Calcium sensitivity of dicarboxylate transport in cultured proximal tubule cells

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Hering-Smith KS, Schiro FR, Pajor AM, Hamm LL. Calcium sensitivity of dicarboxylate transport in cultured proximal tubule cells. *Am J Physiol Renal Physiol* 300: F425–F432, 2011. First published December 1, 2010; doi:10.1152/ajprenal.00036.2010.—Urinary citrate is an important inhibitor of calcium nephrolithiasis and is primarily determined by proximal tubule reabsorption. The major transporter to reabsorb citrate is Na\(^{+}\)-dicarboxylate cotransporter (NaDC1), which transports dicarboxylates, including the divalent form of citrate. We previously found that opossum kidney (OK) proximal tubule cells variably express either divalent or trivalent citrate transport, depending on extracellular calcium. The present studies were performed to delineate the mechanism of the effect of calcium on citrate and succinate transport in these cells. Transport was measured using isotope uptake assays. In some studies, NaDC1 transport was studied in *Xenopus* oocytes, expressing either the rabbit or opossum ortholog. In the OK cell culture model, lowering extracellular calcium increased both citrate and succinate transport by more than twofold; the effect was specific in that glucose transport was not altered. Citrate and succinate were found to reciprocally inhibit transport, indicating that NaDC1 could not likely account for the inhibition. The inhibition varied progressively at intermediate concentrations of substrate, was increased by calcium removal. In contrast, in oocytes expressing NaDC1, citrate did not have a similar effect on transport, indicating that NaDC1 could not likely account for the findings in OK cells. In summary, extracellular calcium regulates constitutive citrate and succinate transport in OK proximal tubule cells, probably via a novel transport process that is not NaDC1. The calcium effect on citrate transport parallels in vivo studies that demonstrate the regulation of urinary citrate excretion with urinary calcium excretion, a process that may be important in decreasing urinary calcium stone formation.

Urinary citrate is an important inhibitor of calcium nephrolithiasis (11, 27). The extent of citrate excretion is determined by proximal tubule reabsorption of filtered citrate. This reabsorption of citrate occurs primarily via an apical dicarboxylate transporter, Na\(^{+}\)-dicarboxylate cotransporter (NaDC1) (25). This transporter reabsorbs a variety of Krebs cycle intermediates, with succinate as the substrate usually studied based on its high affinity. Citrate reabsorption occurs via this dicarboxylate transport process, despite the fact that trivalent citrate (cit\(^{-3}\)) is the predominant ionic species at pH 7.4. The highest pK\(_{a}\) of citric acid is 5.6 (11) and, therefore, the cit\(^{-3}\)-to-divalent citrate (cit\(^{-2}\)) ratio is ~60:1 at pH 7.4.

In previous studies to investigate proximal tubule citrate transport, our laboratory has used the OK (opossum kidney) proximal tubule cell line (13, 21). Our initial cell culture studies demonstrated that citrate uptake in normal calcium-containing solutions (calcium concentration 1.2 mM) occurred via an apparent tricarboxylate transporter resembling the basolateral uptake of citrate in isolated basolateral membrane vesicles (21, 35). However, in contrast, our subsequent study demonstrated that cit\(^{-2}\) uptake increased markedly as extracellular calcium concentration was lowered; this was indicated by both an increased total uptake and by an increased percentage of uptake that could be inhibited by succinate, a dicarboxylate (13). Lowering extracellular magnesium produced a similar increase in cit\(^{-2}\) transport. Most of the effects of low extracellular calcium or magnesium were also found for succinate transport. The findings with succinate were particularly important to clarify the role of divalent cation complexation with citrate. Both calcium and magnesium form complexes with citrate, thus reducing “free” cit\(^{-2}\) concentrations and hence citrate transport. In this manner, increasing calcium concentrations could lower citrate transport simply by forming nontransported complexes. However, succinate has ~100-fold less complexation with calcium and magnesium than citrate (30). Yet transport of both succinate and citrate changes similarly with calcium concentration, and, therefore, the mechanism(s) of the effects of calcium must include factors other than complexation.

