Succinate Transport in Rabbit Renal Basolateral Membrane Vesicles

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ABSTRACT

Properties of succinate transport were examined in basolateral membrane vesicles (BLMV) isolated from rabbit renal cortex. An inwardly directed Na^+ gradient stimulated succinate uptake and led to a transient overshoot. K^+, Li^+, Rb^+ and choline could not substitute for Na^+ in the uptake process. The dependence of the initial uptake rate of succinate on Na^+ concentration exhibited sigmoidal kinetics, indicating interaction of more than one Na^+ with transporter. Hill coefficient for Na^+ was calculated to be 2.0. The Na^+-dependent succinate uptake was electrogenic, resulting in the transfer of positive charge across the membrane.

The succinate uptake into BLMV showed a pH optimum at external pH 7.5～8.0, whereas succinate uptake into brush border membrane vesicles (BBMV) did not depend on external pH. Kinetic analysis showed that a Na-dependent succinate uptake in BLMV occurred via a single transport system, with an apparent Km of 15.5 ± 0.94 μM and Vmax of 16.22 ± 0.25 nmole/mg protein/min. Succinate uptake was strongly inhibited by 4～5 carbon dicarboxylates, whereas monocarboxylates and other organic anions showed a little or no effect. The succinate transport system preferred dicarboxylates in *trans*-configuration (furmarate) over *cis*-dicarboxylates (maleate).

Succinate uptake was inhibited by the anion transport inhibitors DIDS, SITS and furosemide, and Na^+-coupled transport inhibitor harmaline. These results indicate the existence of a Na^+-dependent succinate transport system in BLMV that may be shared by the other Krebs cycle intemediates. This transport system seems to be very similar to the luminal transport system for dicarboxylates.

Key Words: dicarboxylate transport; sodium dependent; basolateral membrane; rabbit kidney

INTRODUCTION

Krebs cycle intermediates, including succinate and citrate, are reabsorbed from the tubular filtrates in the proximal tubules of the kidney (Sheridan et al, 1983). Studies with isolated brush border membrane vesicles (BBMV) (Wright et al., 1980) and intact proximal tubules (Sheridan et al, 1983) demonstrate that reabsorption across brush border membranes occurs via a Na^+ cotransport process with a high degree of structural specificity for Krebs cycle intermediates.

It is observed in the dog kidney that a greater amount of citrate is extracted from blood than is filtered (Herndon & Freeman, 1958). Infusion of Krebs cycle intermediates also led to the net secretion of other carboxylates such as malate or 2-oxoglutarate into the tubular urine (Balagure & Stone, 1967; Vishwakarma & Lotspeich, 1959). These findings

which are confirmed by others (Balagura-Baruch et al, 1973; Cohen & Wittmann, 1963; Gold et al, 1979; Selleck & Cohen, 1965) indicate that a net uptake of dicarboxylates takes place from blood into the tubular cells across basolateral membranes. Microperfusion studies in rat proximal tubules showed a net active transtubular secretion of methylsuccinate (Ullrich et al, 1984). These experiments revealed that the basolateral membrane possesses a Na^+-dependent transport system for dicarboxylates with substrate specificity which is very similar, but not identical to that of the luminal dicarboxylate transport system.

A more direct examination of dicarboxylate transport has been investigated in the basolateral membrane vesicles (BLMV) of rat (Kahn et al., 1984; Burckhardt, 1984) and rabbit (Jörgensen et al, 1983; Sheikh et al, 1982; Wright & Wunz, 1987). kidneys However, the data on the substrate specificity for a Na^+-dependent dicarboxylate transport system of the basolateral membrane are inconsistent. Wright & Wunz (1987) found no inhibition of maleate, *cis*-isomer of succinate, on a Na^+-dependent succinate transport of renal BLMV, inconsistently with the result observed by Burckhardt (1984) who observed a strong inhibition (72.6%). Ullrich et al, (1984, 1987) demonstrated in the microperfused rat proximal tubule that succinate transport across the antiluminal side was inhibited by p-aminohippurate (PAH), and vice versa, suggesting the interaction of organic anion and succinate on the dicarboxylate transporter. In contrast, Burckhardt (1984) found that organic anions such as PAH and probenecid had no effect on the methylsuccinate transport in experiments using the renal BLMV.

The present studies were undertaken to determine the general characteristics of the dicarboxylate transport system using succinate as a substrate in BLMV isolated from the rabbit kidney proximal tubules. The data confirm the presence of a Na^+-stimulated, carrier-mediated electrogenic succinate uptake mechanism in the basolateral membrane. This system is likely not identical to the organic anion transport system.

