 33]. In addition, some of the NaDC-1 and NaDC-3 orthologs carry the amino acids aspartate and glutamate, although with lowered efficiency [7, 8, 51].

Cations

All of the members of the NaDC/NaSi family are sodium-coupled transporters. The coupling stoichiometry is 3 sodium ions for each divalent anion substrate, with the net transfer of one positive charge across the membrane. Substrate-dependent currents in the presence of sodium have been measured in all of the transporters that have been tested (Table 2, [6]). The voltage-dependent step in transport appears to be the binding of sodium and substrate translocation, whereas substrate binding is relatively unaffected by voltage. The coupling stoichiometry has been measured directly for the rat NaDC-1 by the coupled measurement of radiolabeled substrate uptake and inward charge movements under voltage-clamp conditions [8, 40].

One characteristic property of the Na^+/dicarboxylate cotransporters previously identified in functional studies is their sensitivity to inhibition by millimolar concentrations of lithium, even in the presence of 100 mM sodium [49]. In rbNaDC-1, lithium inhibits transport with an apparent K_i of 2 mM [36]. Lithium acts as a competitive inhibitor of sodium, and binds with high affinity at one of the three sodium binding sites [33]. Lithium can also substitute for sodium, but the conformational change produced by lithium binding does not produce the optimal substrate binding site, and the K_m for succinate in lithium is about 30 mM [33]. However, there appear to

be species and isoform differences in interactions with lithium. Some of the transporters, such as rNaDC-3 and rNaDC-1, are inhibited by lithium but lithium does not substitute for sodium (Table 2). The human NaDC-1 does not appear to interact with lithium at all. Finally, xNaDC-2 is not inhibited by lithium, probably because lithium can drive transport almost as effectively as sodium [4].

Studies with isolated brush border membrane vesicles indicate that an additional low affinity Na^+/dicarboxylate cotransporter cDNA remains to be isolated. In the rat, the Na^+/dicarboxylate cotransporter in the small intestine, rNaDC-1, is sensitive to inhibition by lithium whereas the transporter found in colon, which has not been cloned yet, is insensitive to inhibition by lithium [47].

pH Effects

The NaDC-1, NaDC-2 and NaDC-3 transporters exhibit differences in their response to pH. Since the preferred substrates of these transporters are dicarboxylates, and tricarboxylates are carried in protonated form, pH changes can alter transport activity by changing the concentration of preferred substrate. The alterations in pH may also affect the transport proteins. In the NaDC-1 orthologs, the predominant effect of pH is on the concentration of substrate. The transport of succinate is insensitive to changes in pH, whereas the transport of citrate is stimulated at acidic pH, indicating that citrate is carried preferentially as a divalent anion [8, 36]. There is some evidence that trivalent citrate inhibits transport in the human and rat NaDC-1 (SDCT1), because the V_{max} at pH 7.5 is lower than at 5.5 [8, 36]. In contrast, the rabbit NaDC-1 has the same V_{max} at pH 7.5 and 5.5, and the only effect of pH is on the K_m for citrate (0.8 mM at pH 7.5 and 0.3 mM at pH 5.5) [36]. The *Xenopus* NaDC-2, has a similar lack of response to pH changes when succinate transport is measured, but unlike the NaDC-1 orthologs, there is very little effect of pH on the transport of citrate [4].

The high affinity Na^+/dicarboxylate cotransporter in renal basolateral membrane vesicles exhibits a distinct pH response. The transport of succinate shows a pH optimum around 7.5 and the transport of citrate is somewhat stimulated at acidic pH but the effect is not as marked as in brush border membranes [5, 50]. The cloned high affinity transporters, NaDC-3, also show a pH response that is different from the NaDC-1 orthologs. In NaDC-3, the transport of succinate is low at acidic pH and highest at basic pH [7, 17]. Interestingly, there appear to be different effects of pH on citrate transport by rNaDC-3 (SDCT2) depending on the expression system. When rNaDC-3 (SDCT2) is expressed in *Xenopus* oocytes, the effect of pH is similar to the results of studies with renal basolateral membrane vesicles [7]. However, the same rNaDC-3 protein expressed in mammalian cells has a pH optimum for citrate transport between 6.5–7, and the lowest transport rates at pH 5.5 or 8 [17]. The inhibition of succinate transport by citrate, however, is more pronounced at acidic pH, suggesting that the preferred species is protonated citrate [17].