The present studies were aimed to further delineate the effect of calcium on citrate and succinate transport as a first step in determining the mechanisms of this effect. These experiments demonstrate that extracellular calcium clearly changes several properties of citrate and succinate transport, not just the magnitude of transport. The level of extracellular calcium appears either to change the properties of a single transporter or to rapidly induce the expression of another transport process that is not apparent at higher calcium concentrations.

METHODS

OK cells between passages 90 and 100 were maintained in MEM containing 26 mM HCO\(_3\)\(^{-}\) and supplemented with 10% fetal bovine serum (Gibco-BRL), 25 mM HEPES, 11 mM l-glutamine, 100 IU/ml penicillin, and 100 mg/ml streptomycin in a humidified atmosphere of 5% CO\(_2\)-95% air at 37°C. Cell monolayers were grown on 24-well plates (Corning-Costar), wells = 2.0 cm\(^2\), with media changes every 2 days. After reaching confluence, cell monolayers were changed to serum-free media for a minimum of 24 h before study.

Citrate transport was measured by the uptake of radiolabeled (14C) citrate into cell monolayers. Just before uptake measurement, the cells were rinsed free of media and equilibrated for 2 min at 37°C in a

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buffer containing either normal (1.2 mM) or “0” calcium (nominally calcium-free, and measured as <60 μM, but no chelating agent). The remaining components of the buffer were as follows (in mM): 109 NaCl, 3 KCl, 2 KH₂PO₄, 1 MgSO₄, 5 alanine, 8.3 glucose, 1 sodium acetate. 25 HEPES; osmolality was 290 mmol/kgH₂O and pH 7.4.

Ionized calcium was measured to be 1.14 mM in the calcium-containing and <60 μM in the nominally calcium-free solution.

Uptake was performed at 37°C and started by adding 0.4 ml of uptake buffer, the above-mentioned solution, with 0.5 mM/ml [1,5,14C]citrate (Sigma-Aldrich) added to the individual wells. The final concentration of citrate was ~0.014 mM. The uptake solution also contained [3H]mannitol (Perkin Elmer) to determine the residual extracellular volume. After 3 min, the uptake solution was removed, and the wells were rinsed three times with ice-cold 0.1 M MgCl₂; the monolayers were then lysed with 1 ml of 0.1 N NaOH. The lysate was transferred to vials for liquid scintillation counting. In some experiments, the transport of [1,4-14C]succinate (Perkin Elmer) was determined; in these experiments, the succinate concentration was ~0.004 mM.

Uptake was measured from the measured 14C radioactivity per well; appropriate windows and crossover calculations were used to distinguish [3H]mannitol and 14C. Uptake was further factored for the residual extracellular volume that was not removed by the triplicate rinsing; the residual extracellular volume was calculated from the residual [3H]mannitol. Experiments that had a residual extracellular volume that was not removed by the triplicate rinsing were not included in the results presented.

In a small series of separate experiments, succinate and citrate radioisotope uptake were measured in Xenopus oocytes, expressing either opossum or rabbit NaDC1 using methods as previously described (2, 24). Stage V and VI oocytes from Xenopus laevis were dissected, treated with collagenase, and cultured. Transport of radiolabeled isotopes was measured 2–3 days after oocyte injection of the respective cRNA. Transport was initiated by replacement of a choline buffer containing either normal (1.2 mM calcium) or “0” calcium (nominally calcium-free solution). The [1,4-14C]citrate transport was stopped by addition of ice-cold 0.1 M MgCl₂; three additional washes in cold choline buffer. Radioactivity was measured by scintillation counting.

