Sodium and Lithium Interactions with the Na⁺/Dicarboxylate Cotransporter*

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The two-electrode voltage clamp was used to study the currents associated with transport of succinate by the cloned Na⁺/dicarboxylate cotransporter, NaDC-1, expressed in Xenopus oocytes. The presence of succinate induced inward currents which were dependent on the concentrations of succinate and sodium, and on the membrane potential. At −50 mV, the K_{succinate} was 180 μM and the K_{Na⁺} was 19 mM. The Hill coefficient was 2.3, which is consistent with a transport stoichiometry of 3 Na⁺:1 divalent anion substrate. Currents were induced in NaDC-1 by a range of di- and tricarboxylates, including citrate, methylsuccinate, fumarate, and tricarballylate. Although Na⁺ is the preferred cation, Li⁺ was also able to support transport. The K_{Li} was approximately 10-fold higher in Li⁺ compared with Na⁺. In the presence of Na⁺, however, Li⁺ was a potent inhibitor of transport. Millimolar concentrations of Li⁺ resulted in decreases in apparent succinate affinity and in the I_{max}. Furthermore, lithium inhibition under saturating sodium concentrations showed hyperbolic kinetics, suggesting that one of the three cation binding sites in NaDC-1 has a higher affinity for Li⁺ than Na⁺. We conclude that NaDC-1 is an electrogenic anion transporter that accepts either Na⁺ or Li⁺ as coupling cations. However, NaDC-1 contains a single high affinity binding site for Li⁺ that, when occupied, results in transport inhibition, which may account for its potent inhibitory effects on renal dicarboxylate transport.

The active transport of Krebs cycle intermediates, such as succinate and citrate, is mediated by a specific sodium-coupled transporter found on the apical membrane in epithelial cells of the kidney proximal tubule (1, 2). The Na⁺-dicarboxylate cotransporter reabsorbs a wide range of di- and tricarboxylic acids in the form of divalent anions. This transporter is sensitive to inhibition by lithium (3), and patients receiving therapeutic doses of lithium exhibit increased renal excretion of α-ketoglutarate and glutarate (4). The cDNA coding for the rabbit renal Na⁺/dicarboxylate cotransporter, NaDC-1, has been cloned and sequenced (5), and the protein has been identified in renal brush border membranes (6). NaDC-1 belongs to a distinct gene family of sodium-coupled anion transporters that includes the Na⁺/dicarboxylate cotransporters, hNaDC-1, from human kidney (7), and NaDC-2, from Xenopus intestine (8), and the renal Na⁺/sulfate cotransporter, NaSi-1 (9).

The transport mechanism of NaDC-1 is thought to involve the ordered binding of four charged substrates: 3 Na⁺ ions and 1 divalent anion substrate (10–12), resulting in one net inward positive charge across the membrane per cycle. Experiments with rabbit renal brush border membrane vesicles support this hypothesis: sodium-dependent transport of succinate was affected by changes in membrane potential, and transport of succinate also caused a depolarization of membrane potential (12–14). However, the dependence of transport kinetics on membrane voltage is not known, and there have been no direct measurements of currents associated with Na⁺/dicarboxylate cotransport.

In this study, we have used a two-electrode voltage clamp to study the kinetics of succinate transport by NaDC-1 expressed in Xenopus oocytes. The results show that NaDC-1 is electrogenic, with a stoichiometry of 3 Na⁺ per succinate. Negative membrane potentials increase succinate transport. The apparent affinity of the transporter for Na⁺ is increased by negative membrane voltage, whereas succinate binding is relatively voltage-independent. The cation selectivity of NaDC-1 is unique. Although Na⁺ is the preferred cation, Li⁺ can support transport. However, succinate transport is inhibited when one of the three cation binding sites in NaDC-1 is occupied by Li⁺.

In conclusion, this study provides new insights into the transport mechanism of NaDC-1 and the inhibitory action of lithium on renal dicarboxylate transport.

EXPERIMENTAL PROCEDURES

Xenopus oocytes—Stage V and VI oocytes from Xenopus laevis (NASCIO) were dissected and defolliculated as described previously (5, 11). The oocytes were injected with 50 nl of NaDC-1 cRNA (0.5 μg/μl) 1 day following isolation. Currents were measured 3–5 days later. The oocytes were maintained at 18 °C in Barth’s medium supplemented with 5% heat-inactivated horse serum, 2.5 mM sodium pyruvate, 50 mg/ml gentamicin. For experiments, oocytes were superfused with sodium buffer containing (in mM): 100 NaCl, 2 KCl, 1 CaCl₂, 1 MgCl₂, and 10 HEPES-Tris, pH 7.5. For cation replacement experiments, sodium was replaced with an equimolar concentration of other cations, as chloride salts. The results reported are for single experiments that are representative of experiments repeated with oocytes from at least three donor frogs.