Inhibitors

A high-affinity inhibitor of Na^+/dicarboxylate cotransport has not yet been identified and there are species differences in the response to the low-affinity inhibitors. The rabbit NaDC-1 is inhibited by flufenamate (IC_{50} 250 μM) and furosemide (IC_{50} 1.5 mM) but the human and rat NaDC-1 are insensitive to these compounds [36, 8]. The rat NaDC-1 (SDCT1) is sensitive to high concentrations of phloretin (0.5–1 mM), which inhibits both substrate-dependent and substrate-independent currents [8]. In contrast, phloretin specifically inhibits the Na^+-leak currents in the absence of substrate but not the substrate-dependent currents in hNaDC-1 [51]. Other transport inhibitors, such as DIDS, amiloride, and probenecid, have no effect on the members of the NaDC/NaSi family [8, 36].

Water Transport

Similar to many other transporters, there is evidence that NaDC-1 transports water [24]. The presence of rbNaDC-1 protein in the plasma membrane results in an increased passive water permeability that is dependent on the osmotic gradient. However, when rbNaDC-1 is actively transporting, a second type of water permeability appears that is independent of the osmotic gradient across the membrane. Water transport by rbNaDC-1 is stoichiometrically coupled to substrate transport, with the movement of 176 water molecules, 3 sodium ions and 1 dicarboxylate across the membrane for each transport cycle. Transport and volume changes occur simultaneously, and both can be inhibited by the addition of lithium [24].

Structure-Function Studies of Na^+/Dicarboxylate Cotransporters

The human and rabbit NaDC-1 exhibit differences in their relative affinities for citrate, sodium and lithium [30]. Chimera studies have shown that the differences in citrate K_m depend on transmembrane domains 7, 10, 11 (and their associated cytoplasmic/extracellular loops) [16] (Fig. 1). All three transmembrane domains must be present for the full change in K_m to occur and partial effects are seen when only one or two transmembrane

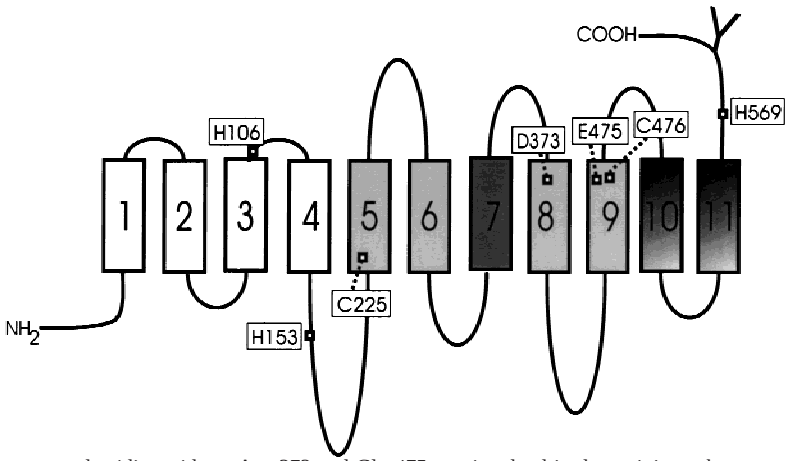

Fig. 1. Secondary structure model of NaDC-1 with 11 transmembrane domains (TMD) (numbered 1–11) and an extracellular carboxy terminus containing the N-glycosylation site. The shaded areas represent functionally important regions: TMD 5–11 contains the substrate recognition domain [38]; TMD 7, 10, 11 contain residues that determine the differences in citrate affinity [16]; the differences in sodium affinity are determined by residues in TMD 10, 11 and the differences in sensitivity to lithium inhibition are determined by residues in TMD 11 [16]. The histidines involved in binding diethylpyrocarbonate (DEPC) are His-153 and His-569; His-106 affects protein trafficking [39]. The cysteines that mediate inhibition by p-chloromercuribenzene sulfonate (pCMBS) are at positions 476 and 225 [34]. Two conserved acidic residues, Asp-373 and Glu-475, are involved in determining substrate and cation affinity [12].

