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Transport of L-ascorbic acid and dehydro-L-ascorbic acid across renal cortical basolateral membrane vesicles

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The uptake of L-ascorbic acid and dehydro-L-ascorbic acid into renal cortical basolateral membrane vesicles has been characterized. The uptake systems for both solutes demonstrate saturation kinetics. The presence of structural analogs of L-ascorbic acid and dehydro-L-ascorbic acid results in cis-inhibition and trans-stimulation. Uptake of each substrate is Na^+-independent, proceeding to an endpoint of substrate equilibrium across the vesicular membrane. The transport mechanism(s) for L-ascorbic acid and dehydro-L-ascorbic acid appears to be facilitated diffusion.

Introduction

L-Ascorbic acid is filtered in the mammalian kidney and reabsorbed across cells of the renal proximal tubule by a two-stage process: (1) entry of the compound into the epithelium across the brush-border membrane, and (2) efflux across the basolateral membrane toward the blood. The transport mechanism in the brush-border membrane has been characterized through flux measurements in isolated vesicles [1]. In kidneys of guinea pig, rat and rabbit, L-ascorbic acid uptake occurs against an electrochemical gradient and is driven by the sodium electrochemical potential gradient across the membrane. Transport of solutes across the basolateral plasma membrane has received less attention due to the inaccessibility of the serosal pole in intact tissue. Techniques of isolating pure basolateral membranes have allowed transport mechanisms to be studied. We report the characterization of the transport systems for L-ascorbic acid and its readily interconvertible, antiscorbutic metabolite, dehydro-L-ascorbic acid in isolated basolateral membranes from rat superficial renal cortex.

Materials and Methods

Preparation of basolateral membranes

Membranes were prepared by a procedure slightly modified from that described by Sacktor et al. [2]. Briefly, the kidneys from two stunned male Sprague-Dawley rats (150–250 g) were used for each preparation. The kidneys were quickly excised and chilled in ice-cold medium containing 0.25 M sucrose, 20 mM Tris, pH 7.6. All steps were performed at 0–4°C. The kidneys were decapsulated and the superficial cortex was removed and minced. The tissue was homogenized by 20 strokes of a glass-Teflon homogenizer. A sample of crude homogenate was reserved for protein and

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Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

enzyme determinations. The crude homogenate was centrifuged at 2500 × *g* for 15 min in a Beckman Model J21B centrifuge. The supernatant was reserved and centrifuged at 24000 × *g* for 20 min. The fluffy upper layer of the resultant pellet was resuspended in 30 ml of 0.25 M sucrose, 20 mM Hepes/Tris (pH 7.2) by 20 strokes of a glass-Teflon homogenizer. The suspension was made 12.5% (v/v) in Percoll (colloidal silica) and mixed by inversion.

The Percoll suspension was centrifuged at 30000 × *g* for 35 min, during which a self-orienting density gradient of Percoll forms. After the centrifugation, two diffuse bands were observed. 7 ml from the middle of the upper band were removed and added to 23.0 ml of 0.25 M sucrose, 20 mM Hepes/Tris (pH 7.2) and 4.3 ml of Percoll. The suspension was mixed by inversion and centrifuged at 30000 × *g* for 35 min. At the end of this centrifugation, a single sharply defined band was observed near the top of the tube. This band was removed in its entirety. The suspension was then diluted with 4 mol. of 100 mM KCl, 100 mM mannitol, 20 mM Hepes/Tris (pH 7.0) and centrifuged for 20 min at 38000 × *g*. The Percoll formed a glass-like pellet with the vesicles above it. The membranes were easily removed by dislodging them with the KCl-containing medium. The membranes were resuspended in the same medium and recentrifuged at 38000 × *g* for 20 min. The resulting membrane pellet was resuspended in 300 mM mannitol, 20 mM Hepes/Tris (pH 7.0) and centrifuged at 38000 × *g* for 20 min. The final pellet contained purified basolateral membranes, which were resuspended in a small volume of 300 mM mannitol, 20 mM Hepes/Tris (pH 7.0).

Enzyme assays

Protein levels in the tissue homogenates and membrane vesicle preparations were assayed by the method of Lowry et al. [3] with bovine serum albumin used as reference. Specific membrane marker enzymes were assayed in homogenates and final preparations in order to determine the extent of enrichment of the desired membrane fractions. These asays also served to indicate the level of cross-contamination by intracellar organelles or cellular membranes.