Electrophysiology—Oocyte currents were measured using the two-electrode voltage clamp method at 22 °C (15, 16). The microelectrodes were filled with 3 M KCl and had resistances <1 megohm, usually between 0.4 and 0.8 megohm. The voltage pulses were controlled with the pClamp6 program suite (Axon Instruments). Current-voltage relationships were obtained from a pulse protocol consisting of test voltages applied for 100 ms between +50 and −150 mV (in 20-mV decrements), with a holding potential of −50 mV. The results of three runs were averaged for each trial.

Data Analysis—Steady state substrate-dependent currents were fit-
ted to the Hill/Michaelis-Menten equations using SigmaPlot software (Jandel Scientific)

\[ I = I_{\text{max}} \frac{[S]^n}{K_{0.5}^\text{succinate} + [S]^n} \]

where \( I \) is the current, \( I_{\text{max}} \) is the maximum current observed at saturating substrate concentrations, \( K_{0.5} \) is the substrate concentration at half-maximal current, and \( n \) is the Hill coefficient. For the Michaelis-Menten equation, \( n = 1 \). The error bars for kinetic data represent errors of the fit.

RESULTS

Succinate-induced Currents in NaDC-1—Current traces obtained in an oocyte injected with NaDC-1 cRNA are shown in Fig. 1. The addition of substrate in the presence of sodium induced inward currents (Fig. 1B) compared with sodium buffer alone (Fig. 1A). Inward currents of up to 3000 nA were measured in oocytes expressing NaDC-1, but not in control, uninjected oocytes (not shown). In Fig. 1C, the steady state currents from Fig. 1, A and B, are plotted as a function of membrane potential. Fig. 1D shows the substrate-dependent currents in NaDC-1, which are calculated from the difference between the currents in the presence and absence of succinate. In this experiment, the I–V curves were linear, although the I–V curves often showed saturation with more negative membrane potentials. In response to step changes in membrane voltage, NaDC-1 also exhibited pre-steady state charge movements (Fig. 1A), which were reduced in the presence of substrate (Fig. 1B). However, the pre-steady state charge movements in NaDC-1 were very rapid (time constants approximately 2–6 ms) and did not show saturation at the positive potentials tested. The pre-steady state charge movements in NaDC-1 were observed only in oocytes exhibiting very high expression of the transporter.

Kinetics of Activation by Succinate—The magnitude of the steady state currents in NaDC-1 was dependent on the concentration of succinate (Fig. 2A and B). For each voltage, the data were fit to Equation 1, with \( n = 1 \). The maximal current, \( I_{\text{max}} \), increased and saturated at hyperpolarizing membrane potentials (Fig. 2C). At negative membrane potentials, the \( K_{0.5}^\text{succinate} \) was voltage-independent (Fig. 2D). For example, at \(-50 \) mV, the \( K_{0.5}^\text{succinate} \) was 180 \( \mu \)M and at \(-150 \) mV the \( K_{0.5}^\text{succinate} \) was 140 \( \mu \)M (Fig. 2D). However, there was an increase in \( K_{0.5}^\text{succinate} \) to 270 \( \mu \)M at \(-10 \) mV.

Substrate Specificity of NaDC-1—The substrate specificity of NaDC-1 was examined by measuring currents in the presence of 10 mM concentrations of test substrates. As shown in Fig. 3, four of the substrates tested produced larger currents than did succinate: methylsuccinate, citrate, fumarate, and tricarballylate. The currents seen in the presence of \( \alpha \)-ketoglutarate and glutarate were about 50% of those reported for succinate, which could reflect a lower affinity of NaDC-1 for these substrates. For example, the \( K_m \) for glutarate in NaDC-1 is approximately 6 mM (11), close to the 10 mM substrate concentration used in these studies. Small currents were measured in dimethylsuccinate, sulfate and pyruvate (Fig. 3). Finally, there were no

FIG. 1. Succinate-dependent currents in an oocyte expressing NaDC-1. Current traces were recorded in an oocyte in the absence (A) and presence (B) of 10 mM succinate. The holding potential was \(-50 \) mV and the test potentials were applied for 100 ms and ranged from \(+50 \) to \(-150 \) mV, in 20-mV decrements (as described under “Experimental Procedures”). In C is shown the steady state currents from A and B plotted as a function of membrane potential. The difference between the two curves (D) is the succinate-dependent current.
Detectable substrate-dependent currents with lactate, consistent with previous uptake studies (5).