domains are substituted. Interestingly, the differences in the relative affinity for sodium in the two transporters are determined by residues in transmembrane domains 10 and 11. The difference in sensitivity to lithium is determined by transmembrane domain 11, which could help to identify the location of the high affinity cation binding site. The results of this study suggest that the residues making up the cation and substrate binding sites must be located close together in the carboxy-terminal part of the protein. Chimeras between the dicarboxylate and sulfate transporters, rbNaDC-1 and NaSi-1, also show that the last 7 transmembrane domains contain the substrate recognition domain [38] (Fig. 1).

The chimera studies help to identify regions of the protein responsible for differences in function. In an alternate approach, conserved acidic amino acids were mutated in rbNaDC-1 to try to identify amino acids responsible for common functions in the members of this family [12]. The functionally important residues were also located in the carboxy terminal half of the protein (Fig. 1). Asp-373 and Glu-475, in transmembrane domains 8 and 9, respectively, appear to have dual roles in determining sodium and substrate affinity [12].

The cysteine at position 476, adjacent to Glu-475, was found to mediate the inhibition of transport by the cysteine-specific reagent, p-chloromercuribenzene sulfonate (pCMBS) [34]. Cys-476 is located near the extracytoplasmic face of transmembrane domain 9 (Fig. 1). The results suggest that Cys-476 is accessible from the outside of the membrane, in a water-filled channel or exposed on the surface of the transporter. A second residue, Cys-225, found close to the inner part of the membrane in transmembrane domain 5, is also sensitive to pCMBS inhibition (Fig. 1). This result suggests either that the location of Cys-225 in our model is incorrect, or that an aqueous-filled pore traverses the membrane to allow pCMBS to reach Cys-225 from the outside of the cell. Unlike other transport proteins, rbNaDC-1 does not tolerate complete substitution of all of the cysteines. Replacement of cysteines in rbNaDC-1 results in a decrease in cell-surface expression [34].

The rbNaDC-1 is also sensitive to inhibition by diethylpyrocarbonate (DEPC), a membrane-permeable reagent with high selectivity for histidine residues [39]. Binding of DEPC to His-153, located in the large intracellular loop between transmembrane domains 4 and 5, and His-569, at the carboxy terminus, results in inhibition of transport (Fig. 1). The results of this study suggest that DEPC binding to His-153 and His-569 impairs the mobility of the transporter or changes the accessibility of functionally important sites. The histidine at position 106, a residue that is conserved in all members of this family, appears to be important for targeting or stability in NaDC-1 [39].

Regulation of Na^+/Dicarboxylate Cotransporters

The activity of the Na^+/dicarboxylate cotransporter of the renal proximal tubule is increased in metabolic acidosis, potassium depletion or starvation [15, 21, 44]. The increased activity is characterized by an increased V_{max} with no change in K_m. Recent studies have shown that both metabolic acidosis and potassium depletion result in an increase in NaDC-1 mRNA and protein abundance in the kidney [2, 3]. Experiments with the OKP cell line, derived from opossum kidney proximal tubule, show similar increases in citrate transport activity after medium acidification for 48 h, suggesting that cellular responses mediate the increased activity [14].