Data were expressed as means ± SE. Each mean is derived from six or more individual wells studied on at least 3 separate days (and from separate batches of cells). Student’s t-test or ANOVA for multiple groups statistical comparisons were made. Statistical significance was defined as P < 0.05. Unless noted otherwise, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

RESULTS

Our laboratory has previously shown that 1 mM succinate inhibits citrate transport in OK cells when studied in low-calcium solutions (13). To further characterize the calcium-sensitive citrate transport in OK cells reported in our previous study, we first examined the concentration dependence of inhibition with succinate (Fig. 1). These experiments were done with no calcium added to the uptake solution (<60 μM), as previously reported. Succinate inhibited citrate uptake in a concentration-dependent manner, consistent with competitive inhibition. (Unfortunately, formal kinetic analysis demonstrating competitive interaction could not be performed; subsequent kinetic analysis demonstrates the difficulties.) Succinate inhibited citrate uptake by almost 50% even at 0.1 mM concentration. This indicates that succinate has a relatively high affinity (≈ 0.1–0.5 mM) for this transport process.

Further studies examined succinate inhibition of citrate uptake with varying levels of extracellular calcium from <60 μM to 1.2 mM calcium. Results are shown in Fig. 2A; the <60 μM data are the same as in Fig. 1. Succinate inhibited citrate uptake in the lower calcium concentration solutions (<60 μM to 0.6 mM calcium), but did not alter citrate uptake in the highest calcium solution (1.2 mM calcium). Figure 2A demonstrates progressively less inhibition of citrate uptake by succinate at increasing extracellular calcium (inhibition decreasing from 0 calcium to 0.3 mM calcium, etc.). If progressive inhibition by succinate indicates dicarboxylate transport (in this case cit-3), then dicarboxylate transport is sensitive to extracellular calcium, decreasing as calcium concentration is raised. Indeed the prior studies (13) indicated that succinate transport increases with decreasing calcium concentration. In contrast, there is a significant trend for that portion of citrate uptake uninhibited by 1 mM succinate (Fig. 2A, far right) to increase with increasing calcium concentrations. Our laboratory has previously proposed that, with 1.2 mM extracellular calcium, most citrate uptake in these cultured cells is tricarboxylate transport, cit-3, based predominantly on the lack of succinate inhibition and the relative insensitivity to extracellular pH (21). This transport process may correspond to citrate transport across the basolateral membrane of the proximal tubule.

To determine whether the effect of lowering extracellular calcium is specific for sodium dicarboxylate transport, or if this occurs with other sodium-dependent transport processes in the proximal tubule, [14C]glucose uptake studies were performed. Glucose uptake was not changed by varying extracellular calcium (0.095 ± 0.001 and 0.094 ± 0.001 nmol/well in the 0 and 1.2 mM calcium groups), indicating that the stimulation of citrate and succinate transport by lowering extracellular calcium is specific.

1 Note that, as in prior studies, the <60 μM solutions contained micromolar concentrations of calcium from trace amounts in other chemicals, and therefore monolayer integrity was not disrupted.
To further examine dicarboxylate transport in low calcium solutions, additional experiments examined succinate transport. In the low-calcium solutions, succinate transport is inhibited by citrate in a concentration-dependent manner (Fig. 2B). However, in 1.2 mM extracellular calcium, succinate transport is not inhibited by citrate (Fig. 2B). These results (Fig. 2, A and B) are consistent with succinate and citrate sharing a transport process in these cells at low extracellular calcium concentrations, but not with 1.2 mM extracellular calcium. In the <60 μM calcium solutions, 0.1 mM citrate inhibits >50% of succinate transport (that component that is not inhibited by 1 mM citrate), consistent with a $K_i$ of ~0.1 mM.

Increasing calcium to 2 mM did not further decrease either citrate or succinate uptake compared with that at calcium of 1.2 mM.

