Substrate specificity of the human renal sodium dicarboxylate cotransporter, hNaDC-3, under voltage-clamp conditions

Birgitta C. Burckhardt, Julia Lorenz, Christoph Kobbe, and Gerhard Burckhardt

Zentrum Physiologie und Pathophysiologie, Abt. Vegetative Physiologie und Pathophysiologie, Georg-August-Universität Göttingen, Göttingen, Germany

Submitted 24 September 2004; accepted in final form 20 November 2004

Burckhardt, Birgitta C., Julia Lorenz, Christoph Kobbe, and Gerhard Burckhardt. Substrate specificity of the human renal sodium dicarboxylate cotransporter, hNaDC-3, under voltage-clamp conditions. Am J Physiol Renal Physiol 288: F792–F799, 2005. First published November 23, 2004; doi:10.1152/ajprenal.00360.2004.—Proximal tubule cells extract dicarboxylates from filtrate and blood, using cotransporters located in the brush border [sodium dicarboxylate cotransporter (NaDC-1)] and basolateral cell membrane (NaDC-3). We expressed the human NaDC-3 (hNaDC-3) in Xenopus laevis oocytes and characterized it by the two-electrode voltage-clamp technique. At −60 mV, succinate (4 carbons) and glutarate (5 carbons) generated inward currents due to translocation of three sodium ions and one divalent dicarboxylate, whereas oxalate (2 carbons) and malonate (3 carbons) did not. The cis-dicarboxylate maleate produced currents smaller in magnitude, whereas the trans-dicarboxylate fumarate generated currents similar to succinate. The substituted succinate derivatives, maleate, 2,2- and 2,3-dimethylsuccinate, and 2,3-dimercapto succinate elicited inward currents, whereas aspartate and glutathionosuccinate showed hardly detectable currents. The C-5 dicarboxylates glutarate and α-ketoglutarate produced larger currents than succinate; glutamate and fumarate failed to cause inward currents. Kinetic analysis revealed, at −60 mV, K<sub>0.5</sub> values of 25 ± 12 μM for succinate and 45 ± 13 μM for α-ketoglutarate, values close to the plasma concentration of these compounds. For both compounds, the K<sub>0.5</sub> was independent of voltage, whereas the maximal current increased with hyperpolarization. As opposed to the rat and flounder orthologs, hNaDC-3 was hardly inhibited by lithium concentrations up to 5 mM. In the absence of sodium, however, lithium can mediate succinate-dependent currents. The narrow substrate specificity prevents interaction of drugs with dicarboxylate-like structure with hNaDC-3 and ensures sufficient support of the proximal tubule cells with α-ketoglutarate for anion secretion via organic anion transporter 1 or 3.

α-ketoglutarate; folate; kidney; tricarboxylic acid cycle intermediates; lithium sensitivity; succinate

KIDNEY PROXIMAL TUBULE CELLS are involved in the uptake and metabolism of tricarboxylic acid cycle intermediates, which include a variety of di- and tricarboxylates such as succinate, citrate, and α-ketoglutarate. In the proximal tubule epithelium, the di- and tricarboxylates are taken up from both the luminal and the basolateral side through sodium-coupled transport systems, thereby allowing maintenance of intracellular concentrations of dicarboxylates three- to fourfold higher than those in the plasma (7, 11, 14, 15, 26). In conjunction with the organic anion transporters 1 and 3 (OAT1, OAT3; for reviews, see Refs. 2, 7, 26), sodium dicarboxylate cotransporter (NaDC-3) indirectly drives the secretion of a variety of endogenous and exogenous organic anions. By Na<sup>+</sup>-coupled uptake, NaDC-3 provides roughly half of the intracellular α-ketoglutarate used for organic anion/dicarboxylate exchange through OAT1 and OAT3 (7).

In recent years, the luminal as well as basolateral dicarboxylate transporters from different species including humans have been cloned (for reviews, see Refs. 11, 14, 15) and designated as NaC1 or the aliases NaDC-1 and SDCT1, and NaC2 or the aliases NaDC-3 and SDCT2 (for a review, see Ref. 11). NaDC-1 was localized to the luminal membrane as demonstrated by Western blot analysis using luminal membrane fractions and a polyclonal antibody directed against a 60-amino-acid peptide from NaDC-1 (19). Basolateral localization of human (h) NaDC-3 was by a polyclonal antibody raised against the fusion protein of glutathione S-transferase (GST)-hNaDC-3 (25).

