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Sodium-dependent dicarboxylate transport in rat renal basolateral membrane vesicles

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Abstract. Dicarboxylate transport in basolateral membrane vesicles prepared from rat kidney cortex was studied using ^{3}H-methylsuccinate as a substrate. A sodium gradient (out > in) simulated methylsuccinate uptake and led to a transient overshoot. Lithium inhibited methylsuccinate uptake in the presence of sodium. The dependence of methylsuccinate uptake on sodium concentration indicated the interaction of more than one sodium ion with the transporter. Half-maximal stimulation was observed at 24 mmol/l sodium. Sodium-driven methylsuccinate uptake was electrogenic carrying a net positive charge. The basolateral dicarboxylate transport system exhibited an optimum at pH 7.0–7.5. In contrast, the sodium-dependent dicarboxylate transport system of brush border membranes depended much less on pH and had no optimum in the tested range. Cis-inhibition studies showed a preference of the system for dicarboxylates in the trans-configuration (fumarate) over cis-dicarboxylates (maleate). Citrate was accepted but oxalate and L-glutamate were not. DIDS exhibited a small inhibition. Among the monocarboxylates, gluconate and pyruvate inhibited methylsuccinate uptake whereas probenecid and p-aminohippurate (1 mmol/l) were without effect. The data indicate the presence of a sodium-dependent transport system in the basolateral membrane which accepts tricarboxylic acid cycle intermediates. This system is most likely not identical to the transport system responsible for organic anion secretion.

Key words: Dicarboxylate transport – Sodium – Lithium – Basolateral membrane – Rat kidney

Introduction

Tricarboxylic acid cycle intermediates are reabsorbed from the glomerular filtrate in the proximal tubules of the kidney [12, 32–35; for a review see 29]. Reabsorption across the luminal membrane of proximal tubular epithelial cells occurs by symport with sodium ions. The substrate specificity of the sodium-dependent dicarboxylate transport system has been investigated in great detail in vivo [28] and in isolated brush border membrane vesicles [38]: the system prefers dicarboxylic acids in the trans-configuration with a 4–5 carbon backbone. Tricarboxylic acids like citrate are transported in their divalent form [39]. As always three sodium ions are cotransported with each divalent anion, the transport process is electrogenic [18, 19, 24, 27, 40, 41].

Herndon and Freeman [13] found that the dog kidney extracts a greater amount of citrate from the blood than is filtered. This finding which was confirmed by others [3, 7, 11] indicated dicarboxylate uptake into renal cells not only across the luminal but also across the contraluminal cell membrane. The assumption of a transport system in the contraluminal membrane is also in accordance with data showing secretion of malate or 2-oxoglutarate during infusion of citric acid cycle intermediates [2, 33, 34]. An active net secretion of a slowly metabolized dicarboxylate, methylsuccinate, has been directly demonstrated by microperfusion experiments in rat proximal tubules [31]. These experiments revealed a sodium-dependent dicarboxylate transport system in the basolateral membrane of proximal tubular cells with a substrate specificity which is comparable, but not identical to that of the luminal transport system. Sheikh et al. [27] and Jørgensen et al. [15] demonstrated sodium-driven L-malate and citrate transport in basolateral membrane vesicles isolated from rabbit kidney cortex. L-Malate transport was electrogenic [27] whereas citrate transport was electroneutral [15]. In this contribution I would like to present evidence for a sodium-dependent dicarboxylate transport system in rat renal basolateral membrane vesicles. Sodium-driven methylsuccinate uptake was inhibited by lithium, was sensitive to alterations in potential and exhibited a pH optimum at pH 7.0–7.5.

Methods

Basolateral membrane vesicles from rat kidney cortex were prepared using a Percoll density gradient centrifugation method [25]. For each experiment the kidneys of 4–6 male Wistar rats were removed and decapsulated. Approximately 1 mm thick cortex slices were cut off and homogenized in 70 ml of a buffer containing 300 mmol/l mannitol, 12 mmol/l Hepes titrated with Tris to pH 7.4. Homogenization was performed by 20 strokes (900 rpm) of a tight-fitting Potter-Elvehjem apparatus and four 30 s bursts of an Ultra-Turrax operated at 180 V (Janke and Kunkel, Staufen, FRG). The crude plasma membrane fraction (for details see [25]) was resuspended in the aforementioned buffer and 13% (w/w)