Since extracellular calcium appears to change the inhibitory interaction between citrate and succinate transport, we next tested the interaction of both citrate and succinate transport with a variety of other potential transporter substrates. The affinities for various substrates can usually be used to identify, distinguish, and classify transport proteins or processes. Historically, the dicarboxylate transporters were first characterized in this manner using membrane vesicles (34); currently, a key property of cloned transporters is the substrate affinities in heterologous expression systems such as oocytes. In OK cells, we tested substrates that have previously been shown to compete with citrate and succinate transport in various systems: malate, α-ketoglutarate, oxaloacetate, glutamate (all dicarboxylates), and the tricarboxylate isocitrate. We have previously used such potential competitive substrates to characterize citrate transport in both normal and low extracellular calcium solutions (13, 21); in the present experiments, 1 mM concentrations of malate, isocitrate, α-ketoglutarate, oxaloacetate, and glutamate were used to examine both citrate and succinate transport, each tested with both <60 μM and normal (1.2 mM) extracellular calcium. In particular, these experiments were designed to com-
pare the inhibitory interaction at the two different calcium concentrations; therefore, the plots are depicted as fraction of control without potential inhibitor, since the low-calcium solutions stimulate absolute uptake of both citrate and succinate, as shown previously (13) and in Fig. 2, A and B, respectively. As shown in Fig. 3A for inhibition of citrate uptake, the relative inhibition in 1.2 mM or <60 μM calcium differed substantially for malate and oxaloacetate (and for succinate shown in Fig. 2A) and differed to some extent for isocitrate, but not for α-ketoglutarate and glutamate. Both α-ketoglutarate and glutamate completely inhibited uptake in either calcium solution. For succinate transport (Fig. 3B), again there were substantial differences in the relative inhibition in 1.2 mM or <60 μM calcium, in this case for all substrates tested (and citrate in Fig. 2B), with the exception of oxaloacetate. These sets of experiments (Fig. 3, A and B) indicate that extracellular calcium changes the relative inhibition by various substrates on the citrate and succinate transport processes. As discussed below, the qualitative differences between the interactions with citrate and succinate may result from multiple transport processes, particularly for succinate transport.

To further study the effects of calcium on dicarboxylate transport, kinetics of succinate transport were examined. As discussed below, citrate kinetics were previously addressed (13). [14C]succinate isotope transport and total succinate uptake were measured with varying concentrations of total succinate, ranging from 0.004 to 10 mM. Results are shown in Fig. 4, A and B. In contrast to citrate uptake, which saturates at low total citrate concentrations in these cells, total succinate uptake did not saturate in this concentration range, whether in <60 μM or normal (1.2 mM) calcium-containing solutions (Fig. 4A). However, at lower concentrations of total succinate (Fig. 4A, inset), succinate uptake was significantly higher with the low-calcium solutions, i.e., calcium inhibits succinate transport.

Although total succinate uptake appeared to be nonsaturable, Figs. 2B and 3B illustrate that citrate and a variety of dicarboxylates inhibit succinate transport in 0 mM calcium when succinate concentration is low. This is consistent with two modes of succinate transport, a nonsaturable mode (up to 10
mM), and a smaller saturable component inhibited by citrate and various dicarboxylates.\(^2\)

Total succinate transport can then be considered as:

\[
V_T = V_{NS} + V_S
\]

where \(V_T\), \(V_{NS}\), and \(V_S\) are total, nonsaturable, and saturable succinate transport, respectively. To analyze the latter saturable component, the nonsaturable component was estimated and subtracted from the total succinate uptake. The nonsaturable component appears to be approximately linearly related to succinate concentration, so the rate of this component could be described as

\[
V_{NS} = K \cdot S
\]

where \(S\) is the succinate concentration, and \(K\) is a linear rate constant. Since \(V_S\) will be a smaller proportion of total transport at high succinate concentrations, \(V_T \approx V_{NS}\) at high \(S\) (\(\geq 1\) mM), \(K\) can be estimated from \(V_T\) at high concentrations of succinate (\(K \approx V_T/S\)). Then at low concentrations of \(S\)

\[
V_S = V_T - V_{NS} = V_T - KS
\]

\(K\) was estimated to be 0.151 and 0.119 with 0 and 1.2 mM calcium-containing solutions, respectively. With this analysis, a saturable component of succinate was estimated, as suggested by the inset of Fig. 4A. This was analyzed separately for <60 \(\mu\)M and 1.2 mM calcium-containing solutions using the
data points up to 5 mM cold succinate (the 10 mM data introduced excess variability). The saturable component was then analyzed using nonlinear regression analysis to the Michaelis-Menton equation.