**Sodium Activation of Succinate-dependent Currents—**$\text{Na}^{+}$ is thought to be an essential activator of NaDC-1 (10, 12). Sodium activation of succinate-dependent currents was measured under voltage-clamp conditions. The succinate-dependent currents in NaDC-1 were sigmoidal functions of $\text{Na}^{+}$ concentration and showed saturation (Fig. 4A). The maximum succinate-dependent current at saturating $\text{Na}^{+}$ concentrations, $I_{\text{max}}$, was dependent on membrane potential, becoming larger and saturating at more negative membrane potentials (Fig. 4B). The $K_{0.5}$ was strongly affected by membrane potential, decreasing from 19 mM at $-50$ mV to 9.7 mM at $-150$ mV (Fig. 4C). The apparent Hill coefficient, $n$, was insensitive to voltage; $n$ was 2.3 at $-50$ mV and 2.4 at $-150$ mV (Fig. 4D).

**Cation Selectivity of NaDC-1—**The largest succinate-dependent currents in NaDC-1 were seen in the presence of $\text{Na}^{+}$ (Fig. 5). When $\text{Na}^{+}$ was replaced by choline or Cs$^{+}$, the inward currents were abolished. However, substrate-dependent inward currents were observed in Li$^{+}$ (approximately 6–25% of the currents seen in $\text{Na}^{+}$, depending on membrane potential), suggesting that Li$^{+}$ supports succinate transport. H$^{+}$ appears to be a very poor substitute for $\text{Na}^{+}$ since only small (<30 nA) substrate-induced currents were observed in choline at pH 5.5 (Fig. 5). There was no difference between the succinate-induced currents measured in $\text{Na}^{+}$ at pH 7.5 and 5.5 (results not shown).

**Succinate Kinetics Measured in Lithium—**The largest succinate-dependent currents in NaDC-1 were seen in the presence of $\text{Na}^{+}$ (Fig. 5). When $\text{Na}^{+}$ was replaced by choline or Cs$^{+}$, the inward currents were abolished. However, substrate-dependent inward currents were observed in Li$^{+}$ (approximately 6–25% of the currents seen in $\text{Na}^{+}$, depending on membrane potential), suggesting that Li$^{+}$ supports succinate transport. This result verifies previous studies done with renal brush border membrane vesicles (3). H$^{+}$ appears to be a very poor substitute for $\text{Na}^{+}$ since only small (<30 nA) substrate-induced currents were observed in choline at pH 5.5 (Fig. 5). There was no difference between the succinate-induced currents measured in $\text{Na}^{+}$ at pH 7.5 and 5.5 (results not shown).

**Sucinate Kinetics Measured in Lithium—**The succinate-induced currents in lithium were measured as a function of increasing succinate concentrations (Fig. 6). As seen in $\text{Na}^{+}$, there was saturation of the substrate-induced lithium currents with increasing concentrations of succinate. For comparison, the currents produced by 1 mM succinate in $\text{Na}^{+}$ in the same oocyte were $-185$ nA at $-150$ mV and $-140$ nA at $-50$ mV (not found).

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**Fig. 2.** Steady state succinate-dependent currents as a function of succinate concentration in an oocyte expressing NaDC-1. A, the steady state substrate-dependent currents were measured at succinate concentrations between 10 $\mu$M and 10 mM and are plotted against the membrane potential. B, the steady state currents are plotted as a function of substrate concentration for four sample membrane potentials. The data were fit to Equation 1. C, the effect of membrane potential on $I_{\text{max}}$. D, the effect of membrane potential on the $K_{0.5}$.
shown). As seen with Na\(^+\), the \(I_{\text{max}}^{\text{succinate}}\) values from the data shown in A are plotted as a function of membrane potential. The error bars represent standard errors of the fit.