METHODS

Preparation of membrane vesicles

Basolateral membranes were prepared by a Percoll density gradient centrifugation method according to Scalera et al (1981) with some modifications (Kim et al, 1987). New Zealand White rabbits were killed by a blow to the neck. The kidneys were perfused with an ice-cold solution containing 140 mM NaCl, 10 mM KCl, and 1.5 mM $CaCl_2$. The cortices were dissected, minced, and homogenized in 10% (wt/vol) sucrose buffer (250 mM sucrose and 10 mM triethanolamine, pH 7.6) with a tissue homogenizer for 10 strokes. The homogenate was centrifuged at 1,200 g for 10 min. The supernatant was collected and recentrifuged at 9,500 g for 15 min. The supernatant was combined with the upper layer of the pellet and centrifuged at 25,000 g for 30 min. The white, upper fluffy layer was gently separated from underlying, dark lower layer and resuspended in 26.5 ml of sucrose buffer by homogenizing with a tissue homogenizer for 10 strokes. Percoll (3.5 ml) was added, mixed vigorously and the mixture was centrifuged at 31,000 g for 45 min. The top 5 ml of the resulting gradient were discarded. The next 8 ml, having a very high Na-K-ATPase activity, was collected as basolateral membrane vesicles (BLMV) and the last 17 ml as brush border membrane vesicles (BBMV). BBMV were further purified by the Mg^{2+}-precipitation procedure by Kinsella et al (1979). Each membrane fraction was diluted with an equal volume of sucrose buffer and centrifuged at 65,000 g for 1 h to remove the Percoll. After centrifugation, the Percoll formed a very dense glassy pellet, and the membranes formed a loose fluffy layer above the Percoll. The membranes were resuspended in intravesicular buffer containing usually 100 mM mannitol, 100 mM KCl, 20 mM N-2-hydroxyethyl-

piperazine-N'-2-ethanesulfonic acid (Hepes)/Tris (pH 7.4) by passing it several times through a 25 -gauge needle and centrifuged again at 65,000 g for 1 h to remove the remaining Percoll. The final membrane pellet was resuspended in vesicle buffer and frozen in liquid nitrogen until used. Before experiments the membrane vesicles were preincubated at 37℃ for 30 min.

Marker enzyme assays

Na-K-ATPase activity was assayed according to Jørgensen and Skou (1971). Alkaline phosphatase activity was determined as described by Linhardt and Walter (1963). Protein concentration was determined according to Bradford (1976) using bovine serum albumin as a standard.

Measurement of uptake

Uptake of [^{14}C] succinate was determined by the rapid filtration method. Uptake was initiated by mixing 10 μl of vesicle suspension (10 mg protein/ml) with 190 μl of incubation medium maintained at 25℃. The composition of the experimental solutions was given in the appropriate figure legends. Transport was stopped after a specified time interval by addition of 2 ml of stop solution, and filtered immediately on 0.45 μm HAWP Millipore filters, prewetted with the distilled water, under vacuum pump suction. The stop solution had the same composition as the uptake solution but not contain labeled substrate. The filters were washed three additional times with 2 ml of stop solution, dissolved in 1 ml of 2-methoxyethanol (Eastman) and counted by standard liquid scintillation techniques.

All uptakes were corrected for nonspecific binding measured in the absence of vesicles. All experiments were performed in at least three separate membrane preparations. Statistical significance was determined with Student's t test for unpaired data and a one-way analysis of variance.

Chemicals

[^{14}C]succinate (50~70 mCi/mmol) was purchased from New England Nuclear (Boston, MA). Valinomycin, PAH, probenecid and ouabain were obtained from Sigma Chemical Co (St. Louis, MO). Percoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). DIDS (4, 4'-diisothiocyano-2, 2'-disulfonic stilbene) was obtained from Pierce (Rockford, IL) and SITS (4-acetamido-4'-isothiocyano-stilbene-2, 2'-disulfonic acid) from ICN Biomedicals Inc. (Plainview, NY). Other chemicals were of the highest purity available from chemical sources.

RESULTS

Purity of membrane fractions

The degree of purification was determined by assaying the activity of Na-K-ATPase, the marker enzyme for BLMV, and of alkaline phosphatase, the marker enzyme for BBMV. BLMV fraction was enriched in Na-K-ATPase specific activity 22.97 -fold over crude homogenate and in alkaline phosphatase activity 1.13-fold. BBMV fraction was enriched in alkaline phosphatase activity 24.20-fold over crude homogenate, while the Na-K-ATPase was decreased to 0.70-fold.

Cation dependence of succinate uptake

Fig. 1 shows the time course of 5 μM succinate uptake by BLMV and BBMV. Uptake was determined in the presence of an inwardly directed Na^+ or K^+ gradients. A 100 mM Na^+ gradient makedly stimulated the initial rate of uptake as compared to a 100 mM K^+ gradient and caused a transient overshoot in which the intravesicular succinate concentration exceeded that at the equilibrium (60 min).