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Abbreviations. DIDS, 4,4′-diisothiocyano-2,2′-disulfonic acid; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; Hepes, N-2-hydroxyethylpiperazine-N-ethanesulfonic acid; Mes, N-morpholinoethanesulfonic acid; Tris, tris(hydroxymethyl)-aminomethane

of Percoll were added. After 30 min centrifugation at 48,000 *g* the first 4 ml from the top of the gradient were discarded, the following 5 ml (basolateral membrane vesicles) were pooled and incubated for 30 min at room temperature in "transport buffer". Basolateral membrane vesicles were washed once more in transport buffer and used for the experiments. If not stated otherwise, the transport buffer contained 150 mmol/l tetramethylammonium chloride, 50 mmol/l KCl, 12 mmol/l Hepes/Tris, pH 7.4. The purity of the membranes was estimated from the enrichment of the specific activities of marker enzymes for basolateral [$(Na^+ + K^+)$-ATPase, EC 3.6.1.3] and brush border membranes (leucine aminopeptidase, EC 3.4.11.–). Compared to the starting homogenate the enrichment factors were 14.1 ± 1.1 for the $(Na^+ + K^+)$-ATPase and 1.1 ± 0.1 for the leucine aminopeptidase (means ± SEM for 23 preparations). Enzyme determinations were performed according to Berner and Kinne [4] and Tuppy et al. [30].

The uptake of ^{3}H-methylsuccinate was studied by a rapid filtration method [14]. Usually, 20 µl of membrane vesicles were added to 80 µl of prewarmed (30°C) incubation medium containing labeled methylsuccinate. The uptake was terminated by diluting 20 µl aliquots into 1 ml iced stop solution. The stop solution had the same composition as the incubation medium but did not contain labeled methylsuccinate. For short time (4 s) incubations, 5 µl vesicles were added to 50 µl of incubation medium and uptake was terminated by pipetting 1 ml of stop solution directly into the test tube [37]. The quenched suspensions were immediately pipetted onto the middle of prewetted filters (cellulose nitrate, 0.65 µm pore size, Sartorius, Göttingen, FRG) kept under suction. The filters were washed once with 5 ml stop solution and the radioactivity remaining on the filters was counted by standard liquid scintillation techniques. Corrections were made for the radioactivity bound to filters in the absence of vesicles. All experiments were performed at least in triplicate with freshly prepared membranes. Absolute solute uptake is expressed in pmol/mg protein and shown as means ± SEM for representative experiments.

Chemicals. ^{3}H-Methylsuccinate was kindly provided by Dr. Fasold. The synthesis of this compound is described in [9]. The salts were purchased from Merck, Darmstadt, FRG, and were of analytical grade. FCCP and valinomycin were from Boehringer, Mannheim, FRG, Percoll from Pharmacia Fine Chemicals, Uppsala, Sweden.

Results

The first experiments were performed to test for a sodium-driven uptake of methylsuccinate into rat renal basolateral membrane vesicles. Figure 1 shows that a sodium gradient (120 mmol/l outside, 0 mmol/l inside) stimulates the uptake of methylsuccinate compared to the complete absence of sodium and leads transiently to an intravesicular methylsuccinate concentration 17.5 times greater than at the equilibrium ("overshoot"). This experiment was performed in the presence of potassium (50 mmol/l outside and inside) and valinomycin to minimize a possible sodium diffusion potential. Therefore, stimulation of methylsuccinate uptake and transient intravesicular accumulation indicates sodium-coupled methylsuccinate transport in basolateral membrane vesicles. Figure 1 also shows that 5 mmol/l lithium inhibits

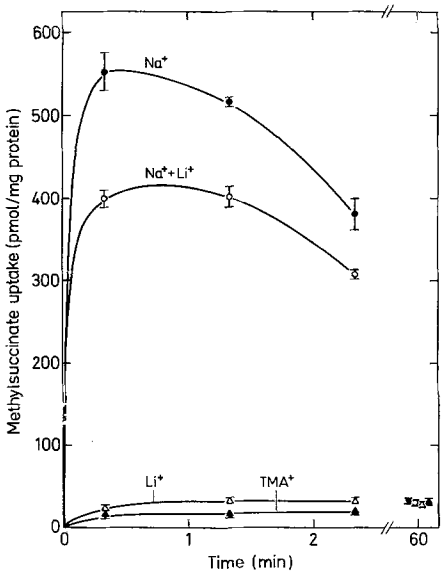

Fig. 1. Effects of sodium and lithium on methylsuccinate uptake into basolateral membrane vesicles. Basolateral membrane vesicles preloaded with 150 mmol/l tetramethylammonium chloride, 50 mmol/l KCl and pretreated with 62.5 µg/ml valinomycin were incubated in buffers containing 7.8 µmol/l labeled methylsuccinate, 50 mmol/l KCl, 30 mmol/l tetramethylammonium Cl and in addition 120 mmol/l NaCl (●), 115 mmol/l NaCl + 5 mmol/l LiCl (○), 120 mmol/l LiCl (△), or 120 mmol/l tetramethylammonium Cl (▲). The uptake is shown as means ± SEM of 4 determinations

sodium-driven methylsuccinate uptake considerably (115 mmol/l sodium outside). If all sodium in the incubation medium is replaced by lithium, methylsuccinate uptake is slightly stimulated when compared to the uptake in the presence of tetramethylammonium. Thus lithium may replace sodium though with much lower efficiency.