**DISCUSSION**

The overexpression of NaDC-1 in *Xenopus* oocytes has provided a means of characterizing its electrophysiological properties. In oocytes expressing NaDC-1, the preference of substrate induced inward currents that were dependent on the concentration of substrate and sodium, and on the membrane potential. Although Na\(^-\) is the preferred cation, Li\(^+\) can also support transport in NaDC-1. However, the poor activation of transport by Li\(^+\) produces inhibition when Na\(^+\) is present. In many
respects, the electrophysiological characteristics of substrate-induced currents in NaDC-1 resemble those of transporters of neutral substrates, including the Na\(^+\)/glucose cotransporter, SGLT1 (15).

The apparent affinity constants for succinate in NaDC-1 measured under voltage-clamp conditions agree well with those obtained from radiotracer uptake studies in oocytes. The \(K_m\) for succinate transport in NaDC-1 is around 0.5 mM (5, 11). In this study, the \(K_{0.5}\) for succinate-induced currents was approximately 0.2 mM at \(-50\) mV. Although there was a marked effect of voltage on \(K_{0.5}\) at more negative membrane potentials, \(K_{0.5}\) at negative membrane potentials was relatively insensitive to voltage, indicating voltage independence of succinate binding. This result was somewhat surprising considering that succinate is transported as a divalent anion. However, similar results have been reported for other sodium-coupled transporters, irrespective of substrate charge, including the Na\(^+\)/glucose cotransporter, SGLT1 (15), the Na\(^+\)/\(\Gamma^-\) cotransporter, NIS (17), and the Na\(^+\)/phosphate cotransporter, NaPi-5 (18). This voltage independence of substrate binding may be a general feature of some sodium-coupled transporters and suggests that the substrate binding site does not sense the electric field. The steep voltage dependence at depolarizing potentials could be a consequence of voltage-dependent steps in Na\(^+\) binding. In contrast, the Na\(^+\)/Cl\(^-\)/\(\gamma\)-aminobutyric acid transporter, GAT-1, and Na\(^+\)/glutamate transporter, HEAAC1, exhibit increases in \(K_{0.5}\) with more negative membrane potentials (19, 20) indicating that the substrate binding site senses membrane potential in this group of anion transporters. The stimulation of \(I_{\text{max}}\) with more negative membrane potentials is seen in all of the sodium-coupled transporters studied to date, including NaDC-1 (15, 17, 20), suggesting that voltage-dependent steps in substrate turnover are a general property of sodium-coupled transporters.

The effects of voltage on sodium binding in NaDC-1 were more pronounced than on substrate binding. The \(K_{0.5}\) \(\text{Na}^+\) decreased by 50% between \(-50\) and \(-150\) mV. As suggested for other sodium-coupled transporters, this effect of voltage on sodium binding supports an “ion-well” hypothesis (15). Activation by cations in NaDC-1 is also affected by voltage since the

**Fig. 6. Steady state succinate-dependent currents as a function of succinate concentration measured in Li\(^+\).** A, the steady state substrate-dependent currents were measured at succinate concentrations between 1 and 50 mM and are plotted against the membrane potential. B, the steady state currents are plotted as a function of substrate concentration for four sample membrane potentials. The data were fit to Equation 1. C, the effect of membrane potential on \(I_{\text{max}}\) for the data from B. D, the effect of membrane potential on the \(K_{0.5}\) measured in Li\(^+\).

**Fig. 7. The effect of lithium on succinate kinetics.** Steady state currents were measured at increasing concentrations of succinate in either 95 mM sodium, 5 mM choline (Control) or in 95 mM sodium, 5 mM lithium (+5 mM Li\(^+\)). A, the succinate \(I_{\text{max}}\) at \(-50\) mV in the absence and presence of lithium. B, The \(K_{0.5}\) succinate at \(-50\) mV in the absence and presence of lithium.
Electrophysiology of NaDC-1

The preferred cation carried by NaDC-1 is Na\(^+\). Unlike other sodium-coupled transporters, such as the Na\(^+\)/glucose cotransporter (25) and the Na\(^+\)/Cl\(^-\)/serotonin cotransporter (26), protons are not effective activators of succinate transport in NaDC-1. However, Li\(^+\) can substitute for Na\(^+\) to produce substrate-dependent currents, which also indicates that the stoichiometry of Li\(^+\)-dependent succinate transport is likely to be 3:1. Li\(^+\) is a poor activator of succinate transport, most likely because binding of Li\(^+\) to NaDC-1 produces a less favorable conformation for substrate binding compared with binding of Na\(^+\). The apparent substrate affinity in NaDC-1 is about 15-fold lower in lithium (\(K_{\text{app}}\) succinate 3 mM) compared with sodium (\(K_{\text{app}}\) succinate 0.2 mM), consistent with the idea that cations are essential activators of transport by producing an increased affinity for substrate. The exact conformational change produced by cation binding is determined by the identity of the cation. The steep voltage dependence of \(K_{\text{app}}\) in Li\(^+\) suggests that hyperpolarizing membrane potentials have a greater effect on one or more steps in the transport cycle in the presence of Li\(^+\) compared with Na\(^+\).