\[ V_s = \frac{V_{max} \cdot S}{S + K_m} \]

where \( V_{max} \) is the maximum rate of just the saturable component. With this analysis, \( V_{max} \) was 0.049 and 0.055 nmol/well in <60 \( \mu M \) and 1.2 mM calcium-containing solutions and \( K_m \) values were 0.22 and 0.36 mM, respectively. The fairly marked difference in isotope uptake at low-succinate concentrations is illustrated better by an analysis similar to that previously done for glucose uptake in membrane vesicles (Fig. 4B) (22). In Fig. 4B, fractional isotope uptakes, not total uptake, are shown so that the large differences at very-low-succinate concentrations can be easily seen. This analysis suggests that calcium does change the kinetic characteristics of succinate transport at low-succinate concentrations.

In oocytes expressing opossum NaDC1, citrate uptake was increased significantly in nominally calcium-free media (282 ± 35 to 705 ± 125 pmol-oocyte\(^{-1}\)·h\(^{-1}\)); however, succinate uptake was decreased significantly (60.7 ± 5.8%). With rabbit NaDC1, succinate uptake again decreased in nominally calcium-free media (243 ± 17 to 159 ± 15 pmol-oocyte\(^{-1}\)·h\(^{-1}\)), but there was no significant change in citrate uptake (29 ± 2 to 33 ± 4 pmol-oocyte\(^{-1}\)·h\(^{-1}\)).

**DISCUSSION**

The present studies extend our laboratory’s previous studies that citrate and succinate transport in the OK cell proximal tubule model are modulated by extracellular calcium (13). The studies here demonstrate that the mutual inhibition of citrate and succinate for transport depends on the level of extracellular calcium. This suggests that lowering extracellular calcium stimulates a dicarboxylate transport process. Succinate is a dicarboxylate ion at physiological pH levels; citrate is predominantly a tricarboxylate at physiological pH, but is transported across the apical membrane of the proximal tubule in the dicarboxylate form (10, 25). This stimulation by low extracellular calcium is specific, since glucose transport is not altered by extracellular calcium. Additional studies examining the effect of other potential transport substrates (Fig. 3) suggest more than just the absolute rate is altered by extracellular calcium. In other words, the qualitative characteristics, in this case, the inhibitory interaction with a variety of potential transport substrates, differ between the 1.2 mM and <60 \( \mu M \) calcium solutions. Furthermore, the affinity for succinate, the \( K_m \), appears to change with extracellular calcium, particularly at low succinate concentrations (Fig. 4B), as discussed further below.

Calcium modulation of citrate reabsorption in the proximal tubule, if confirmed, is potentially physiologically important in preventing calcium stone formation. As luminal and urinary calcium increase, decreasing citrate reabsorption would maintain higher luminal citrate concentrations, which, in turn, could complex more calcium, preventing calcium nephrolithiasis. Some previous studies suggested that urinary citrate varies with urinary calcium under normal conditions. Several earlier human studies addressed with mixed results whether increasing urinary calcium increased urinary citrate; the results were mixed (6, 9, 16, 29). A recent epidemiological study by Taylor and Curhan (31) showed an independent positive relationship between urinary calcium and urinary citrate. These investigators pointed out that high protein/high acid content diets should be associated with increased urinary calcium, but decreased urine citrate, based on the acid content; therefore, the finding of a parallel increase in urine calcium with urinary citrate is unexplained by acid base homeostasis.