Functional characterization of heterologously expressed transporters has been primarily performed in human retinal pigment epithelial cells or Xenopus laevis oocytes using radio-tracer flux or two-electrode voltage-clamp techniques (for reviews, see Refs. 11, 14, 15). These experiments confirmed the results obtained in the intact rat kidney, isolated, perfused tubules, and vesicles from either luminal or basolateral membranes and revealed that all members perform Na<sup>+</sup>-coupled transport of various tricarboxylic acid cycle intermediates. The stoichiometry of sodium-anion symport is 3:1. The coupling of three Na<sup>+</sup> to one generally divalent anion renders the symport electrogenic, with a net transfer of one positive charge across the cell membrane. Transport is thought to be ordered with sodium binding to the transporter before the anion, and lithium can act as a competitive inhibitor to sodium.

As tested with various NaDC-1 orthologs, NaDC-1 has a substrate preference for succinate (K<sub>0.5</sub> 0.18–1.1 mM) and citrate (K<sub>0.5</sub> 0.32–6.8 mM). Significant cis-inhibition of radio-labeled succinate uptake was observed in decreasing potency with succinate, fumarate, malate, oxaloacetate, and citrate. Substrate-associated currents were measured with succinate, α-ketoglutarate, glutarate, oxaloacetate, fumarate, and malate (11, 14, 15). NaDC-3 prefers 2,2-dimethylsuccinate over succinate and cis-inhibition of succinate uptake was demonstrated with 2,2- and 2,3-dimethylsuccinate, succinate, glutarate, α-ketoglutarate, fumarate, citrate, malonate, glutamate, maleate, and pyruvate. Significant currents (>20% of control) were evoked by α-ketoglutarate, fumarate, maleate, succinate, and the dimethylsuccinates.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Despite many similarities, NaDCs show species differences. Rat renal NaDC-1 (SDCT1, rNaCl1), located in the brush-border membrane, has a much higher affinity for succinate (5) than the orthologs from rabbit and human kidneys (18). Moreover, lithium only slightly inhibited human NaDC-1 (18) but strongly decreased transport by NaDC-1 and NaDC-3 from other species (5, 6, 17, 24). Although lithium is used for treatment of bimodal disorders in humans (21), it is not known whether hNaDC-3 and, hence, the supply of proximal tubular cells with dicarboxylates for metabolism and organic anion secretion are impaired at pharmacological lithium concentrations. Finally, endogenous derivatives of dicarboxylates, like folate, guanidinosuccinate, and quinolinate, have not yet been tested as putative substrates of NaDC-3. In uremic patients, guanidinosuccinate and quinolinate concentrations in plasma are increased (23) with possible side effects in renal dicarboxylate transport.

The aim of the present study was to determine the specificity of human NaDC-3 for di- and tricarboxylates and its interaction with lithium.

MATERIALS AND METHODS

In vitro transcription of cRNA. Sodium-dependent dicarboxylate transporter NaDC-3 cDNAs from human (24) kidney were used as a template for cRNA synthesis. Plasmids were linearized with NolI, and in vitro cRNA transcription was performed using T7 mMessage mMachiné Kit (Ambion, Austin, TX). The resulting cRNA was suspended in purified, RNAsé-free water to a final concentration of 1 µg/µl.

Oocyte preparation and storage. Stage V and VI oocytes from X. laevis (Nasco, Fort Atkinson, WI) were separated by treatment with collagenase (Typ CLS II, Biochrom, Berlin, Germany) and maintained afterwards at 16–18°C in control solution (in mM): 110 NaCl, 3 KCl, 2 CaCl2, 5 HEPES/Tris, pH 7.5. One day after removal from the frog, oocytes were injected with 30 nl of cRNA coding for hNaDC-3 and maintained at 16–18°C in control solution supplemented with 50 µM gentamycin and 2.5 mM pyruvate. After 3 to 4 days of incubation with daily medium changes, oocytes were used for electrophysiological studies. Oocytes injected with water served as controls.