These results agree with previous findings on dicarboxylate transport across the luminal membrane [36, 38]. Therefore, sodium-stimulated methylsuccinate uptake may be due to a contamination of our preparation with brush border membrane vesicles. However, D-glucose uptake in the same basolateral membrane vesicles was stimulated by sodium only by 19% and no overshoot was found indicating a negligible contamination with brush border membrane vesicles (data not shown). Therefore, the existence of a sodium-driven dicarboxylate transport system in rat renal basolateral membrane vesicles must be assumed.

In order to find out which cations besides sodium can activate methylsuccinate uptake into basolateral membrane vesicles, NaCl in the incubation medium was replaced isoosmotically by chloride salts of various cations. As can be seen from Table 1, sodium is by far the best cation to stimulate 4 s methylsuccinate uptake followed by lithium, ammonium, tetramethylammonium, potassium, cesium, rubidium, and choline. Compared to Fig. 1, sodium stimulates less effectively which is due to the higher methylsuccinate concentration used in the experiment shown in Table 1 (100 versus 7.8 µmol/l).

The dependence of stimulation of methylsuccinate uptake on sodium concentration is shown in Fig. 2. In order to approach unidirectional influx conditions, 4 s uptake was determined. Sodium in the incubation medium was replaced isoosmotically by mannitol. Four seconds uptake is stimu-

Table 1. The influence of cations on methylsuccinate uptake into basolateral membrane vesicles. Basolateral membrane vesicles preloaded with 150 mmol/l tetramethylammonium (TMA^+) Cl were incubated for 4 s in media containing 150 mmol/l chloride salts of the indicated cations and 0.1 mmol/l labeled methylsuccinate. Uptake is shown as means ± SEM of 4 determinations

Cations (150 mmol/l)	Uptake (pmol/mg protein 4 s)	% of uptake in the presence of sodium
Na^+	133.1 ± 3.7	100
Li^+	47.3 ± 0.57	35.6
K^+	24.8 ± 6.5	18.6
Rb^+	21.9 ± 2.8	16.4
Cs^+	21.9 ± 4.4	16.5
NH_4^+	30.9 ± 3.8	23.3
$Choline^+$	18.6 ± 3.8	15.9
TMA^+	26.8 ± 1.6	20.1

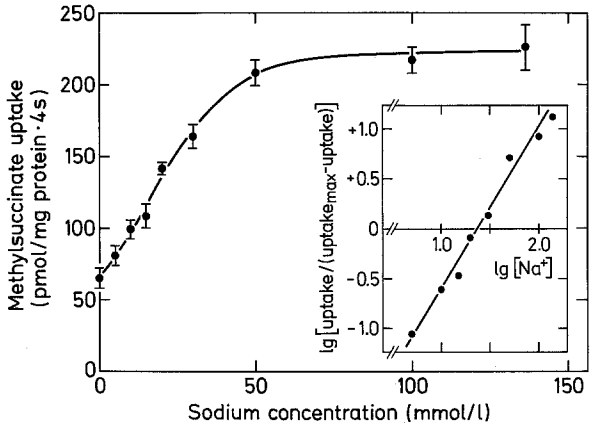

Fig. 2. Sodium concentration-dependent stimulation of methylsuccinate uptake into basolateral membrane vesicles. Vesicles preloaded with 150 mmol/l tetramethylammonium Cl were incubated for 4 s in media containing 100 µmol/l ^{3}H-methylsuccinate and the indicated sodium concentrations, sodium being isoosmotically replaced by mannitol. The uptake is shown as means ± SEM for 8 determinations (2 preparations). The inset shows a Hill plot of the data corrected for sodium-independent uptake

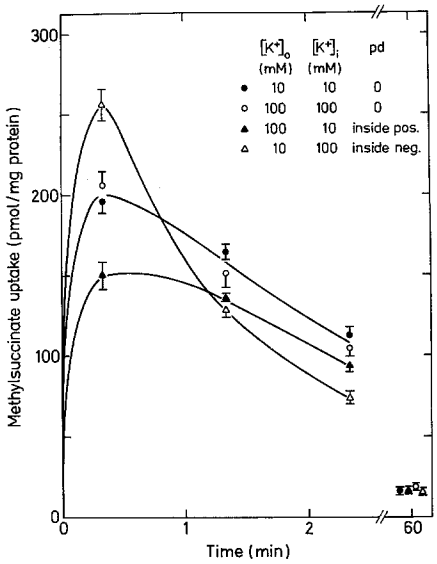

Fig. 3. The influence of a K^+-diffusion potential on methylsuccinate uptake into basolateral membrane vesicles. Vesicles have been preloaded with 10 (●, ▲) or 100 (○, △) mmol/l KCl, 190 (●, ▲) or 100 (○, △) mmol/l tetramethylammonium Cl and were pretreated with 62.5 µg/ml valinomycin. They were incubated in media containing 135 mmol/l NaCl, 10 (●, △) or 100 (○, ▲) mmol/l KCl, and 145 (●, △) or 55 (○, ▲) mmol/l tetramethylammonium Cl. Means ± SEM of 10 determinations (2 preparations) are shown