The sequence of the 5′ promoter region of hNaDC-1 (SLC13A2 gene) contains several potential binding sites for *cis*-acting elements [1]. When various constructs containing portions of the 1.9 kb upstream of the translation initiation site of hNaDC-1 were expressed, cell-specific expression was seen. Activity of a luciferase reporter gene was observed in the OKP renal cell line but not in the NIH3T3 fibroblast cell line. Peak promoter

activity was seen with a construct containing only 142 bp. Interestingly, none of the promoter constructs were sensitive to media acidification, suggesting that the pH sensor is located outside the 1.9 kb region, or that activation of another gene is required first [1].

Acute regulation of rbNaDC-1 expressed in *Xenopus* oocytes occurs in response to activation of protein kinase C (PKC) with phorbol ester [37]. The stimulation of PKC activity results in almost complete inhibition of rbNaDC-1 activity, which is independent of the two consensus sites for PKC phosphorylation. The inhibitory effect of phorbol ester treatment on rbNaDC-1 activity was found to have two components: some of the inhibition (a maximum of 30%) was due to endocytosis of the transporter from the plasma membrane, but most of the inhibition is likely to be due to a direct inhibitory effect on the transporter itself [37]. The mechanism of this inhibition is not yet known.

There is also evidence that the activity of the Na^+/dicarboxylate cotransporters is modulated by calcium. Since citrate is an endogenous chelator of calcium, changes in calcium concentrations may affect the concentration of free citrate, which is the transported form. However, calcium also affects the activity or expression of Na^+/dicarboxylate cotransporters. OKP cells, from opossum kidney, express a low affinity Na^+/tricarboxylate cotransporter that does not interact with succinate when the transport is measured at normal calcium concentrations [20]. However, acute reduction of calcium concentrations in the medium results in a change from tricarboxylate to dicarboxylate transport activity. At low calcium, the transport of succinate and citrate has the properties of the NaDC cotransporters [14]. It is not known yet whether calcium modulates the substrate selectivity of the transporter or if calcium affects the expression of transport proteins on the plasma membrane.

Conclusion

In the past year, considerable advances have been made in our knowledge of the Na^+/dicarboxylate cotransporters. The cDNAs coding for the major low affinity (NaDC-1) and high affinity (NaDC-3) Na^+/dicarboxylate cotransporters have been identified and sequenced. The functional properties and tissue distribution of the cloned transporters matches previous functional characterizations in isolated membrane vesicles or cells. In addition, the tissue distribution of the cloned transporters has identified Na^+/dicarboxylate cotransporters in organs that had not previously been characterized. The genome sequencing project has contributed sequences of human chromosomes 17 and 20, containing the genes for hNaDC-1 and hNaDC-3, respectively. Now that many of the tools are in place, it should be possible to make progress in several areas related to the structure and function of the members of the NaDC/NaSi family. For example, there is still very little information about the relationship between NaDC-1 activity and the development of kidney stones. It is known that hypocitraturia contributes to stone formation through the loss of the chelating properties of citrate. In many patients it appears that the hypocitraturia is secondary to other disturbances, such as metabolic acidosis, in which case the regulation of NaDC-1 is very important. It is not known whether idiopathic hypocitraturia as a result of a mutation of the transporter could also be involved in the development of kidney stones. There are additional questions related to regulation that still need to be answered, such as the identity of the pH sensor in cells expressing NaDC-1. At a fundamental level, the secondary structure of the NaDC transporters has still not been tested, and further structure-function studies are needed to identify the mechanism of transport. Finally, the identification of the two transporters found on opposite membranes of renal proximal tubule cells, NaDC-1 and NaDC-3, should help to identify membrane targeting signals in this family of transporters.

Thanks to Dr. Lee Hamm for providing a copy of his manuscript prior to publication. This research was supported by National Institutes of Health grants DK46269 and DK02429.