In experimental animal studies, Bushinsky et al. (5) showed that increases in urine calcium were accompanied by increases in urine citrate; this was demonstrated in both normal and hypercalcuiac rats by varying dietary calcium. In other studies using brush-border membrane vesicles, Barac-Nieto (4) demonstrated that very high concentrations of calcium (up to 10 mM) inhibit citrate transport, presumably secondary to complexation. However, the observations herein on proximal tubule cell transport cannot be accounted for predominantly by complexation, since succinate transport was similarly altered by calcium, and succinate is complexed by calcium almost 100-fold less than citrate (30). Our laboratory’s previous studies (13) and the present results have been the first, to our knowledge, to demonstrate a mechanism other than complexation, whereby urine citrate would vary with urine calcium.

As discussed below, the mechanism(s) of the effects of calcium are unknown but appear to involve more than just an increase in the rate of transport of citrate and succinate. This conclusion is based on three sets of results, which demonstrate changes with extracellular calcium: 1) changes in the magnitude of the mutual inhibition between citrate and succinate uptake with variations in calcium (Fig. 2, A and B); 2) changes in other substrate inhibition of citrate and succinate transport with calcium (Fig. 3, A and B); and 3) changes in succinate kinetics (\( K_m \)) with calcium at low-succinate concentrations (Fig. 4). Analysis and understanding of these changes are undoubtedly complicated by the presence of multiple dicarboxylate transport processes in the intact proximal tubule cell, but qualitative changes in dicarboxylate transport do occur with variations in extracellular calcium.

Both apical and basolateral dicarboxylate transporters have been characterized in intact proximal tubules and in membrane vesicles and have been cloned (26). Previously, none of the cloned dicarboxylate transporters have been shown to be calcium sensitive, although this has not been tested in most cases. In oocytes expressing either rabbit or opossum NaDC1, removal of external calcium decreases succinate transport (see results above), opposite of that found in the OK cells. The increase in citrate transport in oocytes expressing opossum NaDC1 (but not with rabbit NaDC1) may be secondary to calcium-citrate complexation (see discussion of NaDC1 below). In the proximal tubule, citrate is probably transported as a dicarboxylate ion on the basolateral membrane (10, 19, 35). In fact, our laboratory has previously suggested that OK cells exhibit predominantly this mode of transport in 1.2 mM calcium solutions (21). The basolateral dicarboxylate transporter has not been molecularly identified. NaDC3 (discussed below), a dicarboxylate transporter, is on the basolateral membrane, but is not known to be calcium sensitive. A distinct sodium-coupled citrate transporter has been found in brain and liver, but not in kidney (17, 18); the calcium sensitivity has not been reported.
The present studies do not distinguish whether a single transporter accounts for all of the citrate and succinate transport in the range of calcium concentrations used, or, alternatively, whether extracellular calcium modulates the relative amount of transport occurring via two or more transporters. If a single transporter accounts for the findings here, then not only must the rate of dicarboxylate transport change with calcium, but the characteristics (possibly the affinity for different substrates) must change.

Although the presence of multiple possible transporters complicates straightforward utility of the OK proximal tubule cell model, few if any other cell culture models of citrate transport are available. Woost et al. (33) described dicarboxylate stimulation of current in a proximal tubule cell line, but this has not been extended or applied to analysis of citrate transport. Aruga et al. (2) recently described use of the related OK clone P (OKP) cell line to study citrate transport. However, to be able to measure citrate transport, they had to transfect the cloned transporter into Xenopus oocytes or OKP cells (a subclone of OK cells), i.e., citrate transport was not measurable in the native (nontransfected) state.

Is the calcium-sensitive transport of citrate and succinate a unique feature of opossum proximal tubule cells? Although this is possible, with the paucity of studies of citrate transport in proximal tubule cells in vivo or in culture, the alternative possibility in that the calcium-sensitive citrate (and succinate) transport process observed is present in other mammalian proximal tubule cells and just has not been adequately explored.