lated by sodium maximally 3–4 times compared to the uptake in the absence of sodium. The concentration dependence does not obey hyperbolic kinetics but seems to be S-shaped. When the data were replotted in a Hill plot (inset of Fig. 2) a straight line was obtained with a slope of 1.63. This suggests the interaction of more than one sodium ion with the dicarboxylate transporter. The half-maximal activation of methylsuccinate uptake is observed at 24 mmol/l sodium.

If two sodium ions are cotransported with the doubly negatively charged methylsuccinate, the symport should be electroneutral. Sodium-driven methylsuccinate transport would be electrogenic, if three sodium ions (or two sodium ions and one proton) are cotransported with each methylsuccinate molecule. Figure 3 shows an experiment to test for the electrogenicity of sodium-driven methylsuccinate uptake into basolateral membrane vesicles. The electrical potential difference across the vesicle membrane was varied by imposition of potassium gradients (10 mmol/l outside, 100 mmol/l inside or 100 mmol/l outside, 10 mmol/l inside) in the presence of valinomycin. Controls were run also in the presence of valinomycin but without a potassium gradient (10 mmol/l or 100 mmol/l in- and outside). Under control condition, sodium-driven methylsuccinate uptake showed an overshoot above the equilibrium which was not significantly different for $K_o^+ = K_i^+ = 10$ mmol/l and $K_o^+ = K_i^+ = 100$ mmol/l indicating that potassium itself had no effect on methylsuccinate uptake. In the presence of an inside negative membrane potential ($K_o^+ < K_i^+$) methylsuccinate uptake showed a higher overshoot which dissipated faster than under the control condition. In the presence of an inside positive membrane potential ($K_o^+ > K_i^+$) methylsuccinate uptake was retarded with respect to the controls. Stimulation of methylsuccinate uptake at inside negative and inhibition at inside positive potassium diffusion potentials indicates an electrogenic sodium-methylsuccinate cotransport with a surplus of positive charges in basolateral membrane vesicles.

A possible symport of methylsuccinate uptake with protons (or an antiport with hydroxyl ions) was tested by imposition of pH-differences across the vesicular membrane. Basolateral membrane vesicles were preloaded with buffers of pH 6.5 and incubated in media of pH 6.5 or 8.0. Likewise, vesicles preloaded with a buffer of pH 8.0 were incubated at pH 6.5 or 8.0 (Table 2). If a symport of methylsuccinate with protons occurs, and inward H^+ gradient should stimulate and an outward H^+ gradient should inhibit methylsuccinate uptake. At pH_0 6.5, 4 s methylsuccinate uptake is higher at pH_i 8.0 than at pH_i 6.5 suggesting a symport with protons. At pH_0 8.0, however, methylsuccinate uptake is nearly unaffected by the intravesicular pH arguing against a cotransport with protons. Thus the symport with protons may depend on the extravesicular pH and/or on the degree of dissociation of methylsuccinate (pK_{a2} 5.9 [38]) and has to be tested in greater detail.

Table 2. The influence of a pH-difference on methylsuccinate uptake into basolateral membrane vesicles. Basolateral membrane vesicles were preloaded with 150 mmol/l tetramethylammonium Cl, 50 mmol/l KCl, and either 30 mmol/l Mes/Tris, pH 6.5 or 30 mmol/l Hepes/Tris, pH 8.0. They were incubated for 4 s in buffers containing sodium instead of tetramethylammonium, 10 μmol/l labeled methylsuccinate and 30 mmol/l Mes or Hepes titrated with Tris to pH 6.5 or 8.0, respectively

pH		Uptake (pmol/mg protein 4 s)	*n*
Outside	Inside		
6.5	6.5	61.4 ± 3.7	12
6.5	8.0	85.5 ± 6.7	12
8.0	6.5	98.5 ± 4.4	10
8.0	8.0	95.4 ± 7.0	12