Demonstration of the overshoot phenomenon pro-

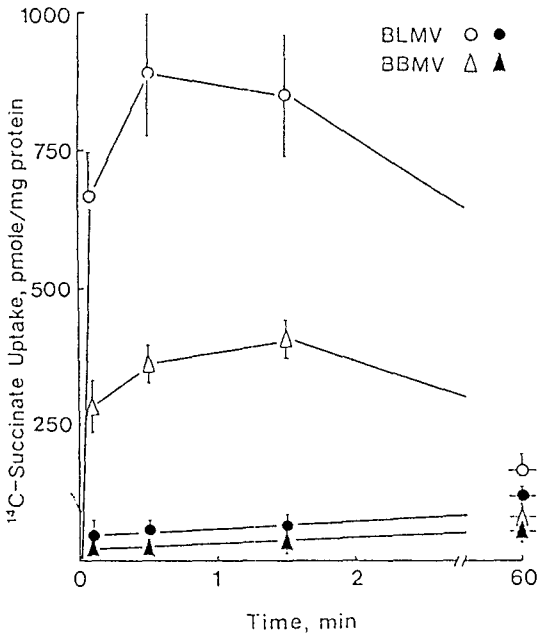

Fig. 1. Effect of Na^+ gradient on succinate uptake in rabbit renal BLMV (○, ●) and BBMV (△, ▲). Membrane vesicles were preloaded with 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.4). Uptake of 5 μM [^{14}C] succinate was measured in buffers containing 100 mM mannitol, 100 mM NaCl (○, △) or 100 mM KCl (●, ▲) and 20 mM Hepes/Tris (pH 7.4). Each point represents mean±SEM of four determinations.

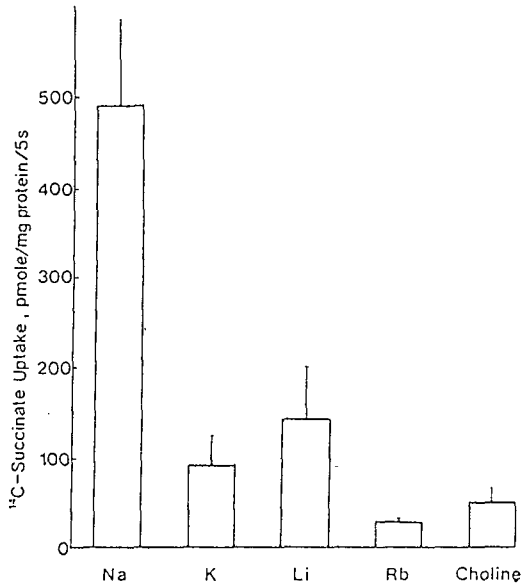

Fig. 2. Effects of monovalent cations on succinate uptake in BLMV. Membrane vesicles were preloaded with 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.4). Uptake of 5 μM [^{14}C]succinate was measured for 5s in buffers containing 100 mM mannitol, 100 mM chloride salts of indicated cations and 20 mM Hepes/Tris (pH 7.4). Each point represents mean±SEM of 12 determinations.

vides strong evidence for the cotransport process (Heinz & Weinstein, 1984), in this case, the coupling of Na^+ and succinate transport across both brush border and basolateral membranes. However, the initial rate of succinate uptake into BLMV exceeds by>50% that in BBMV. This latter observation indicates that the Na^+-dependence of succinate transport into BLMV observed in the present study was not merely due to a contamination of BBMV.

Effects of various monovalent cations on succinate uptake are shown in Fig. 2. When 100 mM Na^+ gradient was replaced by gradients of K^+, Li^+, Rb^+, or choline, succinate uptake was markedly reduced. Thus, no monovalent cations tested were effective as replacement ions for Na^+ in the succinate transport into BLMV.

Effect of Na^+ concentration

The initial rate (5s) of succinate uptake was measured as a function of Na^+ concentrations over the range of 0~150 mM. The Na-dependent succinate uptake is plotted vs. Na^+ concentration in Fig. 3. It is clear from this figure that the uptake data show a sigmoidal dependence on Na^+ concentration, which is indicative of multiple Na^+ binding sites. An estimation of the number of Na^+ binding site can be obtained by fitting the data to Hill equation (Segel, 1975), flux=Vmax $[Na]^n/(K_{0.5}^n+[Na]_{0.5}^n)$. A least-square analysis of this plot yields Vmax of 17.04 nmole/mg protein/min, $K_{0.5}$ of 38.48 mM, and n of