The activity of leucine aminopeptidase was chosen as the microvillous membrane marker. The assay for this enzyme was achieved by the use of a commercially available procedure (bmc Single Vial LAP, Cat. No. 124869, Biodynamics, Indianapolis, IN 46250, U.S.A.).

The basolateral membrane marker enzyme chosen was $(Na^+ + K^+)$-ATPase (EC 3.6.1.3). The activity of this enzyme was assayed by the method of Scharschmidt et al. [4].

The level of contamination of the final vesicle preparations by cytosolic enzymes was represented by the activity of D-glucose-6-phosphate dehydrogenase (EC 1.1.1.49). This cytosolic enzyme was assayed by a commercially available procedure from Sigma Chemical Company, St. Louis, MO (Sigma No. 345-UV 1982).

The level of lysosomal enzyme and intact lysosome contamination of the final membrane vesicle preparation was indicated by the specific activity of the total acid phosphatase (EC 3.1.3.2). The level of acid phosphatase was colorimetrically determined by commercially available reagent kit (Sigma No. 104 1982). The presence of the intracellular organelle endoplasmic reticulum was determined by assaying for NAD oxidoreductase (EC 1.1.2.3) by the method of Sottocasa et al. [5].

The presence of mitochondria was monitored by assaying the level of succinate cytochrome *c* reductase (EC 1.6.99.3) according to Fleischer and Fleischer [6].

Transport measurements

Uptake of D-[^{3}H]glucose, L-[^{14}C]ascorbic acid or dehydro-L-[^{14}C]ascorbic acid was measured by a filtration technique (nitrocellulose, 0.45 μm, Whatman). 20 μl of basolateral membrane suspension (50–60 mg protein/ml) were incubated in 100 μl of transport buffer of the following composition: 100 mM mannitol, 20 mM Hepes/Tris (pH 7.0), and either 100 mM NaCl or 100 mM KCl.

Aliquots of 20 μl were removed at preselected times and added to 2.0 ml of ice-cold stop solution of the same composition as the incubation buffer except for the absence of substrate. The suspension was placed on a pre-wetted, chilled filter, and drawn through by vacuum. The filter was rinsed with an additional 2.0 ml of ice-cold stop solution. Total filtration time was less than 2 s.

Chemicals

D-[^{3}H]Glucose and L-[^{14}C]ascorbic acid (8.4 mCi/mmol) were obtained from Amersham. Valinomycin was obtained from Sigma Chemical Company. All other chemicals were of reagent grade from commercial sources. Water was deionized and glass-distilled. Dehydro-L-ascorbic acid was freshly prepared for each study by Br_2 oxidation of L-ascorbic acid [7]. All solutions were filtered through 0.22 μm Millipore filters prior to use.

The identity of the radiolabelled substrates in the incubation media was routinely analyzed by liquid chromatography [8].

Results

Evaluation of the isolated renal basolateral membranes

The specific enzyme membrane marker chosen for the basolateral membrane was ($Na^+ + K^+$)-ATPase. The activity of this marker increased 8.7 $\pm$ 0.33-fold ($n = 6$) relative to the crude homogenate (Table I). Leucine aminopeptidase activity decreased to 0.05 of the homogenate activity. The activities of other cellular marker enzymes were also decreased in the final pellet when compared to the starting homogenate.

The membrane preparation was also functionally evaluated to determine whether there was contamination by a microvillous component. If microvillar vesicles were present at a significant level, then Na^+-gradient dependent D-glucose transport would be demonstrated [9]. We saw no concentrative D-glucose uptake in the presence of an inward directed Na^+-gradient and no significant difference in initial rates between Na^+-medium or Na^+-free media (data not shown). Unless the Percoll selectively inactivates Na-dependent transport of contaminating brush-border membranes, it appears that the membranes used currently are of basolateral origin.

Uptake of L-ascorbic acid and dehydro-L-ascorbic acid

The uptake of L-ascorbic acid and dehydro-L-ascorbic acid at 65 μM as a function of time is shown in Fig. 1. There was no concentrative 'overshoot' evident in Na^+-medium for either substrate. Additionally, when the extravesicular medium contained K^+ rather than Na^+, there was no change in the initial rate of transport of either L-ascorbic acid or dehydro-L-ascorbic acid. Similar results were obtained with choline substituting for sodium or mannitol for NaCl. The substrates appeared to reach osmotic equilibrium by 5 min as there was little difference between 2 min and 5 min values. Similar results were observed with guinea pig membranes.