Electrophysiological studies. Electrophysiological studies were performed by the conventional two-microelectrode voltage-clamp method (3, 4). Oocytes were superfused with control solution and after stabilization of the membrane potential, single oocytes were clamped at −60 mV and the current induced by 1 mM succinate was measured to demonstrate functional expression of the hNaDC-3 protein. Voltage pulses between −90 and +10 mV, in 10-mV increments, were applied for 5 s each and steady-state currents were recorded to obtain current-voltage (I-V) relationships. In general, the I-V protocol was applied first under control conditions and then 30 s after changing the superfusion to the test solution. The difference between the steady-state currents measured in the presence and absence of substrates was considered as substrate-induced current, ΔI. Water-injected controls showed no substrate-dependent currents in response to mono-, di-, and tricarboxylates.

Chemicals. All chemicals were purchased from Sigma (Taufkirchen, Germany) or Merck (Darmstadt, Germany). Control solution was prepared as a five times concentrated stock solution. Appropriate dilution and addition of the test substrate were performed at the day of the experiment. Sodium-free solutions were obtained either by equimolar substitution of all sodium by N-methyl-d-glucamine or lithium.

RESULTS

Interactions of lithium with hNaDC-3. Application of succinate (1 mM) to hNaDC-3-expressing oocytes yielded potential-dependent inward currents that were larger in amplitude at negative than at positive clamp potentials (Fig. 1A, ●). These succinate-associated currents were, in contrast to succinate-induced currents observed in oocytes expressing rat or mouse NaDC-3, only marginally affected by increasing lithium concentrations. As measured in paired experiments in five oocytes from three donors, in the absence of lithium, currents induced by 1 mM succinate were −56 ± 6, −44 ± 5, −32 ± 4, and −22 ± 3 nA at −90, −60, −30, and 0 mV, respectively (Fig. 1A, ●). These currents only slightly decreased in response to increasing concentrations of lithium. In the presence of 5 mM lithium, succinate-induced currents decreased to −41 ± 5, −32 ± 4, −26 ± 3, and −18 ± 3 nA (Fig. 1A, △). When at a holding potential of −90, −60, and −30 mV (Fig. 1B), the

![Fig. 1. Influence of increasing lithium concentrations on succinate-induced inward currents. A: current-voltage (I-V) relationships as obtained in 5 oocytes from 3 donors on application of either 1 mM succinate (●) or succinate and 0.1 mM LiCl (○), 0.5 mM LiCl (□), 1 mM LiCl (△), 2.5 mM LiCl (▲), and 5 mM LiCl (●) were shown. Error bars were omitted for clarity. B: succinate-associated currents, as measured in A, were plotted as a function of external lithium concentration at 3 representative clamp potentials: Vc = −90, −60, and −30 mV. Data are means ± SE from 5 oocytes from 3 donors.](http://ajprenal.physiology.org/Download/fig1.png)
Succinate-induced currents were plotted as a function of increasing lithium concentrations, residual lithium-insensitive currents of 73, 72, 81, and 81% of the initial current persisted.

Total replacement of sodium by lithium, as performed in paired studies with four hNaDC-3-expressing oocytes from four donors, revealed the following results. In the presence of sodium, steady-state currents showed in the absence (Fig. 2A, ■) and presence (1 mM, Fig. 2A, □) of succinate near-to-linear I-V relationships throughout the tested potential range. Succinate-induced currents (Fig. 2C, ●) were inwardly directed within the potential range between −90 and 0 mV, in support of the proposed coupling of three sodium ions for each divalent succinate. When all sodium was replaced by lithium (Fig. 2B), steady-state currents in the absence (Fig. 2B, △) and presence of succinate (1 mM, Fig. 2B, ▲) showed again near-to-linear I-V relationships. The succinate-associated currents (Fig. 2C, ○), however, differed markedly from those obtained in the presence of sodium. These currents increased in amplitude as the potential depolarized (Fig. 2C, ○). For comparison, the succinate-induced currents in the presence of sodium decreased from −44 ± 5 nA at −90 mV to −30 ± 7 nA at −30 mV (Fig. 2C, ●) and changed in the presence of lithium from 3 ± 2 nA at −90 mV to −7 ± 2 nA at −30 mV (Fig. 2C, ○). When all sodium was replaced by N-methyl-D-glucamine, succinate-induced currents were completely abolished (data not shown).