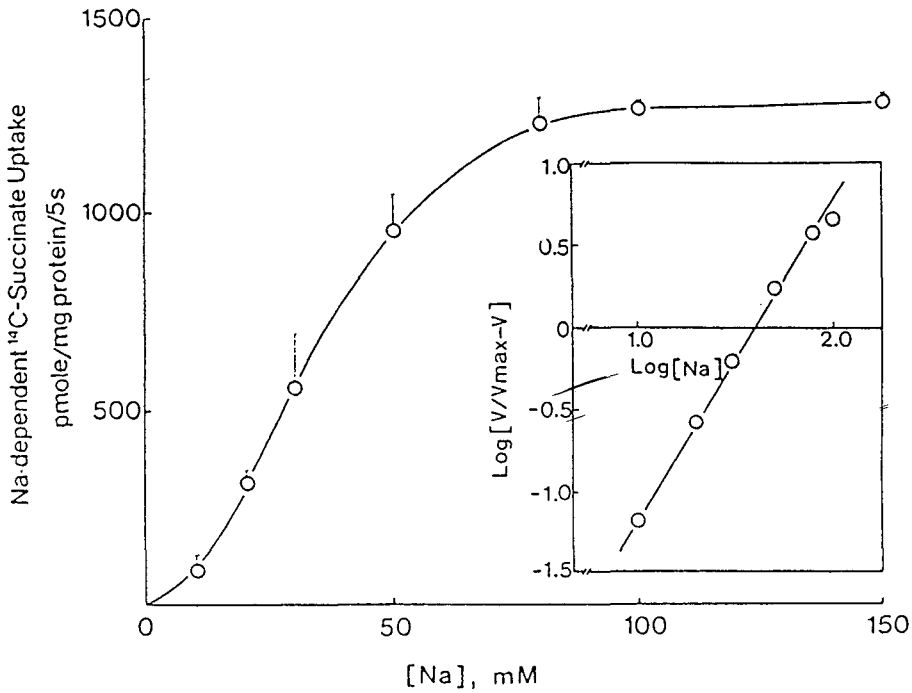

Fig. 3. Dependence of succinate uptake on Na^+ concentrations in BLMV. Membrane vesicles were preloaded with 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.4). Uptake of succinate was measured for 5s in buffers containing 100 μM [^{14}C]succinate, 20 mM Hepes/Tris (pH 7.4) and indicated Na^+ concentration. The concentration of mannitol in the uptake buffer was adjusted to maintain osmolality. A Na-dependent succinate uptake was obtained by subtracting uptake measured in the absence of Na^+ from total uptake. Each point represents mean±SEM of six determinations. The inset shows a Hill plot of the data.

2.0. A Hill plot of these data using the above parameters is linear with r=0.998 (Fig. 4, inset). Although the Hill equation is only an approximate representation of the true Na dependence of succinate transport (Segel, 1975), the n value given above suggests that more than two Na ions (probably 3 Na^+) interact with succinate transporter, where succinate bears two negative charges.

Effect of membrane poteintial

Succinate exists predominantly as a dicarboxylate with two negative charges at pH 7.4. Since the probable stoichiometry of Na^+/succinate cotransport appears to be 3:1 in Fig. 3, succinate uptake should result in transfer of positive charge across the membrane, and as a consequence, should be potential-sensitive.

Therefore, the influence of a valinomycin-induced K^+-diffusion potential on succinate uptake was examined (Fig. 4). The addition of valinomycin in the presence of outwardly directed K^+ gradient, a maneuver which renders the vesicle inside more negative, caused a significant stimulation of the initial rate (5s) of uptake. These results demonstrate that Na^+/succinate cotransport is electrogenic process resulting in a net transfer of positive charge. These data also provide support for a Na^+/succinate stoichiometry of 3:1.

pH Dependence of succinate uptake

To test pH effect on succinate uptake, membrane vesicles were preloaded with buffer of pH 7.4 and

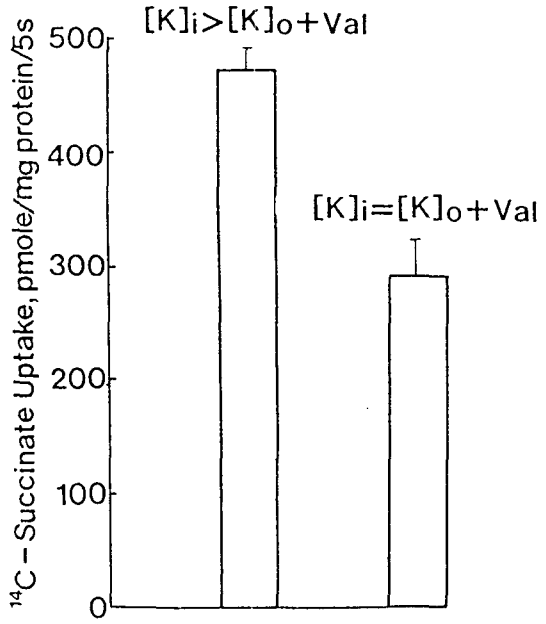

Fig. 4. Effect of K-diffusion potential on succinate uptake in BLMV. Membrane vesicles were preloaded with 300 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.4). Uptake of 5 μM [^{14}C]succinate was measured for 5s in buffer containing 300 mM mannitol or 100 mM mannitol polus 100 mM KCl, 100 mM NaCl, 2 μM valinomycin and 20 mM Hepes/Tris(pH 7.4). Each point represents mean±SEM of six determinations.