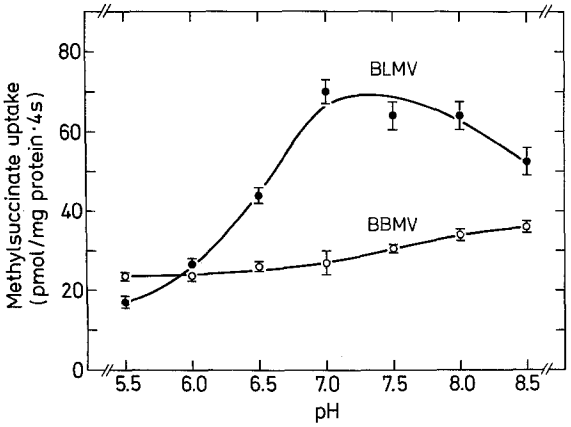

Fig. 4. pH-dependence of methylsuccinate uptake into basolateral (BLMV) and brush border (BBMV) membrane vesicles. Vesicles preloaded with 150 mmol/l tetramethylammonium Cl, 50 mmol/l KCl, 1 mmol/l Hepes/Tris, pH 7.0 and pretreated with 62.5 μg/ml valinomycin and 5 μmol/l FCCP were incubated for 30 min in buffers of identical composition but containing 25 mmol/l Mes or Hepes titrated with Tris to pH 5.5–7.0 or 7.5–8.5, respectively. Then, 10 μl of each vesicle suspension was added to 100 μl buffer of the respective pH ($pH_o = pH_i$) containing 10 μmol/l labeled methylsuccinate, 136.4 mmol/l NaCl, 50 mmol/l KCl, 14.6 mmol/l tetramethylammonium Cl and 25 mmol/l Mes titrated with Tris to pH 5.5–7.0 or 25 mmol/l Hepes/Tris, pH 7.5–8.5. Means ± SEM for 10 determinations are shown (2 preparations)

Then the pH-dependence of sodium-driven methylsuccinate uptake was determined. Vesicles were preequilibrated in buffers with various pH values in the presence of equimolar intra- and extravesicular potassium concentrations, valinomycin and FCCP. This pretreatment ensures the complete absence of transmembrane pH differences during methylsuccinate uptake. The results in Fig. 4 show that sodium-driven methylsuccinate uptake into basolateral membrane vesicles has a pH optimum at pH 7.0–7.5, i. e. at the physiological pH. In contrast, methylsuccinate uptake into brush border membrane vesicles tested under identical conditions is much less influenced by the pH. It shows no optimum in the tested range but increases steadily with increasing pH. The different pH-dependences indicate different transport systems in the luminal and basolateral membrane of proximal tubule cells.

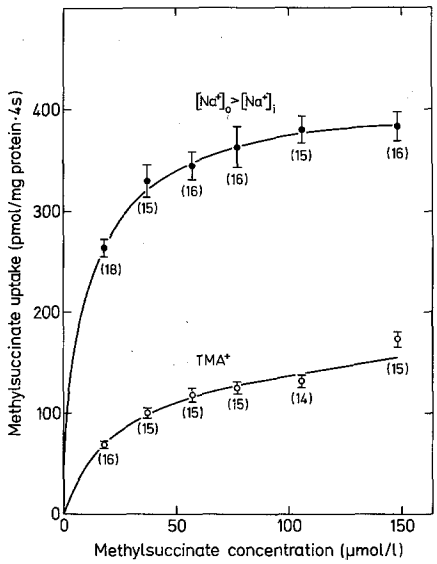

Fig. 5. Concentration dependence of methylsuccinate uptake into basolateral membrane vesicles. Vesicles preloaded with 150 mmol/l tetramethylammonium Cl, 50 mmol/l KCl and pretreated with valinomycin were incubated for 4s in media containing 136.4mmol/l NaCl, 14.6 mmol/l tetramethylammonium Cl (●) or 150 mmol/l tetramethylammonium Cl (○), 50 mmol/l KCl and varying concentrations of methylsuccinate. The figure shows means ± SEM of *n* determinations (*n* is given in brackets) from 4 preparations

The next experiments were performed to find out the methylsuccinate concentration needed for half-maximal uptake (apparent K_m) at saturating sodium concentrations. Methylsuccinate concentrations were varied between 18 and 148 μmol/l. The data from experiments with four different membrane preparations are shown in Fig. 5. It is evident that methylsuccinate uptake into basolateral membrane vesicles saturates in the presence as well as in the absence of sodium. In the presence of a sodium gradient half maximal uptake was observed at 10.1 μmol/l methylsuccinate and V_{max} was 413.4 pmol/mg protein. In the absence of sodium, the apparent K_m was 38.4 μmol/l and V_{max} 200.7 pmol/mg protein. The data indicate that the dicarboxylate transport system has a high affinity for methylsuccinate. Moreover, the transport system seems to function also in the absence of sodium though at lower V_{max} and with a smaller affinity for methylsuccinate.