Stability and metabolism of substrates

Chromatographic analysis of the incubation mixture was necessary to determine the identity of the radiolabel. Metabolism of L-ascorbic acid and dehydro-L-ascorbic acid by endogenous membrane-bound enzymes was always a possibility, but

TABLE I

SPECIFIC ACTIVITIES OF MARKER ENZYMES IN STARTING HOMOGENATE AND FINAL MEMBRANE PREPARATION

The homogenate and final membrane preparation was obtained as described in the text. Enzyme specific activities are reported as mU per mg protein. An enzyme milliunit (mU) is defined as the activity of enzyme which converts 1 nmol of substrate in one minute at standard conditions. The values in parentheses represent the specific activity of the final preparation relative to the starting homogenate specific activity (i.e., the enrichment factor). The values represent the means $\pm$ S.E. for six determinations.

	Leucine amino-peptidase	($Na^+ + K^+$) ATPase	Glucose-6-*P* dehydrogenase	Acid phosphatase	NADH oxidoreductase	Cytochrome *c* succinate oxidoreductase
Homogenate	523 $\pm$ 13	60 $\pm$ 8	245 $\pm$ 51	107 $\pm$ 16	160 $\pm$ 11	520 $\pm$ 37
Vesicles	24 $\pm$ 7	522 $\pm$ 19	17 $\pm$ 4	10 $\pm$ 2	29 $\pm$ 4	21 $\pm$ 3
	(0.05)	(8.7)	(0.07)	(0.09)	(0.18)	(0.04)

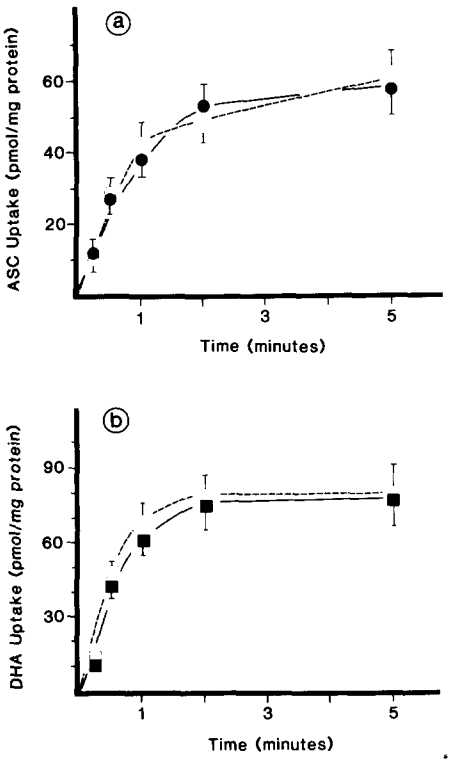

Fig. 1. Uptake of L-ascorbic acid (a) and dehydro-L-ascorbic acid (b) into rat renal basolateral membrane vesicles. Vesicles were pre-equilibrated with 300 mM mannitol, 20 mM Hepes/Tris (pH 7.0), and incubated at 20°C in a solution containing 100 mM NaCl (●, ■) or 100 mM KCl (○, □), 100 mM mannitol, 20 mM Hepes/Tris (pH 7.0) and 65 μM. L-[^{14}C]Ascorbic acid (a) or 65 μM dehydro-L-[^{14}C]ascorbic acid (b). Bars represent the standard error and where not indicated are smaller than the symbol used, in this and subsequent figures.