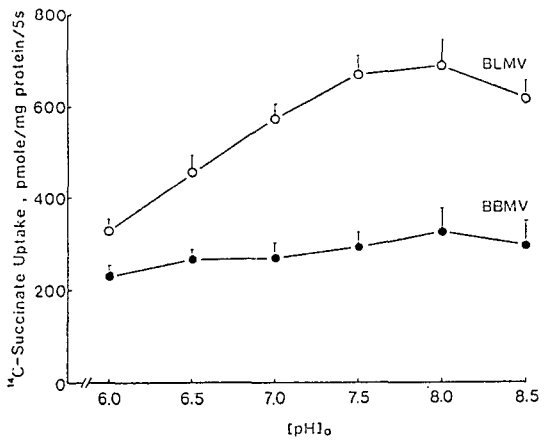

Fig. 5. pH-Dependence of succinate uptake in BLMV (○) and BBMV (●). Membrane vesicles were preloaded with 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.4). Uptake of 5 μM [^{14}C]succinate was measured for 5s in buffer containing 100 mM mannitol, 100 mM NaCl and 20 mM Mes or Hepes titrated with Tris to pH 6.0 ~7.0 or 7.5~8.5, respectively. Each point represents mean±SEM of nine determinations.

incubated in media of various pHs. As shown in Fig. 5, changing external pH from 6.0 to 8.5 produced no change in the initial rate of Na^+-dependent succinate uptake into BBMV. On the other hand, Na^+-dependent succinate uptake into BLMV tested under identical conditions was sensitive to external pH, having a pH optimum at pH 7.5~8.0. These data are in good agreement with observations of Burckhardt (1984) and Wright & Wunz 987).

Kinetics of succinate uptake

In order to determine values for the kinetic parameters of succinate transport into BLMV, the initial rates (5s) of uptake were measured at various substrate concentrations. Data were fitted to saturable, single-binding site model (Fig. 6): V=Vmax [S]/(Km+[S]), where V is the uptake of succinate (S), Vmax is the maximal rate of uptake, and Km is the apparent Michaelis constant. In this plot, Vmax=16.22±0.25 n mole/mg protein/min, Km=15.5±0.94 μM.

Substrate specificity

To determine the substrate specificity of the BLMV dicarboxylate transporter, Na^+-dependent succinate uptake was measured in the presence of various organic anions in the incubation medium. The concentration of the test anions was 1 mM and that of succinate was 5 μM. The effect of monovalent organic anions on succinate uptake is given in Table 1. L-lactate, pyruvate and octanoate showed small, but significant inhibitions. On the other hand,