Finally the substrate specificity of the dicarboxylate transport system in basolateral membranes was tested. All experiments were performed with equimolar intra- and extravesicular potassium concentrations and valinomycin to minimize indirect effects via anion diffusion potentials. The concentration of the test anions was 1 mmol/l, that of methylsuccinate 10 μmol/l. In Table 3 some selected dicarboxylates are tested as cis-inhibitors of methylsuccinate uptake. 2-Oxoglutarate and fumarate are the strongest inhibitors. Maleate, an unsaturated dicarboxylate in the cis configuration inhibited less than the unsaturated trans-dicarboxylate fumarate. The dicarboxylate transport system obviously also accepts the tricarboxylate citrate, but not the dicarboxylate oxalate. The dicarboxylic amino acid L-glutamate seems not to be accepted. These data indicate

Table 3. The influence of selected di- and tricarboxylates on methylsuccinate uptake. Basolateral membrane vesicles were preloaded with 150 mmol/l tetramethylammonium Cl, 50 mmol/l KCl, 62.5 μg/ml valinomycin, and incubated for 4 s in buffers containing 10 μmol/l labeled methylsuccinate, 150 mmol/l NaCl, 50 mmol/l KCl, and 1 mmol/l of the indicated anions. Uptake is given as means ± SEM, n is the number of determinations. P values are from a t-test against uptake in the absence of test anions as a reference; NS denotes not significant

Anion (1 mmol/l)	Uptake (pmol/mg protein 4 s)	Inhibition (%)	n	P
–	101.8 ± 5.01	0	8	
2-Oxoglutarate	3.49 ± 0.53	95.6	8	< 0.001
Fumarate	2.69 ± 0.20	97.4	7	< 0.001
Maleate	27.8 ± 3.89	72.6	8	< 0.001
Oxalate	102.6 ± 4.28	0	8	NS
L-Glutamate	96.0 ± 3.55	5.7	8	NS
Citrate	40.9 ± 2.24	59.9	8	< 0.001

Table 4. Effect of monocarboxylates and DIDS on methylsuccinate uptake. The experimental details are the same as for the experiment shown in Table 3

Anion (1 mmol/l)	Uptake (pmol/mg protein 4 s)	Inhibition (%)	n	P
–	105.4 ± 6.7	0	7	
L-Lactate	96.5 ± 3.6	8.4	8	NS
Pyruvate	70.2 ± 1.8	33.4	8	< 0.001
p-Aminohippurate	99.8 ± 3.9	5.3	8	NS
Probenecid	98.3 ± 2.8	6.7	8	NS
DIDS	80.2 ± 4.3	23.9	7	< 0.01

that the transport system prefers dicarboxylates in the trans configuration and that the two carboxyl groups must have a certain distance within the molecule. A third carboxyl group does not exclude the interaction with the transport system but an amino group does.

Table 4 shows the influence of 1 mmol/l of monocarboxylates and DIDS on sodium-driven methylsuccinate uptake. Only pyruvate and DIDS showed significant inhibitions of methylsuccinate uptake. 1 mmol/l L-lactate, para-aminohippurate and probenecid showed small, but not significant inhibitions. This indicates that methylsuccinate uptake does not occur to a significant extent through the probenecid-inhibitable transport system for PAH.

In preliminary experiments a reduction of the overshoot of methylsuccinate uptake was noticed in media containing gluconate instead of chloride. Therefore, the influence of various sodium salts on methylsuccinate uptake into basolateral membrane vesicles was tested and the result is shown in Table 5. Sodium-driven methylsuccinate uptake was comparable in the presence of chloride, bicarbonate, nitrate, sulfate, phosphate and cyclamate whereas it was considerably reduced in the presence of thiosulfate and gluconate. Whether this inhibition reflects an interaction of these anions with the dicarboxylate transport system is not clear at present.

Table 5. The influence of different sodium salts on methylsuccinate uptake. Basolateral membrane vesicles were proloaded with 150 mmol/l tetramethylammonium gluconate, 50 mmol/l K gluconate and pretreated with 62.5 μg/ml valinomycin. They were incubated for 4 s in media containing 0.1 mmol/l methylsuccinate, 50 mmol/l K gluconate, 150 mmol/l Na^+ and the indicated anions (150 mmol/l monovalent or 75 mmol/l divalent anions). Uptake is shown as means ± SEM, n = number of determinations. P values from Student's t-test are against uptake in the presence of 150 mmol/l NaCl. NS = not significant

Anion	Uptake (pmol/mg protein 4 s)	Inhibition (%)	n	P
Cl^-	142.6 ± 9.5	0	8	
HCO_3^-	133.7 ± 4.6	6.3	8	NS
NO_3^-	145.2 ± 4.4	0	8	NS
SO_4^{2-}	127.4 ± 5.9	10.7	8	NS
SSO_3^{2-}	102.3 ± 4.5	28.3	8	< 0.005
$H_2PO_4^-/HPO_4^{2-}$	146.1 ± 8.5	0	8	NS
Gluconate	76.2 ± 3.6	46.6	7	< 0.001
Cyclamate	143.8 ± 4.6	0	8	NS