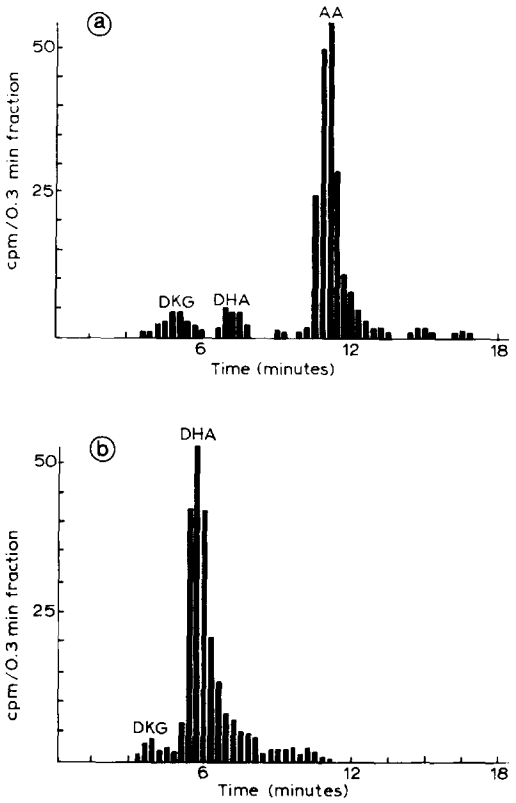

Fig. 2. Total distribution of the ^{14}C radiolabel after 5 min incubation with basolateral membranes at 20°C. Aliquots of incubation medium containing 100 mM NaCl, 100 mM mannitol, 20 mM Hepes/Tris (pH 7.0), basolateral membrane vesicles and 65 μM L-[^{14}C]ascorbic acid (AA) (a) or 65 μM dehydro-L-[^{14}C]ascorbic acid (DHA) (b) were stabilized in 10% metaphosphoric acid. A 20 μl protein-free aliquot of both media was injected into a C_{18} reversed-phase column and eluted by a potassium phosphate buffer (pH 2.3) at 0.5 ml/min. The representative histograms shown above indicate the distribution of the L-[^{14}C]ascorbic acid label (a) or dehydro-L-[^{14}C]ascorbic acid (b) after 5 min incubation at 20°C. DKG, 2,3-diketo-L-gulonic acid.

a more likely occurrence was chemical degradation. L-Ascorbic acid undergoes autooxidation, catalyzed by transition metal ions [10]. Dehydro-L-ascorbic acid is known to be rapidly hydrolyzed to 2,3-diketo-L-gulonic acid at physiological pH and temperatures [11]. Representative studies illustrated in Fig. 2 indicate that neither L-ascorbic acid nor dehydro-L-ascorbic acid undergoes significant metabolism in the presence of membrane vesicles for 5 min. Additionally, only 5.6% of dehydro-L-ascorbic acid is hydrolyzed to 2,3-diketo-L-gulonic acid. It is concluded that there is no significant effect on the experimental results from either metabolism by endogenous enzymes or from chemical degradation of L-ascorbic acid or dehydro-L-ascorbic acid.

Transport of substrate vs. binding

The total uptake of a solute by an isolated membrane system can be due to transport into an intravesicular space and/or binding to membrane surfaces. One way to distinguish between these two phenomena is to analyze the osmotic sensitivity of solute uptake by an isolated membrane

preparation. The amount of solute transported into an internal space should be in direct proportion to the intravesicular volume at equilibrium. The intravesicular volume may be modified by addition of impermeant solutes to the external medium [12]. The results of such a study for L-ascorbic acid and dehydro-L-ascorbic acid uptake in basolateral membranes are illustrated in Fig. 3. It is concluded from the abscissa intercepts that the binding of L-ascorbic acid or dehydro-L-ascorbic acid to membrane sites contributes insignificantly to total uptake of either substrate. Therefore, the total uptake of L-ascorbic acid and dehydro-L-ascorbic acid may be considered to be due to transport into an intravesicular space.

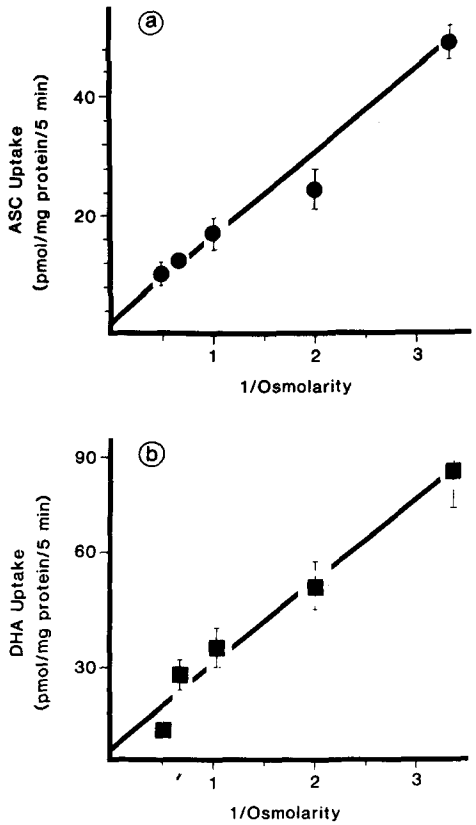

Fig. 3. Uptake of L-ascorbic acid (ASC) (a) and dehydro-L-ascorbic acid (DHA) (b) into rat renal basolateral membrane vesicles as a function of incubation medium osmolarity. The vesicles, pre-equilibrated in 300 mM mannitol, 20 mM Hepes/Tris (pH 7.0) were incubated for 5 min at 20°C in 65 μM L-[^{14}C]ascorbic acid (a) or 65 μM dehydro-L-[^{14}C]ascorbic acid (b), 100 mM NaCl, 100 mM mannitol, 20 mM Hepes/Tris (pH 7.0), and varying concentrations of sucrose.