Substrate specificity: Carbon chain length and different substitutions at C-2 and C-3. Citric acid cycle intermediates consist of carboxylates with a chain length of four to six carbons connected either by single or double bonds and different substitutions at C-2 or C-3. The substrate specificity of tricarboxylic acid cycle intermediates irrespective whether they are mono-, di-, or tricarboxylates at physiological pH was tested by measuring currents induced by these compounds at a holding potential of −60 mV (Fig. 3). The data are expressed as a percentage of the succinate-induced currents in the same oocytes. The substrate concentration used was 1 mM, which should yield maximal-inducible currents, at least in the case of succinate. Succinate, α-ketoglutarate, fumarate, malate, and oxaloacetate produced currents of similar amplitude. Currents of <20% of control were observed in the presence of citrate, isocitrate, and cis-aconitate. These substances are predominantly

---

**Fig. 2.** Comparison of the succinate-induced currents in the presence of sodium (A) and during total replacement of sodium by lithium (B). In paired experiments on 4 oocytes from 4 different frogs, steady-state currents were first measured in control, sodium-containing solution in the absence (■) and then in the presence (□) of 1 mM succinate. Afterwards, total sodium was replaced by equimolar concentration of lithium and currents were obtained in the absence (▲) and presence of 1 mM succinate (○). The respective succinate-induced currents are shown in (C), where the ● represents the currents obtained by subtraction of the currents shown in A and the ○ those in B.

**Fig. 3.** Substrate-dependent currents evoked by tricarboxylic acid cycle intermediates. Substrate-dependent currents induced by tricarboxylic acid cycle intermediates (each 1 mM) at −60 mV were expressed as a percentage of the current evoked by succinate (100%) in the same oocyte. Data are reported as means ± SE from at least 4 different oocytes from 4 different frogs.
trivalent at physiological pH, and the small currents observed may be due to the low concentration of divalent substrate present at pH 7.5.

Succinate- and α-ketoglutarate-induced currents were examined to identify the substrate concentration at half-maximal current ($K_{0.5}$) for these substrates under voltage-clamp conditions in the range between −90 and 0 mV. Succinate as well as α-ketoglutarate showed potential-dependent inward currents that saturated at concentrations >0.5 mM. In the case of succinate, application of higher concentrations decreased substrate-dependent currents (data not shown) most probably due to intracellular accumulation. As determined in four oocytes from three donors, the $K_{0.5}$ for succinate was ~25 μM independent of membrane voltage (Fig. 4A). The succinate-induced maximum current at saturating concentrations ($I_{\text{max}}$) increased as the membrane voltage was made more negative (Fig. 4B). Similar results regarding $K_{0.5}$ and $I_{\text{max}}$ were obtained for α-ketoglutarate. $K_{0.5}$ was ~35 μM independent of membrane voltage (Fig. 4C), and $I_{\text{max}}$ again increased as the oocytes became hyperpolarized (Fig. 4D, 4 determinations from 3 donors).

The ratio $I_{\text{max}}/K_{0.5}$ indicates the transport efficiency of the cotransporter for a given substrate (Table 1). $I_{\text{max}}/K_{0.5}$ decreased in the order succinate ∼ glutarate > α-ketoglutarate, indicating that transport efficiency was not dependent on the length of the carbon backbone, and substitution at C-2 influenced transport efficiency.