Discussion

Effect of sodium

Using methylsuccinate as a substrate a sodium-driven transport system for tricarboxylic acid cycle intermediates was identified in basolateral membranes from rat kidney cortex. The stimulation of methylsuccinate uptake showed an S-shaped dependence on sodium concentration with a Hill coefficient of 1.6 indicating an interaction of more than one sodium ion with the transporter. Wright et al. [40] found a Hill coefficient of 1.8 in studies with rabbit renal brush border membrane vesicles. When these authors fitted their data to a model [10] assuming an independent interaction of "n" sodium ions with the luminal dicarboxylate transport system, the best results were obtained with $n = 3$. This stoichiometry agreed with the observation of an electrogenic, sodium-coupled dicarboxylate transport in rabbit renal brush border membrane vesicles [19, 41]. The Hill coefficient therefore seemed to underestimate the true stoichiometry. An analysis of our data according to Garay and Garahan [10] gave equally good fits for $n = 2$ and $n = 3$ making a decision on the stoichiometry impossible. Stimulation of sodium-dependent methylsuccinate uptake by inside negative membrane potentials and inhibition by inside positive potentials, however, indicate a symport of three cations (2 sodium ions plus one proton or 3 sodium ions) with one divalent methylsuccinate anion.

The half-maximal stimulation of methylsuccinate uptake into basolateral membrane vesicles was found at 24 mmol/l sodium. This datum is close to that found by Wright et al. [40] for succinate uptake into rabbit renal brush border membrane vesicles. Thus, both systems are fully activated at physiological sodium concentrations. The dependence of luminal and contraluminal dicarboxylate transport on sodium was also found in experiments in vivo [28, 31]. In sodium-free perfusates, active transtubular absorption of 2-oxoglutarate and active secretion of methylsuccinate were abolished indicating that the sodium gradient across the plasma membranes provides the driving force for transtubular active transport.

Effect of lithium

5 mmol/l lithium inhibited methylsuccinate uptake into basolateral membrane vesicles to a considerable extent although sodium was present at a 23 fold greater concentration. This finding indicates a high affinity of the contraluminal dicarboxylate transport system for lithium ions. Inhibition of sodium-driven methylsuccinate transport by lithium was also found in vivo and occurred at the contraluminal [31] and at the luminal membrane of proximal tubular epithelial cells [28]. In rabbit renal brush border membrane vesicles lithium inhibited sodium-dependent succinate uptake with a K_i of 1.3 mmol/l. The inhibition is probably due to replacement of one of three sodium ions by lithium on that cation site which has a high affinity for lithium [36]. In contrast, the stimulation of dicarboxylate uptake by lithium in the absence of sodium (36, and this study) seems to occur only if all three cation sites are occupied with lithium. As high lithium concentrations were required to fully activate the system, two of three cation binding sites have been assumed to have a small affinity for lithium. Moreover, lithium decreased the affinity of the transport system for succinate [36] explaining the small stimulation of methylsuccinate uptake by lithium as compared to sodium. Therapeutic doses of lithium enhanced the excretion of 2-oxoglutarate, but not that of citrate in man [6]. In rats, lithium increased the excretion of a variety of dicarboxylates including citrate [1, 5]. Thus there may be species differences in the sensitivity of renal transport systems to lithium. As lithium inhibits dicarboxylate transport across both, the luminal and the contraluminal membrane, an enhanced excretion of dicarboxylate in lithium-treated rats must be due to a stronger inhibition of the luminal system. This assumption is supported by data of Sheridan et al. [28] who found a K_i of 1.6 mmol/l lithium for the luminal transport system which is smaller than the K_i for the contraluminal system (5.5 mmol/l [31]).

Dependence on substrate concentration

In basolateral membrane vesicles methylsuccinate uptake saturates in the presence as well as in the absence of sodium. This finding suggested carrier-mediated methylsuccinate transport with and without sodium. The methylsuccinate concentrations required for half-maximal transport were 10 and 49 μmol/l, respectively. An increase in apparent K_m by removal of sodium is in accordance with Wright et al. [37]. Our results, however, are at odds with kinetic data obtained in vivo and in vitro. Fritzsch et al. [9] found an apparent K_m of 0.12 mmol/l for contraluminal methylsuccinate uptake in intact tubules. Methylsuccinate inhibited dicarboxylate transport through the luminal membrane with K_i = 0.14 mmol/l in vivo and with K_i = 0.16 mmol/l in isolated brush border membrane vesicles from rabbit kidneys [38]. The reason for the discrepancy between our results and those of others is not clear at present.