Kinetic parameters of L-ascorbic acid and dehydro-L-ascorbic acid transport

The effects of increasing substrate concentration on transport of L-ascorbic acid and dehydro-L-ascorbic acid at 15 s are illustrated in Fig. 4. The transport of L-ascorbic acid or dehydro-L-ascorbic acid is seen to be a saturable process. The apparent kinetic constants at 15 s were determined by double-reciprocal plots of the data indicated. The results were $K_m = 649 \pm 35$ μM and $V_{max} = 93.5 \pm 6.8$ pmol $\cdot$ mg$^{-1} \cdot 15$ s^{-1} for the L-ascorbic acid transport system and 147 ± 28 μM and 16.0 ± 1.9 pmol $\cdot$ mg$^{-1} \cdot 15$ s^{-1} for the dehydro-L-ascorbic acid system.

Dehydro-L-ascorbic acid transport in the presence of L-ascorbic acid

Vesicles were incubated in the presence of 1

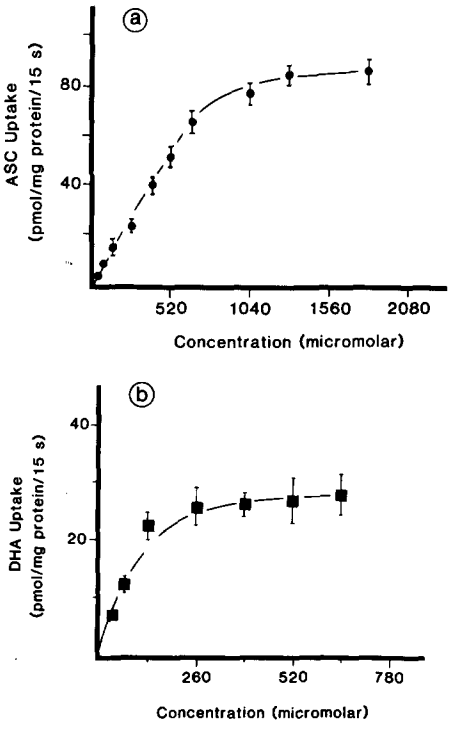

Fig. 4. Uptake of L-ascorbic acid (a) and dehydro-L-ascorbic acid (b) into rat renal basolateral vesicles as a function of the extravesicular substrate concentration. Vesicles pre-equilibrated as Fig. 1 were incubated at 20°C for 15 s in 100 mM NaCl, 100 mM mannitol, 20 mM Hepes/Tris (pH 7.0) and sufficient L-[^{14}C]ascorbic acid or dehydro-L-[^{14}C]ascorbic acid to give the concentrations indicated.

mM L-ascorbic acid to determine whether the reduced form of the vitamin alters uptake of dehydro-L-ascorbic acid present at 65 μM. The results illustrated in Fig. 5 indicate 54% less uptake at 15 s and suggest that L-ascorbic acid inhibits the transport of dehydro-L-ascorbic acid across the basolateral membrane. The effects of increasing L-ascorbic acid concentrations on 15 s uptake values of 65 μM and 130 μM dehydro-L-ascorbic acid into vesicles were analyzed by Dixon plots (Fig. 6). The resultant data gives a K_i value of 2.8 mM and indicates competitive inhibition of dehydro-L-ascorbic acid transport by L-ascorbic acid.

L-Ascorbic acid transport of the presence of D-isoascorbic acid

D-Isoascorbic acid (an epimer of L-ascorbic acid at C-5) has been demonstrated to be an inhibitor of L-ascorbic acid transport in intact surviving intestinal tissue [13] and in the kidney, investigated either in situ [14] or as isolated brush-border membranes [1]. The effect on L-ascorbic acid uptake by D-isoascorbic acid in renal basolateral membranes is shown in Fig. 5b. Uptake at 15 s was reduced by 65%. Dixon plot analysis of D-isoascorbic acid inhibition of L-ascorbic acid uptake during 15 s incubations of vesicles with 65 μM and 130 μM L-ascorbic acid indicates that the competition is competitive and that the K_i of D-isoascorbic acid for L-ascorbic acid transport in this system is 1.97 mM.