In addition to succinate, other C-4 and C-5 dicarboxylates, which are not members of the tricarboxylic acid cycle, were also translocated via hNaDC-3. The data are plotted as percent of the succinate-induced currents in the same oocytes. The introduction of two methyl groups at C-2 (2,2-dimethylsuccinate, 2,2-DMS) increased substrate-dependent current to 152 ± 4% (Fig. 5). However, the substitution at C-2 and C-3 with either methyl-(2,3-dimethylsuccinate), (2,3-DMS) or sulfhydryl-groups (2,3-dimercaptosuccinate, DMSA, succimer) reduced the current to 74.5 ± 5 and 28 ± 7%, respectively, compared with succinate (4 oocytes from 4 frogs). Aspartate and glutamate differ from succinate and glutarate, respectively, by the presence of a positively charged NH$_3^+$ group at C-2. This substitution decreased aspartate- and glutamate-associated currents to 18 ± 12 and 3 ± 1% of control (3 oocytes from 3 donors). Fumarate and maleate are both C-4 dicarboxylates and differ with respect to the trans- and cis-position of their COOH groups. Fumarate exhibited currents comparable in magnitude to succinate (see Fig 3), whereas maleate induced barely detectable currents (Fig. 5).

Divalent dicarboxylates with shorter chain lengths like oxalate (C-2) and malonate (C-3) showed no substrate-induced currents (Fig. 5).

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

![Graph D](image4.png)

**Table 1. Substrate specificity of hNaDC-3**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_{0.5}$, μM</th>
<th>$I_{\text{max}}/K_{0.5}$, nA/μM</th>
<th>n/m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate</td>
<td>25±16</td>
<td>1.38±0.56</td>
<td>4/3</td>
</tr>
<tr>
<td>Glutarate</td>
<td>40±12</td>
<td>1.23±0.33</td>
<td>5/4</td>
</tr>
<tr>
<td>α-Ketoglutarate*</td>
<td>45±13</td>
<td>0.63±0.20</td>
<td>4/4</td>
</tr>
</tbody>
</table>

Values are means ± SE. By definition, $K_{0.5}$ is the substrate concentration where half-maximal currents are achieved, and $I_{\text{max}}$ is the substrate-inducible current at saturating substrate concentrations. $K_{0.5}$ and $I_{\text{max}}$ were calculated from experimental data obtained at −60 mV using similar concentrations for all 3 substrates: 10, 50, 100, 500, and 1,000 μM. n Denotes the number of oocytes and m the number of frogs used in the study. *α-Ketoglutarate was statistically significantly different from succinate and glutarate at P<0.01 as derived from Student’s t-test. hNaDC-3, human renal sodium dicarboxylate cotransporter.
Among the monovalent C-3 carboxylates tested, pyruvate (5 oocytes from 5 donors) and lactate (3 oocytes from 3 donors) expressed currents of 25\% and 19\% of control.

Interactions of other dicarboxylates and related compounds that may be substrates of hNaDC-3: Folate, quinolinate, and guanidinosuccinate.

As shown in Fig. 6A, folate, formally an \( \text{N} \)-substituted glutamate derivative with two COOH groups, seemed not to be a substrate of hNaDC-3. At \(-60\) mV in paired experiments with four oocytes from four donors, succinate (1 mM, Fig. 6A, ●) induced an inward current of \(-26 \pm 1\) nA, whereas the folate-associated current was \(-1 \pm 1\) nA (Fig. 6A, ○). Folate concentrations up to 10 mM did not induce substrate-dependent currents (data not shown). Folate, however, was able to alter succinate-induced currents. Compared with succinate (1 mM, Fig. 6B, ●), simultaneous application of succinate (1 mM) and folate (0.5 mM, Fig. 6B, ○) resulted in small inward currents at potentials more negative than \(-60\) mV and outward currents were seen at potentials more positive than \(-60\) mV (paired experiments with 4 oocytes from 4 donors).

Quinolinolate, a cyclic dicarboxylate derived from tryptophan metabolism, showed compared with succinate (Fig. 7A, ●) small potential-dependent inward currents (Fig. 7A, ○). However, whereas the succinate-evoked currents reversed at positive potentials, the quinolinolate-induced currents reversed at \(-36 \pm 15\) mV. Picolinolate (1 mM), a cyclic monocarboxylate, did not induce currents at all (Fig. 7A, ■). These experiments were performed with eight oocytes from three donors. In addition, quinolinolate (0.5 mM) was not able to inhibit the currents elicited by succinate. As determined in seven oocytes from three donors, the currents measured in the simultaneous presence of succinate (1 mM) and quinolinolate (0.5 mM; Fig. 7B) were not significantly different from those induced by succinate alone (1 mM, Fig. 7B, ●).