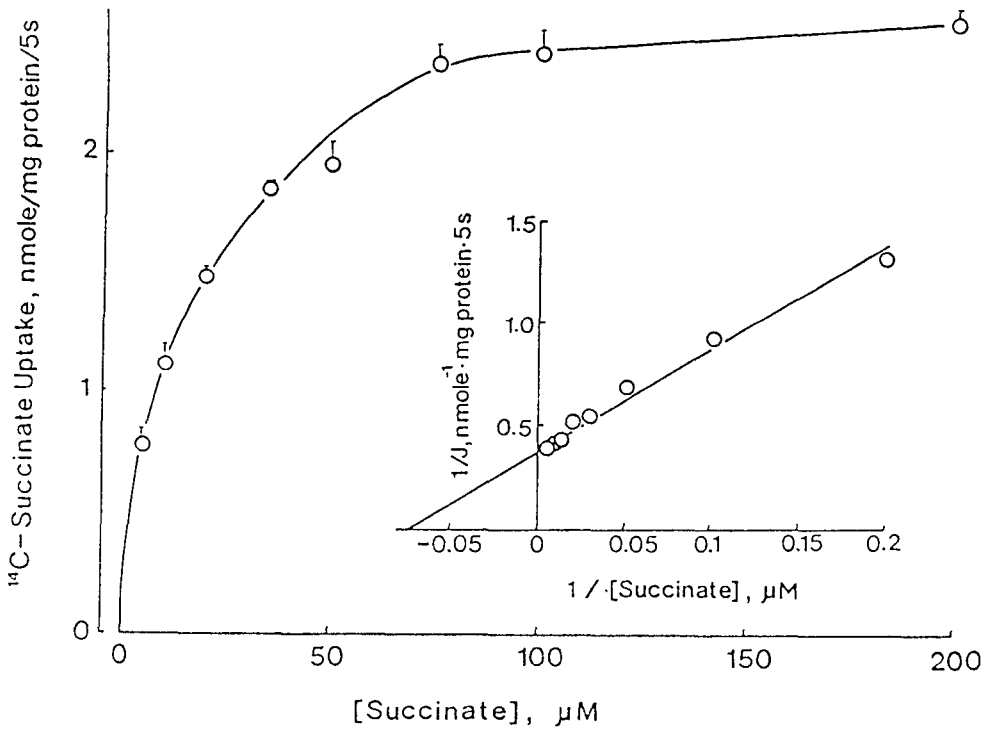

Fig. 6. Concentration dependence of succinate uptake in BLMV. Membrane vesicles were preloaded with 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.4). Uptake was measured for 5s in buffer containing 100 mM mannitol, 100 mM NaCl, 20 mm Hepes/Tris (pH 7.4) and various concentrations of succinate. Each point represents mean±SEM of six determinations.

acetate, propionate, formate and valerate failed to inhibit the succinate uptake. Similarly, organic anions such as PAH and probenecid had no effect on the succinate uptake.

Table 2 shows the inhibition of succinate uptake by di- and tricarboxylates. Unlabelled succinate, 2-oxoglutarate, and fumarate were potent inhibitors for succinate uptake, which caused>90% at 1 mM. Succinate transport was also effectively inhibited by the tricarboxylate citrate. However, oxalate showed a small inhibition and maleate failed to inhibit the succinate uptake.

Effect of inhibitors

Finally, we examined the effect of various transport inhibitors on Na^+-dependent succinate uptake to further characterize the properties of renal basolateral dicarboxylate transport. The 5-s uptake of 5 μM succinate was determined in the presence and absence of 1 mM of inhibitors. As shown in Fig. 7, the anion transport inhibitors furosemide, DIDS and SITS reduced succinate uptake 55%, 58% and 47%, respectively. Harmaline, Na^+-coupled transport inhibitor in renal BBMV (Aronson & Bounds, 1980) and BLMV (Grassl et al., 1987), was observed to inhibit Na^+-dependent succinate uptake into BLMV 30%.

DISCUSSION

Na^+ dependence of succinate uptake

A Na^+-dependent succinate transport is extensively characterized in BBMV (Bindslev & Wright, 1984;

Table 1. Effect of Monovalent Organic Anions on Succinate Uptake in BLMV

Anion (1 mmol/L)	Uptake (pmole/mg protein/5s)	Inhibition (%)	n	P
Control	620.23±31.88	0	8	
Lactate	521.81±21.29	15.22±3.43	10	<0.01
Pyruvate	498.80±32.52	19.58±5.24	10	<0.01
Acetate	569.80±14.20	8.13±2.29	10	NS
Propionate	574.25±36.66	7.41±5.91	7	NS
Formate	605.93±24.17	1.45±3.50	7	NS
Valerate	604.20±33.27	2.59±5.36	7	NS
Octanoate	450.16±61.77	27.42±9.96	7	<0.01
PAH	596.65±33.51	3.80±5.40	10	NS
Probenecid	598.79±35.81	5.46±4.69	10	NS

Membrane vesicles were preloaded with 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.4). Uptake of 5 μM [^{14}C]succinate was measured for 5s in buffer containing 100 mM mannitol, 100 mM NaCl, 20 mM Hepes/Tris (pH 7.4) and 1 mM of indicated organic anions. Data are given as mean±SEM, n is the number of determinations.

Table 2. Effects of Di-and Tricarboxylates on Succinate Uptake in BLMV

Anion (1 mmol/L)	Uptake (pmole/mg protein/5s)	Inhibition (%)	n	P
Control	853.56±48.66	0	10	
Succinate	28.87± 1.41	96.43±0.21	8	<0.01
2-Oxoglutarate	43.18± 5.30	94.75±0.57	8	<0.01
Fumarate	53.77± 1.63	94.15±0.38	5	<0.01
Maleate	780.05±68.09	8.99±2.20	7	NS
Oxalate	750.63±81.28	18.77±4.09	7	<0.01
Citrate	213.13± 6.81	73.96±1.99	7	<0.01

Membrane vesicles were preloaded with 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris (pH 7.4). Uptake of 5 μM [^{14}C]succinate was measured in buffer containing 100 mM mannitol, 100 mM NaCl, 20 mM Hepes/Tris (pH 7.4) and 1 mM of indicated carboxylates. Data are given as mean±SEM, n is the number of determinations.