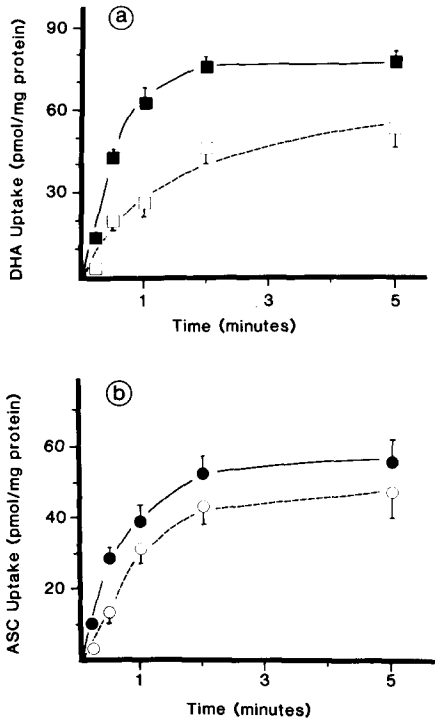

Fig. 5. Uptake rates of dehydro-L-ascorbic acid in the presence of L-ascorbic acid (a), and L-ascorbic acid in the presence of D-isoascorbic acid (b) into rat renal basolateral membrane vesicles. Vesicles were pre-equilibrated as in Fig. 1. Incubation of vesicles was at 20°C in media containing 300 mM mannitol, 20 mM Hepes/Tris (pH 7.0) and 65 μM dehydro-L-[^{14}C]ascorbic acid with (□) or without (■) 1 mM L-ascorbic acid (a) or 65 μM L-[^{14}C]ascorbic acid with (○) or without (●) 1 mM D-isoascorbic acid (b).

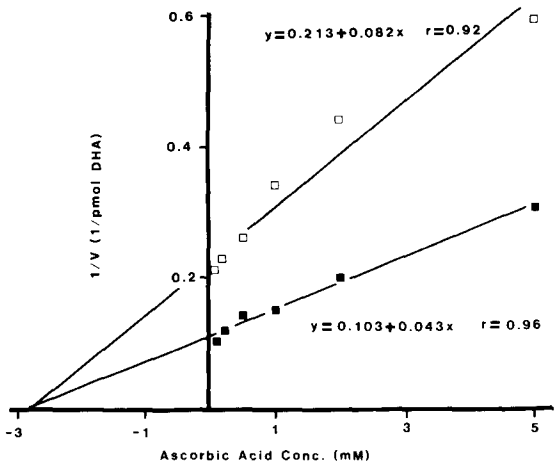

Fig. 6. Single-reciprocal plots of initial velocity (15 s) or dehydro[^{14}C]ascorbic acid uptake at 65 μM (■) and 130 μM (●) with respect to external L-ascorbic acid. $n = 6$.

Trans-stimulation of L-ascorbic acid and dehydro-L-ascorbic acid transport

Isolated basolateral membranes from rat renal cortex were pre-equilibrated with either 300 μM D-isoascorbic acid or 300 μM L-ascorbic acid for transport studies of 65 μM D-[^{14}C]ascorbic acid or 65 μM dehydro-L-[^{14}C]ascorbic acid, respectively. The preloaded vesicles were incubated in mannitol buffers and the resultant data are illustrated in Fig. 7. The 2-fold increase in initial L-ascorbic acid uptake and 3-fold increase in initial dehydro-L-ascorbic acid uptake cannot be ascribed to any ionic coupling effect or to a change in electrical potential as there were no ionic gradients present.

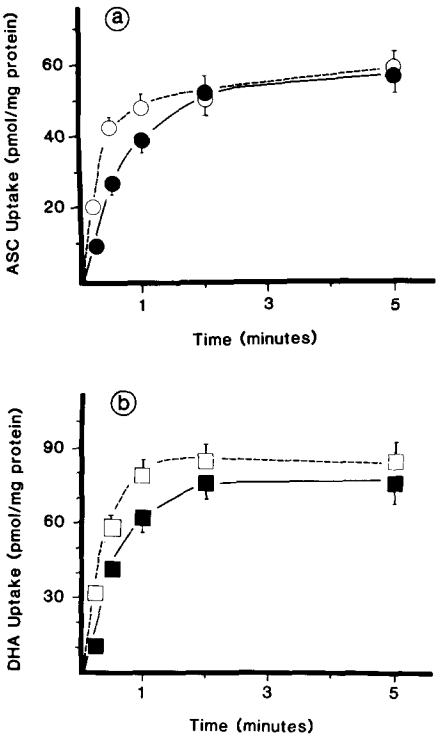

Fig. 7. Trans-stimulation of uptake of L-ascorbic acid (a) and dehydro-L-ascorbic acid (b) into renal cortical basolateral membrane vesicles. Vesicles, pre-equilibrated in 300 mM mannitol, 20 mM Hepes/Tris (pH 7.0), with (○) and without (●) 1 mM D-isoascorbic acid, were incubated at 20°C in 300 mM mannitol, 20 mM Hepes/Tris (pH 7.0), and 65 μM L-[^{14}C]ascorbic acid. Other vesicles, pre-equilibrated in 300 mM mannitol, 20 mM Hepes/Tris (pH 7.0), with (□) and without (■) 1 mM L-ascorbic acid, were incubated at 20°C in 300 mM mannitol, 20 mM Hepes/Tris (pH 7.0), and 65 μM dehydro-L-[^{14}C]ascorbic acid.