Guanidinosuccinate is a toxin present in uremic plasma up to concentrations of 0.35 mM (23). As observed in paired experiments on nine oocytes from seven donors, at \(-60\) mV the currents induced by 1 mM (Fig. 8A, ○) and 10 mM guanidinosuccinate (■) were \(-1 \pm 1\) and \(-3 \pm 1\) nA, respectively, whereas the succinate-induced current (●) reached \(-33 \pm 15\) nA. Guanidinosuccinate was able to inhibit to some extent the currents (\(<20\%\) of control, 5 oocytes from 5 frogs). Among the monovalent C-3 carboxylates tested, pyruvate (5 oocytes from 5 donors) and lactate (3 oocytes from 3 donors) expressed currents of 25 \% and 19 \% of control.
succinate-evoked current (Fig. 8B, ○). At −60 mV and in paired experiments in seven oocytes from three donors, the current determined in the simultaneous presence of guanidinosuccinate (0.5 mM) and succinate (1 mM) was 76.9 ± 15.1% of the current produced by succinate (1 mM) alone.

**DISCUSSION**

Mammalian NaDCs are plasma membrane transporters mediating the cellular entry of a variety of dicarboxylates that are intermediates in the tricarboxylic acid cycle (e.g., succinate, fumarate, malate, and α-ketoglutarate) in an electrogenic sodium-dependent fashion (11, 14, 15). Accordingly, the transport can be visualized by cell membrane depolarization, and, under voltage-clamp conditions, by an inward current. Succinate-induced currents were a linear function of the holding potential, being smaller at less negative potentials and reversing at positive potentials. This behavior is in accordance with an electrogenic cotransport of three sodium ions with one dicarboxylate, a property shared by all NaDCs. However, there are significant functional differences between NaDC-1 and NaDC-3 with regard to the cation effect. When sodium was replaced by choline or cesium, substrate-dependent inward currents were abolished in oocytes expressing the rabbit NaDC-1 (17). During substitution of sodium by lithium, how-

---

**Fig. 7. Effect of quinolinate.** A: comparison of succinate (1 mM, ○), quinolinate (1 mM, ●), and picolinate (1 mM, ■)-evoked currents as derived in paired experiments in 8 oocytes from 3 donors did not support translocation of quinolinate and picolinate by human sodium dicarboxylate cotransporter-3. B: quinolinate (0.5 mM) did not affect currents induced by 1 mM succinate. At all potentials tested, these currents (●) were similar in magnitude to those evoked by succinate (1 mM, ○) alone (paired experiments on 7 oocytes from 3 donors). C: structural formulas represent the dicarboxylate quinolinate and the monocarboxylate picolinate.

**Fig. 8. Influence of guanidinosuccinate.** A: guanidinosuccinate in concentrations of 1 mM (○) and 10 mM (■) did not evoke currents similar in magnitude to succinate (1 mM, ●). 9 oocytes from 7 donors. B: guanidinosuccinate (0.5 mM) slightly inhibited currents induced by succinate (1 mM). In individual experiments, currents in the presence of succinate and guanidinosuccinate were smaller than those evoked by succinate alone with P < 0.01 using paired t-test (7 oocytes from 3 donors). C: structural formula of guanidinosuccinate.
ever, succinate-induced inward currents were observed (17), which decreased as the membrane depolarized. This indicated that the Li\(^+\)-succinate \(-2\) stoichiometry is most likely 3:1 in rabbit NaDC-1. When these studies were repeated in oocytes expressing hNaDC-1, no such succinate-induced currents were detected in the presence of lithium (27), indicating species differences with respect to the ability of Li\(^+\) to support succinate transport.

During total replacement of sodium by lithium, succinate induced outward current deflections at holding potentials that changed into inward current deflections at potentials less negative than \(-50\) mV in oocytes expressing mouse NaDC-3 (16) and hNaDC-3 (this study). These currents were much smaller than the inward currents induced by succinate in the presence of sodium. The nature of the current deflections seen in the presence of lithium is unclear. If three Li\(^+\) would be cotransported with succinate, the currents should be inward at all tested potentials, as found for rbNaDC-1 (17). The cotransport of two Li\(^+\) with one succinate would result in an electroneutral process not visible by the two-electrode voltage-clamp technique. In case of cotransport of one Li\(^+\) with one succinate, outward currents increasing with depolarizing membrane potentials should be observed. \(I-V\) relationships in the presence of lithium revealed a small decrease in conductance when succinate was added. Therefore, succinate appears to inhibit rather than to induce a current in the presence of lithium. The mechanism of this process remains to be determined.