Lithium also acts as a potent inhibitor of succinate transport by NaDC-1 when Na\(^+\) is present, with an apparent \(K_i\) of 2 mM (3, 11). In humans and rodents, treatment with Li\(^+\) leads to rapid increases in urinary concentrations of Krebs cycle intermediates (4, 27). In this study, Li\(^+\) behaved as a mixed-type inhibitor of NaDC-1. Succinate-dependent currents in NaDC-1 were inhibited by millimolar concentrations of Li\(^+\), with a decrease in \(I_{\text{app}}\) max and an increase in \(K_{\text{app}}\) succinate. Mixed-type inhibition is characterized by a combination of competitive inhibition, which would be seen as an increased \(K_{\text{app}}\) due to the mutual exclusion of substrate and inhibitor (i.e. Na\(^+\) and Li\(^+\)), and noncompetitive inhibition, which would be seen as a decrease in \(I_{\text{app}}\) due to the production of an inactive intermediate (22). The proportional change in \(I_{\text{app}}\) is much greater than the change in \(I_{\text{app}}\) in the voltage range used in our studies. The smaller effect on \(I_{\text{app}}\) could explain why studies with brush border membrane vesicles reported an increase in \(K_m\) for succinate in the presence of 2 mM Li\(^+\) but no significant effect on \(V_{\text{max}}\) (3).

The inhibitory effects of Li\(^+\) on NaDC-1 occur at relatively low concentrations, even in the presence of saturating Na\(^+\) concentrations, which confirms previous suggestions that at least one of the cation binding sites in NaDC-1 has a high affinity for Li\(^+\) (3). The hyperbolic kinetics of inhibition by Li\(^+\) are also consistent with one cation binding site which has a higher affinity for Li\(^+\). This result shows that the multiple sodium binding sites in NaDC-1 are not identical. The apparent lithium \(K_i\) of 2.5 mM seen at -10 mV is similar to the \(K_i\) measured in transport experiments in oocytes (11).

One difference between NaDC-1 and other Na\(^+\)-coupled transporters is the difficulty in measuring pre-steady state charge movements in response to voltage jumps. Although pre-steady state currents were observed in oocytes expressing NaDC-1, it was only possible to measure them in oocytes exhibiting high transport expression. The fast time constants, close to the limit of resolution of the two-electrode voltage clamp, and the lack of saturation of charge movements in the voltage range used in our studies made it difficult to obtain reliable fits of the data to the Boltzmann equation in order to estimate \(Q_{\text{max}}\). Interestingly, the properties of the charge movements in NaDC-1 appear to resemble those seen in the unrelated Na\(^+\)/phosphate cotransporter, NaPi-5 (18). The max-
imum time constant, $\tau_{\text{max}}$, in NaPi-5 is around 5 ms, compared with time constants between 9 and 150 ms in other sodium-dependent transporters (28). Pre-steady state charge movements are thought to represent movement of the transporter in response to changes in the electric field, probably representing reorientation of charges or dipoles in the protein or binding and release of Na$^+$ (28). If either of these steps is rate-limiting in NaDC-1, the rapid pre-steady state charge movements could indicate that the turnover number will be relatively high, although this remains to be tested.

In conclusion, NaDC-1 expressed in *Xenopus* oocytes generates substrate-induced currents that are dependent on concentrations of substrate, sodium, and membrane potential. Although NaDC-1 belongs to a distinct gene family, in some respects it behaves very much like other Na$^+$-coupled transporters, including the Na$^+$/glucose cotransporter (15), the Na$^+$/I$^-$ cotransporter (17), and the Na$^+$/phosphate cotransporter (18). The cation selectivity of NaDC-1 is unique, however, with a single high affinity binding site for lithium that, when occupied, results in transport inhibition. This study provides new insights into the mechanism of transport by NaDC-1 and should allow us to design models of NaDC-1 function.

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