The calcium-sensitive citrate and succinate transport demonstrated in OK cells does not appear to be transport via NaDC1. NaDC1, in all species studied, including OKP cells, has a relatively low affinity for citrate (>0.6 mM) and, in most species, for succinate (0.35–0.8 mM). [The Km for succinate of the opossum NaDC1 is lower than in most species at 0.15 mM (2).] In our laboratory’s previous studies of citrate transport in OK cells (13), the Km for citrate was very low, but could not be established precisely: the radioisotope-specific activity was too low to detect citrate transport with increasing total citrate concentration in the uptake solutions. In other words, when cold citrate was added to the uptake solution to increase total extracellular citrate, the isotope uptakes became essentially indistinguishable from background.) The Km could be estimated to be <0.1 mM. The present experiments suggest, however, that the Km for succinate (when studied at low concentrations) is ~0.2–0.4 mM, i.e., the saturable component of succinate discussed above and referred to in Fig. 4. A similar high affinity for citrate and succinate can be inferred from the ~50% inhibition of citrate transport by 0.1 mM succinate (Fig. 1), and by the ~50% inhibition of succinate transport (at least that inhibitable by any concentration of citrate up to 1 mM) by 0.1 mM citrate (Fig. 2B). Moreover, a variety of potential substrates inhibit citrate and succinate substantially at 1 mM concentration, likely indicating a Km for these of <1 mM. In particular, the glutamate sensitivity of succinate transport here (Fig. 3B) differs from that reported for opossum NaDC1 (2). Therefore, the observed calcium-sensitive citrate and succinate transport is likely a high-affinity (low Km) dicarboxylate transporter, more closely resembling NaDC3 than NaDC1. NaDC3 (also called SDCT2 for the rat ortholog) is a high-affinity dicarboxylate transporter presumably located on the proximal tubule basolateral membrane (7, 26, 28). The calcium-sensitive process in OK cells described herein is likely an apical process, as our laboratory has reported in preliminary studies (12).

Therefore, the identity of the transporter responsible for the calcium-sensitive citrate and succinate transport in OK cells is unknown, but likely not NaDC1. Recent studies of NaDC1 knockout mice demonstrate that citrate excretion does increase when NaDC1 is knocked out (15); however, the published studies do not provide sufficient detail (e.g., filtered citrate) to know whether other transporters reabsorb some of filtered citrate in mice (15).

As additional evidence that the calcium-sensitive dicarboxylate transport in OK cells is not NaDC1, succinate transport by NaDC1 in oocytes is altered by calcium in a direction opposite that in OK cells. The effect of calcium on citrate transport in oocytes expressing opossum NaDC1 could be an effect of complexation between calcium and citrate, since succinate was not altered in the same direction. The mechanism of the calcium sensitivity in OK cells is not known. Although some component of complexation between citrate and calcium may contribute to decreased citrate transport with increasing calcium, this cannot account for most of the effect in OK cells, since succinate transport is similarly affected by extracellular calcium (4). Succinate is complexed by calcium and magnesium to a much lower extent than citrate. A direct effect of extracellular calcium on a transporter is possible and has precedent with certain transporters (20, 23); in fact, taurine transport and, to a lesser extent, succinate transport in placental cells are very sensitive to calcium (20). Identification and isolated study of the responsible transporter in proximal kidney cells will likely be necessary to adequately test this possibility. Another possibility is that extracellular calcium “signals” a change in citrate transport. Such a signaling could occur via the calcium-sensing receptor, or possibly via other processes responsive to extracellular calcium (e.g., transient receptor potential channels) (8). Calcium-sensing receptor is expressed in the apical membrane of proximal tubule cells, including OK cells (3, 32). Effects of extracellular calcium on certain channels also occur via changes in membrane charge shielding (1, 14).

In summary, the present studies describe a novel calcium-sensitive dicarboxylate transport process for succinate and citrate in OK proximal tubule cells. Decreasing extracellular calcium increases both succinate and citrate transport and also appears to change the sensitivity to inhibition by various dicarboxylates. Although acid-base status is probably the most important physiological determinant of urinary citrate, calcium excretion is also likely an important signal that may occur in the proximal tubule. The calcium sensitivity of citrate transport in this proximal tubule cell model corresponds with the physiological increase in urinary citrate with increasing urinary calcium, a teleologically protective mechanism against calcium nephrolithiasis.

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