In our experiments on hNaDC-3, up to 5 mM Li\(^+\) only slightly inhibited succinate-induced currents. A comparably small effect of Li\(^+\) was found with hNaDC-1 (18), whereas rodent NaDC-1 and NaDC-3 were strongly inhibited by lithium (6, 10, 17, 18), indicating again species differences. The plasma concentrations of lithium used for the treatment of bimodal disorders are in the range of 0.8–2 mM (21). At these concentrations, neither hNaDC-1 nor hNaDC-3 is inhibited, suggesting that therapeutic concentrations of lithium do not affect reabsorption of succinate and other dicarboxylates either from the tubular lumen or from the peritubular capillaries. The observed increase in urinary citrate excretion during lithium therapy can, therefore, not be attributed to an effect on NaDC-1 and NaDC-3 and suggests that a distinct transporter for citrate is affected. Such a transporter has not yet been identified in the human kidney.

hNaDC-3 has a broad substrate specificity for 4- and 5-carbon, terminal dicarboxylic acids in the \(trans\)-configuration, including the tricarboxylic acid cycle intermediates succinate and \(\alpha\)-ketoglutarate. The kinetic constants for hNaDC-3 as derived from the two-electrode voltage-clamp studies agree quite well with previous data. The \(K_{0.5}\) for succinate was in the range of 30 \(\mu\)M in agreement with the \(K_{0.5}\) of 20 ± 1 \(\mu\)M obtained in human retinal pigment epithelial cells transfected with hNaDC-3 but is lower than the \(K_{0.5}\) of 102 ± 20 \(\mu\)M in hNaDC-3-expressing oocytes (24). In the potential range between \(-90\) and 0 mV, the \(K_{0.5}\) for succinate was not affected by the membrane potential, suggesting that binding of succinate to the transporter is voltage independent. Similar findings were obtained for \(\alpha\)-ketoglutarate showing a potential-independent \(K_{0.5}\) of 40 \(\mu\)M. Importantly, these \(K_{0.5}\) values are in the range of the circulating plasma concentrations of succinate and \(\alpha\)-ketoglutarate, which lie between 10 and 40 \(\mu\)M (26). The transport efficiency, \(\Delta\mu_{\text{max}}\)/\(K_{0.5}\), for succinate and \(\alpha\)-ketoglutarate differed by a factor of 2.1 for hNaDC-3. As opposed, succinate was translocated 15 times more efficiently than \(\alpha\)-ketoglutarate in hNaDC-1 (27), indicating differences in the substrate binding and translocation between hNaDC-1 and hNaDC-3.

Besides succinate and \(\alpha\)-ketoglutarate, hNaDC-3 translocated the dicarboxylic tricarboxylic acid cycle intermediates fumarate, malate, and oxaloacetate, as visualized by the respective substrate-induced currents. With the tricarboxylates citrate, isocitrate, and \(cis\)-aconitate, much smaller currents were observed, indicating a considerably slower translocation. At 25°C, the \(pK\)s of citrate are 3.1, 4.8, and 6.4, respectively. Therefore, at physiological pH, less than 10% of citrate is present as a divalent anion. This implies that at a total concentration of 1 mM citrate, \(\sim\)0.1 mM citrate \(-2\) was responsible for the observed small inward current. The total plasma concentration of citrate is 0.135 mM (26). At pH 7.4, less than 0.014 mM of citrate is present as a divalent anion, a concentration too low to interact significantly with hNaDC-3. Because citrate is excreted in healthy humans, another transporter responsible for translocation of citrate and presumably other trivalent carboxylates such as isocitrate and \(cis\)-aconitate must be present in the kidneys. Similar findings with respect to citrate were observed with hNaDC-1 (27).