References

1. Aruga, S., Moe, O.W., Preisig, P.A., Pajor, A.M., Alpern R.J. 1999. Genomic organization and tissue specific expression of the human NaDC-1 gene. *J. Amer. Soc. Nephrol.* **10:**50*A* (*Abstr*)
2. Aruga, S., Preisig, P.A., Pajor, A.M., Alpern, R.J. 1998. Chronic K^+ deficiency increases NaDC-1 protein and mRNA abundance while alkali feeding has no effect on NaDC-1. *J. Amer. Soc. Nephrol.* **9:**50*A* (*Abstr*)
3. Aruga, S., Wehrli, S., Kaissling, B., Moe, O.W., Preisig, P.A., Pajor, A.M., Alpern, R.J. 1999. Chronic metabolic acidosis increases NaDC-1 mRNA and protein abundance in rat kidney. *Kidney Int.* (*in press*)
4. Bai, L., Pajor, A.M. 1997. Expression cloning of NaDC-2, an intestinal Na^+ or Li^+-dependent dicarboxylate transporter. *Am. J. Physiol.* **273:**G267–G274
5. Burckhardt, G. 1984. Sodium-dependent dicarboxylate transport in rat renal basolateral membrane vesicles. *Pfluegers Arch.* **401:**254–261
6. Busch, A.E., Waldegger, S., Herzer, T., Biber, J., Markovich, D., Murer, H., Lang, F. 1994. Electrogenic cotransport of Na^+ and sulfate in *Xenopus* oocytes expressing the cloned Na^+/SO_4^{2-} transport protein NaSi-1. *J. Biol. Chem.* **269:**12407–12409
7. Chen, X., Tsukaguchi, H., Chen, X.Z., Berger, U.V., Hediger, M.A. 1999. Molecular and functional analysis of SDCT2, a novel rat sodium-dependent dicarboxylate transporter. *J. Clin. Invest.* **103:**1159–1168
8. Chen, X.Z., Shayakul, C., Berger, U.V., Tian, W., Hediger, M.A. 1998. Characterization of rat Na^+-dicarboxylate cotransporter. *J. Biol. Chem.* **273:**20972–20981
9. Edwards, R.M., Stack, E., Trizna, W. 1997. α-ketoglutarate trans-

port in rat renal brush border and basolateral membrane vesicles. *JPET* **281:**1059–1064