The results appear to indicate trans-stimulation of substrate transport in each instance.

The effect of a membrane electrical potential on the transport of L-ascorbic acid and dehydro-L-ascorbic acid

The transport system in the basolateral membrane of the renal proximal tubule for D-glucose is Na-independent, and the same appears to be true for transport of L-ascorbic acid and dehydro-L-ascorbic acid. Therefore, any effect of an electrical gradient on the uptake of D-glucose or dehydro-L-ascorbic acid must be on the transport carrier (mediated through the transport complex) as both these compounds are electrically neutral in solution. However, the transport of L-ascorbic acid may be expected to be potential-sensitive as the bulk of L-ascorbic acid is dissociated at the pH used in this study. When vesicles were incubated at 20°C with substrates and a potassium gradient (out to in) in the presence of valinomycin, the transport of D-glucose (data not shown) and dehydro-L-ascorbic acid were not significantly different from the control condition of no valinomycin. The

TABLE II

THE EFFECT OF AN INWARDLY OR OUTWARDLY DIRECTED POTASSIUM GRADIENT ON THE UPTAKE OF DEHYDRO-L-ASCORBIC ACID OR L-ASCORBIC ACID IN RENAL CORTICAL BASOLATERAL MEMBRANE VESICLES

The vesicles were preequilibrated in 300 mM mannitol, 20 mM Hepes/Tris (pH 7.0) with or without 10 μg valinomycin/mg protein. The vesicles were incubated at 20°C in 100 mM NaCl, 50 mM KCl, 20 mM Hepes/Tris (pH 7.0) and 65 μM dehydro-L-[^{14}C]ascorbic acid or L-[^{14}C]ascorbic acid. Other vesicles were preincubated in 200 mM mannitol, 50 mM KCl, 20 mM Hepes/Tris (pH 7.0) with or without 10 μg valinomycin/mg protein. These vesicles were incubated at 20°C in 100 mM NaCl, 100 mM mannitol, 20 mM Hepes/Tris (pH 7.0) and 65 μM dehydro-L-[^{14}C]ascorbic acid or 65 μM L[^{14}C]ascorbic acid.

Initial external concn. (mM)		Initial internal concn. (mM)	Valinomycin	Expected internal electrical charge	Initial dehydro-L-ascorbic acid rate (pmol/mg per s)	Initial L-ascorbic acid rate (pmol/mg per 15 s)	*n*
NaCl	KCl	KCl					
100	50	0	−		12.9±1.4	9.7±1.2	6
100	50	0	+	+	14.0±2.4	20.5±2.3 *	6
100	8.3	50	−		13.2±2.3	9.0±1.1	6
100	8.3	50	+	−	11.9±2.1	4.8±0.9 *	6

* $P < 0.01$.

presence of the ionophore is expected to result in an exaggerated electrical gradient (inside-positive) due to an enhanced influx of K^+. When L-ascorbic acid was the substrate uptake was doubled at the initial time point (Table II).

When vesicles were pre-equilibrated with potassium and incubated in the presence of dehydro-L-ascorbic acid or L-ascorbic acid with or without valinomycin, only the uptake of L-ascorbic acid was affected (Table II). The presence of valinomycin allows an exaggerated electrical potential (inside-negative) to develop because of enhanced K^+-efflux from the vesicle. This electrical potential manipulation inhibited L-ascorbic acid uptake by 47%.

Discussion

Whereas there have been several reports characterizing the transport of L-ascorbic acid cross the brush-border membrane of the intestine [13,15] and the kidney [1], there have been no reports concerning the transport of L-ascorbic acid or its oxidized form, dehydro-L-ascorbic acid, across the basolateral membranes of these cells. Indeed, the mechanism of dehydro-L-ascorbic acid movement across any membrane has been undecertain. Dehydro-L-ascorbic acid enters several cell types at a higher rate than does L-ascorbic acid [16]. Simple diffusion often has been postulated as the uptake process because dehydro-L-ascorbic acid is electrically neutral in solution at physiological conditions, unlike L-ascorbic acid, and, furthermore, presents an electron-rich area in its structure due to the presence of three vicinal keto-groups.