Among other carboxylates, the monocarboxylate pyruvate showed small substrate-dependent inward currents. This finding fits with another study, in which uptake of radiolabeled pyruvate into hNaDC-3-expressing oocytes was demonstrated (9). In addition, the monocarboxylate lactate (this study) and the monovalent anion dimercapto-1-propane sulfonate showed inward currents of small amplitude in oocytes expressing either hNaDC-3 or NaDC-3 (3), demonstrating that the NaDC-3s from different species are able to handle monovalent anions, at least to a small extent.

hNaDC-3 also interacted with dicarboxylates substituted with two methyl groups (2,2-DMS, 2,3-DMS) or two mercapto groups (2,3-DMSA). 2,2-DMS induced a current larger than that evoked by succinate, whereas 2,3-DMS produced a considerably smaller current. Rat NaDC-3 transported 2,3-DMS with high affinity (\(K_{0.5}\) 27.2 ± 2.2 \(\mu\)M), and 2,3-DMS evoked \(\sim\)60% of the succinate-induced current (6). In contrast, rat NaDC-1 showed low affinity for 2,3-DMS (\(K_{0.5}\) 272 ± 3 \(\mu\)M) and evoked only 17% of the succinate-evoked current (6). Thus NaDC-1 does not appreciably interact with 2,3-DMS. In a previous study on flounder NaDC-3 (3), we showed that the \(K_{0.5}\) for 2,2-DMS was independent of voltage, whereas the \(K_{0.5}\) for 2,3-DMS increased with decreasing voltage, suggesting that the position of substituents at the succinate molecule determines the voltage dependence of the apparent affinity. The second 2,3-disubstituted compound, DMSA, contains a backbone of four carbons, two negatively charged COOH groups and two vicinal sulfhydryl groups, which are highly active in chelating free heavy metals like lead and mercury within cells or blood (1). Heavy metals are predominantly deposited within renal proximal tubule cells (28). Therefore, renal detoxification requires the entry of uncomplexed DMSA via a transport system (28). In the present study, we provide evidence for the translocation of DMSA by human NaDC-3.

Once within the cell, DMSA can chelate heavy metals and thus contribute to detoxification. Thus hNaDC-3 appears to be
instrumental for the increased renal heavy metal excretion during administration of DMSA.

Folate is freely filtered at the glomerulus and undergoes active reabsorption as well as secretion in the proximal tubule (8, 12). OAT1 (20) and OAT3 (22) can facilitate uptake of folate or folate derivatives like methotrexate across the basolateral membrane. Because folate possesses a 5-C backbone similar to glutarate and glutamate, we investigated its interaction with hNaDC-3. Folate, however, did not induce any current most probably due to its bulky substitution at C-2. However, folate markedly interfered with succinate-induced currents, which were no longer a linear function of the holding potential, and reversed at about −60 mV rather than at positive voltages. Such a change in succinate-induced current was observed earlier with flufenamate and was attributed to the occurrence of a K⁺ conductance, the nature of which remained unclear (4).

Circulating uremic toxins have been proposed to facilitate progression of chronic renal failure. The monovalent organic anions indoleacetic, indoxyl sulfate, hippurate, and p-hydroxyhippurate are handled by OAT1 (13). We tested here the dicarboxylic uremic toxins, guanidinosuccinate and quinolinate (23). Neither guanidinosuccinate nor quinolinate was able to induce substrate-dependent currents. Guanidinosuccinate, but not quinolinate, inhibited succinate-induced currents up to 20%. Thus the cyclic dicarboxylate quinolinate is not a substrate of hNaDC-3, whereas guanidinosuccinate may act as an inhibitor in uremia.

Taken together, the human NaDC-3 shows affinities for succinate and α-ketoglutarate matching their plasma concentrations. For translocation, C-4 and C-5 dicarboxylates with the COOH groups in trans-configuration are preferred. Substituted dicarboxylates do not induce currents when a positively charged NH₂ group is present as in aspartate, glutamate, or dicarboxylates do not induce currents when a positively charged NH₂ group is present as in aspartate, glutamate, or dicarboxylates. Molecular and functional analysis of SDCT2, a novel rat sodium-dependent dicarboxylate transporter. J Clin Invest 105: 1159–1168, 1999.