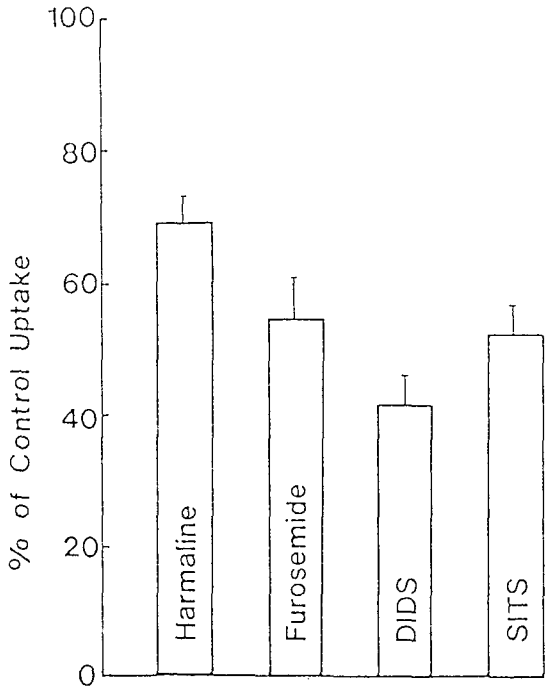

Fig. 7. Effects of various inhibitors on succinate uptake in BLMV. Membrane vesicles were preloaded with 100 mM mannitol, 100 mM KCl and 20 mM Hepes/Tris(pH 7.4). Uptake of 5 μM [^{14}C]succinate was measured in buffer containing 100 mM mannitol, 100 mM NaCl, 20 mM Hepes/Tris (pH 7.4) and 1 mM of inhibitors. Each point represents mean±SEM of eight determinations.

Fukuhara & Turner, 1983; Hirayama & Wright, 1984; Schell & Wright, 1985; Wright et al, 1981). Studies on the transport of dicarboxylates across the basolateral membrane of proximal tubules are limited, but the presence of a Na-dependent transport system for dicarboxylates is proposed in BLMV (Burckhardt, 1984; Kahn et al., 1984; Wright & Wunz, 1987) and in the intact rat proximal tubule (Ullrich et al, 1984, 1987).

In this study, the dicarboxylate transport system was characterized using succinate as a substrate in BLMV isolated from rabbit kidney proximal tubule. The uptake of succinate was stimulated by an inwardly directed Na gradient and showed an over-

shoot phenomenon, a result consistent with the existence of a Na^+/succinate cotransport system in the basolateral membrane. The Na^+-dependency of succinate uptake showed a sigmoidal-shape with a Hill coefficient of 2.0, indicating an interaction of a minimum two Na^+ ions with the transporter. Since the derivation of the Hill equation is based on the assumption of strong cooperativity between Na^+ binding sites, the calc_lated value of n is almost always expected to be less than the actual number of Na binding sites (Segel, 1975). Thus, a n value of 2.0 suggests that the probable stoichiometry of the Na^+/succinate cotransporter is 3:1, a conclusion supported further by the observed stimulation of a Na^+-dependent succinate uptake when inside-negative potential is developed. This result is in good agreement with the observations of Burckhardt (1984) for rat renal BLMV and of Wright et al (1982) for renal BBMV.

Kinetics

Kinetic analysis of the Na-dependent succinate uptake into BLMV showed a saturable, single binding site model with a apparent affinity constant of 15.5 ± 0.94 μM. This value was very close to that observed previously for rat (Burckhardt, 1984) and rabbit (Wright & Wunz, 1987) renal BLMV, but was 70～700 times lower than that reported in renal BBMV ranged from 0.1 to 1 mM (Wright et al, 1980; Nord et al, 1982; Fukuhara & Turner, 1983; Wright & Wunz, 1987). Thus, the basolateral transport system exhibits manifold greater affinity for succinate than does the luminal transport system. This suggests that the transport process of dicarboxylates in the basolateral membrane could play an important role in supplying substrates to proximal tubule cells. Krebs cycle intermediates have been proposed to be important substrates for oxidative metabolism in intact proximal tubules (Klein et al, 1981).

One source of oxidizable substrates for proximal tubule cells is reabsorption of these solutes, and an alternative source from the peritubular fluid. Since blood levels of dicarboxylate Krebs cycle intermediates are less than 50 μM (Diem, 1962), the comparatively high apparent affinity of the basolateral pathway for succinate seems to be well-suited to the efficient transport of these solutes into cells from the blood.

Substrate specificity

The structural specificity of the succinate transport system of rabbit BBMV was examined in detail by Wright et al (1980). They observed that the dicarboxylate transport system is highly specific for 4～5 carbon dicarboxylates *trans*-configuration. Ullrich et al (1984, 1987) proposed that the specific pattern of contraluminal dicarboxylate transport system is very similar with that of the luminal dicarboxylate transport system. In the present study, monocarboxylates tested, but not pyruvate and octanoate, showed no inhibition of succinate uptake, results consistent with those observed in vivo (Ullrich et al, 1984) and in vitro (Burckhardt, 1984).