10. Ganapathy, V., Ganapathy, M.E., Tiruppathi, C., Miyamoto, Y., Mahesh, V.B., Leibach, F.H. 1988. Sodium-gradient-driven, high-affinity, uphill transport of succinate in human placental brush-border membrane vesicles. *Biochem. J.* **249:**179–184
11. Girard, J.P., Baekkevold, E.S., Feliu, J., Brandtzaeg, P., Amalric, F. 1999. Molecular cloning and functional analysis of SUT-1, a sulfate transporter from human high endothelial venules. *Proc. Natl. Acad. Sci. USA* **96:**12772–12777
12. Griffith, D.A., Pajor, A.M. 1999. Acidic residues involved in cation and substrate interactions in the Na/dicarboxylate cotransporter, NaDC-1. *Biochemistry* **38:**7524–7531
13. Hamm, L.L. 1990. Renal handling of citrate. Kidney Int. **38:**728–735
14. Hering-Smith, K., Gambala, C.T., Hamm, L.L. Citrate and succinate transport in proximal tubule cells. *Am. J. Physiol.* (*in press*)
15. Jenkins, A.D., Dousa, T.P., Smith, L.H. 1985. Transport of citrate across renal brush border membrane: effects of dietary acid and alkali loading. *Am. J. Physiol.* **249:**F590–F595
16. Kahn, E.S., Pajor, A.M. 1999. Determinants of substrate and cation affinities in the Na^+/dicarboxylate cotransporter. *Biochemistry* **38:**6151–6156
17. Kekuda, R., Wang, H., Huang, W., Pajor, A.M., Leibach, F.H., Devoe, L.D., Prasad, P.D., Ganapathy, V. 1999. Primary structure and functional characteristics of a mammalian sodium-coupled high affinity dicarboxylate transporter. *J. Biol. Chem.* **274:**3422–3429
18. Khatri, I.A., Kovacs, S.V.B., Forstner, J.F. 1996. Cloning of the cDNA for a rat intestinal Na^+/dicarboxylate cotransporter reveals partial sequence homology with a rat intestinal mucin. *Biochim. Biophys. Acta* **1309:**58–62
19. Kippen, I., Hirayama, B., Klinenberg, J.R., Wright, E.M. 1979. Transport of tricarboxylic acid cycle intermediates by membrane vesicles from renal brush border. *Proc. Natl. Acad. Sci. USA* **76:**3397–3400
20. Law, D., Hering-Smith, K.S., Hamm, L.L. 1992. Citrate transport in proximal cell line. *Am. J. Physiol.* **263:**C220–C225
21. Levi, M., McDonald, L.A., Preisig, P.A., Alpern, R.J. 1991. Chronic K depletion stimulates rat renal brush-border membrane Na-citrate cotransporter. *Am. J. Physiol.* **261:**F767–F773
22. Mann, S.S., Hart, T.C., Pettenati, M.J., von Kap-herr, C., Holmes, R.P. 1999. Assignment of the sodium-dependent dicarboxylate transporter gene (SLC13A2 alias NaDC-1) to human chromosome region 17p11.1–q11.1 by radiation hybrid mapping and fluorescence in situ hybridization. *Cytogenet. Cell Genet.* **34:**89–90
23. Markovich, D., Forgo, J., Stange, G., Biber, J., Murer, H. 1993. Expression cloning of rat renal Na^+/SO_4^{2-} cotransport. *Proc. Natl. Acad. Sci. USA* **90:**8073–8077
24. Meinild, A.K., Loo, D.D.F., Pajor, A.M., Zeuthen, T., Wright, E.M. Water transport by the renal Na^+/dicarboxylate cotransporter. *Am. J. Physiol.* (*in press*)
25. Moseley, R.H., Jarose, S., Permoad, P. 1992. Hepatic Na^+-dicarboxylate cotransport: identification, characterization, and acinar location. *Am. J. Physiol.* **263:**G871–G879
26. Murer, H., Manganel, M., Roch-Ramel, F. 1992. Tubular transport of monocarboxylates, Krebs cycle intermediates, and inorganic sulfate. *In*: Handbook of Physiology—Renal Physiology. E.E. Windhager (editor). Am. Physiol. Soc., New York pp. 2165–2188.
27. Norbis, F., Perego, C., Markovich, D., Stange, G., Verri, T., Murer, H. 1994. cDNA cloning of a rat small intestinal Na^+/SO_4^{2-} contransporter. *Pflugers Arch.* **428:**217–223
28. Ogin, C., Grassl, S.M. 1989. Dicarboxylate transport in human placental brush-border membrane vesicles. *Biochim. Biophys. Acta* **980:**248–254
29. Pajor, A.M. 1995. Sequence and functional characterization of a renal sodium/dicarboxylate cotransporter. *J. Biol. Chem.* **270:**5779–5785
30. Pajor, A.M. 1996. Molecular cloning and functional expression of a sodium-dicarboxylate cotransporter from human kidney. *Am. J. Physiol.* **270:**F642–F648