Electrogenicity

By means of a potential-sensitive cyanine dye Sheikh et al. [27] demonstrated an electrogenic, sodium-dependent L-malate uptake into rabbit renal basolateral membrane vesicles. Our data are in accordance with their findings: stimulation of methylsuccinate uptake by inside negative and inhibition by inside positive potassium diffusion potentials indicates a symport of 3 cations with each divalent dicarboxylate anion. These cations may be 3 sodium ions or 2 sodium ions plus one proton. At pH 8.0 we found no indication for a methylsuccinate-proton symport favouring the hypothesis of 3 sodium ions being cotransported with one methylsuccinate anion. At pH 6.5, however, a symport with sodium ions and protons may occur, because methylsuccinate uptake was stimulated by a pH difference (inside more alkaline). Electrophysiological experiments on rat proximal tubules in vivo [24] and studies with isolated brush border membrane vesicles from rabbit kidney cortex [18, 19, 41] revealed an electrogenic sodium-dicarboxylate cotransport also in the luminal membrane. Interestingly, sodium-citrate cotransport was electrogenic in rabbit renal brush border membrane vesicles [15, 41] but electroneutral in rat proximal tubules in vivo [24]. All authors came to the conclusion that a symport of three sodium ions with one dicarboxylate takes place. Citrate seems to be transported in its divalent form in rabbit renal brush border membrane vesicles [39] but in its trivalent form through the luminal membrane of rat proximal tubule cells [24].

pH Dependence

The dicarboxylate transport systems in the luminal and the contraluminal membrane differ markedly with respect to their pH dependence. Despite an influence of pH on the availability of the divalent form of methylsuccinate (pK_{a2} = 5.9), uptake into brush border membrane vesicles is hardly changed between pH 5.5 and 8.5. This is in agreement with results of Wright et al. [39]. In contrast, methylsuccinate uptake into basolateral membrane vesicles showed a clear optimum at pH 7.0–7.5. Different pH dependences of luminal and contraluminal dicarboxylate transport may explain the enhanced excretion of 2-oxoglutarate and citrate during alkalosis [2, 7, 8]. If net transtubular dicarboxylate transport is the result of two opposing transport systems and if solely the contraluminal system is faster at higher pH, a decrease of net reabsorption (or an increase of net secretion) can be expected at alkalosis. In addition, pH was found to exert an effect on dicarboxylate metabolism, e. g. citrate utilization is decreased at higher pH [3]. Thus, different pH sensitivities of the transport systems and altered metabolism may explain the influence of changes in acid-base balance on renal dicarboxylate handling.

Substrate specificity

The substrate specificity of the dicarboxylate transport system was studied in great detail with rabbit renal brush border membrane vesicles [38]. These studies revealed the preference of the transport system for dicarboxylates in the trans-configuration and a backbone of 4–5 carbons. Similar results were obtained in rat kidney in vivo [28]. Cis-inhibition studies presented in this paper show a similar molecular requirement for the basolateral transport system. The trans-dicarboxylate fumarate exhibited a greater inhibition of methylsuccinate uptake than the cis-dicarboxylate maleate. The lacking inhibition of methylsuccinate transport by oxalate indicated that a certain distance between the two carboxyl groups is required. A third carboxyl group in the molecule (citrate) does not exclude the interaction with the transporter but a positively

charged amino group does, as concluded from the lacking inhibition of methylsuccinate uptake by L-glutamate.

In the proximal tubule a variety of organic anions are transported across the basolateral membrane [21]. Therefore it was of interest whether these anions share a common system. As already mentioned, L-glutamate is not accepted by the dicarboxylate transport system. Yet acidic amino acids are cotransported with sodium across the basolateral membrane ([16, 23], and own unpublished results) indicating the presence of distinct systems for dicarboxylates and acidic amino acids. Furthermore, the lacking inhibition of methylsuccinate transport in basolateral membrane vesicles by probenecid and p-aminohippurate indicates that the system for organic anion secretion is also different from the dicarboxylate transport system. This does not rule out that citric acid cycle intermediates interfere also with the organic anion system as suggested by experiments with isolated proximal tubules [17]. Our data show, however, that dicarboxylate transport across the basolateral membrane can not occur to a significant extent through the system for organic anion secretion. Finally, the lacking inhibition of methylsuccinate transport by sulfate, chloride, bicarbonate and phosphate, i. e. anions which interfere with the common anion exchanger present in this membrane [20] argues against a common transport system for inorganic anions and citric acid cycle intermediates.

In conclusion, the experiments have shown the presence of a sodium-coupled transport system for Krebs cycle intermediates in basolateral membrane vesicles from rat kidney cortex. This system shares a number of characteristics with the dicarboxylate transport system in the luminal membrane but has a different pH-sensitivity. The role of sodium-dependent transport systems in both, luminal and antiluminal membrane may be the support of cells with fuels. Unlike most of the other organs, the kidney can utilize citrate for metabolism [22] and is thus together with the liver the most important organ to regulate blood dicarboxylate levels [26].

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