Furthermore, organic anions such as PAH and probenecid failed to inhibit succinate uptake, which is in agreement with the observation of Burckhardt (1984). These data indicate that dicarboxylate transport system in BLMV is different from the monocarboxylate and other organic anion transport systems. However, Ullrich et al (1984, 1987) found a moderate inhibition of methylsuccinate across the contraluminal cell side by PAH and vice versa, in the rat intact proximal tubule. They concldluded that PAH and dicarboxylates are transported by two systems with ov· ıpping substrate specificities.

This discrepancy in results obtained from membrane vesicles and the intact tubule is difficult to explain at present. However, it is interesting that the dicarboxylate glutarate inhibits strongly succinate transport as well as the PAH transport across the

basolateral membrane in vitro (Burckhardt, 1984; Shimada et al, 1987; Wright & Wunz, 1987) and in vivo (Ullrich et al, 1984, 1987). Although the PAH transport system, therefore, shares some dicarboxylates such as glutarate, PAH and succinate might be transported by different transport system. Low et al (1984) observed also in renal BLMV that sulfate/PAH exchanger did not accept succinate.

The succinate transport system is specific for 4～5 carbon dicarboxylates. The *trans*-dicarboxylate fumarate was a potent inhibitor of succinate transport, whereas the *cis*-dicarboxylate maleate was not (Table ?). This indicates that the dicarboxylate transport system is stereospecific, requiring the carboxyl groups to be in a *trans*-configuration. The lower affinity of oxalate may be because of a short distance between the two carboxyl groups as pointed out by Ullrich et al (1984). Citrate, a tricarboxylate, is an effective inhibitor of succinate uptake, suggesting that the dicarboxylate transport system could accept tricarboxylate.

Finally, a Na^+-dependent succinate transport was reduced by stilbene disulfonates DIDS and SITS (Fig. 8), results consistent with those reported by Burckhardt (1984) and Ullrich et al (1984). These anion transport inhibitors inhibit also the organic anion PAH transport (Hong et al., 1978; Koshier et al, 1980) as well as inorganic anions such as sulfate (Bastlein & Burckhardt, 1986; Grinstein et al 1980; Pritchard & Rentro, 1983) and phosphate transport (Ullrich et al, 1982) across basolateral membranes. Thus, these inhibitors seems to reduce the basolateral transport of both organic and inorganic anions.

The inhibitory effect of harmaline, Na^+-coupled transport inhibitor in BBMV (Aronson & Bounds, 1980) and BLMV (Grassl et al, 1987), most likely reflects an interaction with the Na binding sites on the Na^+-succinate cotransporter.

In conclusion, the existence of electrogenic Na^+-succinate cotransport system has been demonstrated in BLMV isolated from rabbit renal cortex. This system was shared by the dicarboxylates oxoglutarate and furmarate, and tricarboxylate citrate, but not by organic anions such as PAH and probenecid, suggesting that its structural specificity is very similar to those in the luminal membrane. However, a Na^+-dependent succinate transport systems in both membranes, luminal and antiluminal, have different characteristics each other in pH-sensitivity and the apparent affinity for substrate.

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= 국문초록 =

가토 근위세뇨관 Basolateral Membrane Vesicle에서 Succinate 이동 특성

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가토 신장 피질에서 Percoll density gradient 방법으로 분리한 basolateral membrane vesicle (BLMV)에서 rapid filtration technique을 이용하여 succinate의 이동 특성을 관찰하였다.

Na^+은 succinate의 이동을 증가시켜 "overshoot" 현상을 보였으며 이러한 효과는 K^+, Li^+, Rb^+, choline과 같은 다른 양이온들에 의해 나타나지 않았다. Na^+ 농도변화에 따른 succinate의 이동율은 sigmoid 모양을 보였고, Na^+에 대한 Hill coefficient는 2.0이었다. succinate의 이동은 vesicle 내부가 음전압일 때 더욱 증가되었다. BLMV에서 succinate 이동은 용액내 pH 변화에 따라 영향을 받았으나 brush border membrane vesicle (BBMV)에서는 영향을 받지 않았다. 동력학적 분석결과 succinate의 Km 값은 15.5±0.94 μM이었고 Vmax는 16.22±0.25 n mole/mg protein/min이었다. succinate의 이동은 4~5 탄소를 가진 dicarboxylate들에 의해 강력하게 억제되었으나 monocarboxylate나 다른 유기음이온들에 의해 영향을 적게 받거나 받지 않았다. succinate의 이동은 DIDS, SITS, furosemide와 같은 음이온 이동 억제제와 harmaline과 같은 Na^+ 이동 억제제에 의해 억제되었다.

이들 결과들은 BLMV에서 succinate는 Na^+에 의존하여 이동하며 다른 Krebs cycle 중간 산물들과 동일한 운반기전을 이용함을 가르킨다. 또한 BLMV에서 succinate의 이동은 그 기질특이성에 있어서 다른 연구자에 의해 보고된 BBMV에서 이동특성과 유사함을 보였다.