31. Pajor, A.M. 1999. Citrate transport by the kidney and intestine. *Seminars in Nephrology* **19:**195–200
32. Pajor, A.M. 1999. Sodium-coupled transporters for Krebs cycle intermediates. *Ann. Rev. Physiol.* **61:**663–682
33. Pajor, A.M., Hirayama, B.A., Loo, D.D.F. 1998. Sodium and lithium interactions with the Na^+/dicarboxylate cotransporter. *J. Biol. Chem.* **273:**18923–18929
34. Pajor, A.M., Krajewski, S.J., Sun, N., Gangula, R. 1999. Cysteine residues in the Na^+/dicarboxylate cotransporter, NaDC-1. *Biochem. J.* **344:**205–209
35. Pajor, A.M., Sun, N. 1996. Characterization of the rabbit renal Na^+/dicarboxylate cotransporter using anti-fusion protein antibodies. *Am. J. Physiol.* **271:**C1808–C1816
36. Pajor, A.M., Sun, N. 1996. Functional differences between rabbit and human Na^+-dicarboxylate cotransporters, NaDC-1 and hNaDC-1. *Am. J. Physiol.* **271:**F1093–F1099
37. Pajor, A.M., Sun, N. 1999. Protein kinase C-mediated regulation of the renal Na^+/dicarboxylate cotransporter, NaDC-1. *Biochim. Biophys. Acta* **1420:**223–230
38. Pajor, A.M., Sun, N., Bai, L., Markovich, D., Sule, P. 1998. The substrate recognition domain in the Na^+/dicarboxylate and Na^+/sulfate cotransporters is located in the carboxy-terminal portion of the protein. *Biochim. Biophys. Acta* **1370:**98–106
39. Pajor, A.M., Sun, N., Valmonte, H.G. 1998. Mutational analysis of histidines in the Na^+/dicarboxylate cotransporter, NaDC-1. *Biochem. J.* **331:**257–264
40. Sekine, T., Cha, S.H., Hosoyamada, M., Kanai, Y., Watanabe, N., Furuta, Y., Fukuda, K., Igarishi, T., Endou, H. 1998. Cloning, functional characterization and localization of a rat renal Na^+-dicarboxylate cotransporter. *Am. J. Physiol.* **275:**F298–F305
41. Sekine, T., Watanabe, N., Hosoyamada, M., Kanai, Y., Endou, H. 1997. Expression cloning and characterization of a novel multispecific organic anion transporter. *J. Biol. Chem.* **272:**18526–18529
42. Shank, R.P., Bennett, D.J. 1993. 2-Oxoglutarate transport: a potential mechanism for regulating glutamate and tricarboxylic acid cycle intermediates in neurons. *Neurochem. Res.* **18:**401–410
43. Shank, R.P., Campbell, G.L. 1981. Avid Na^+/dependent, high affinity uptake of alpha-ketoglutarate by nerve terminal enriched material from mouse cerebellum. *Life Sciences* **28:**843–850
44. Sheridan, E., Rumrich, G., Ullrich, K.J. 1983. Reabsorption of dicarboxylic acids from the proximal convolution of rat kidney. *Pfluegers Arch.* **399:**18–28
45. Steffgen, J., Burckhardt, B.C., Langenberg, C., Kuhne, L., Muller, G.A., Burckhardt, G., Wolff, N.A. 1999. Expression cloning and characterization of a novel sodium-dicarboxylate cotransporter from winter flounder kidney. *J. Biol. Chem.* **274:**20190–20196
46. Ullrich, K.J., Fasold, H., Rumrich, G., Kloss, S. 1984. Secretion and contraluminal uptake of dicarboxylic acids in the proximal convolution of rat kidney. *Pfluegers Arch.* **400:**241–249

47. Wolffram, S., Badertscher, M., Scharrer, E. 1994. Carrier-mediated transport is involved in mucosal succinate uptake by rat large intestine. *Exp. Physiol.* **79:**215–226
48. Wright, E.M. 1985. Transport of carboxylic acids by renal membrane vesicles. *Ann. Rev. Physiol.* **47:**127–141.
49. Wright, E.M., Wright, S.H., Hirayama, B.A., Kippen, I. 1982. Interactions between lithium and renal transport of Krebs cycle intermediates. *Proc. Natl. Acad. Sci. USA* **79:**7514–7517
50. Wright, S.H., Wunz, T.M. 1987. Succinate and citrate transport in renal basolateral and brush-border membranes. *Am. J. Physiol.* **253:**F432–F439
51. Yao, X., Pajor, A.M., 2000. The transport properties of the human renal Na^+/dicarboxylate cotransporter under voltage clamp conditions. *Am. J. Physiol.* (*in press*)
52. Zimmerli, B., O'Neill, B., Meier, P.J. 1992. Identification of sodium-dependent and sodium-independent dicarboxylate transport systems in rat liver basolateral membrane vesicles. *Pfluegers Arch.* **421:**329–335.