This report presents characteristics of the transport systems for both reduced and oxidized L-ascorbic acid in the basolateral membrane of rat renal superficial cortex. Uptake of both compounds is postulated to be by facilitated diffusion mechanisms. The driving force for net transport in this system is the concentration gradient of the two substrates. The rate of transport is unaffected by a sodium gradient (out to in) as there is no significant effect on the initial rates of uptake for either L-ascorbic acid or dehydro-L-ascorbic acid (Fig. 1). This lack of sodium dependency suggests that there is little contamination of our basolateral preparation by a microvillous membrane, as L-ascorbic acid transport has previously been demonstrated to be mediated by a sodium-dependent mechanism in the renal brush-border membrane [1]. Further evidence of a lack of brush-border material is provided by the fact that D-glucose uptake shows no response to a sodium gradient in our control studies.

The transport processes of L-ascorbic acid and dehydro-L-ascorbic acid are saturable, as demonstrated by the data in Fig. 3. It is recognized that the determination of kinetic parameters of solute transport into a vesiculated membrane system must satisfy some important considerations. Unidirectional flux must be observed rather than net transport, which presents a more complex situation. The intravesicular concentration of the substrate must be small as trans-effects cannot be predicted. Some substrates may exhibit different modifying effects at the cis- and trans-sides of the membrane [17] or may modify transport at only one side of the membrane [18]. The ideal situation is the determination of initial rates. In addition, the driving force should be maintained at a constant level. It therefore follows that the incubation times must be kept as short as possible. In the present study, time points shorter than 15 s gave radioactivity levels too small to yield reliable results. This may be due to both a low system capacity for the ligands and a low specific activity of the radiolabel. The use of 15 s incubations appears to give reasonable values for the apparent kinetic parameters. Since L-ascorbic acid is oxidized to dehydro-L-ascorbic acid, and dehydro-L-ascorbic acid is hydrolyzed to 2,3-diketo-L-gulonic acid, the identity of the radiolabel must be considered in these transport studies. Analysis of L-[^{14}C]ascorbic acid and dehydro-L-[^{14}C]ascorbic acid samples by liquid chromatography after 5 min incubation in the presence of vesicles showed insignificant levels of metabolism or degradation.

Competition between structurally related analogs is observed at the cis-side of the membrane (Fig. 4), whereas these same analogs may induce trans-stimulation under appropriate conditions (Fig. 5). Transport of L-ascorbic acid and dehydro-L-ascorbic acid by facilitated diffusion mechanisms is indicated by saturation kinetics, cis-inhibition and trans-stimulation.

The transport system for dehydro-L-ascorbic

acid is insensitive to an electrical potential (Fig. 7a). Perhaps due to the dissociation of ascorbic acid in solution at physiological pH, its uptake rate does respond to changes in membrane potential (Fig. 7b).

Dehydro-L-ascorbic acid has been reported to be cytotoxic and has been demonstrated to increase cellular permeability to D-mannitol [19]. The interpretation of Fig. 4 might then be equivocal; apparent saturation of uptake at levels of dehydro-L-ascorbic acid above 200 μM might be attributed to permeability alterations induced by the substrate. We have, however, confirmed that short (15 s) incubations of vesicles at dehydro-L-ascorbic acid concentrations at high as 700 μM do not induce an increase in mannitol permeation at 15 s incubations (data not shown).

Previous investigations have demonstrated an effect of D-glucose on both L-ascorbic acid and dehydro-L-ascorbic acid transport in cell membranes of non-polar cells [16,20]. Other investigators have demonstrated that there is no direct inhibition of L-ascorbic acid transport in brush-border membranes [1,19], nor have we observed, in preliminary studies, any inhibition by D-glucose in our preparations of renal basolateral membranes with regard to L-ascorbic acid or dehydro-L-ascorbic acid transport.

Until an inhibitor specific for L-ascorbic acid or dehydro-L-ascorbic acid is identified, the overlapping characteristics of the two transport systems will not allow elucidation of possible pathway multiplicity. This study establishes that the mechanism(s) by which L-ascorbic acid and dehydro-L-ascorbic acid are transported is one of facilitated diffusion.

The overall aspect of the vectorial transport of L-ascorbic acid and dehydro-L-ascorbic acid in vivo cannot be complete until the mechanisms of transport at both poles of renal reabsorptive cells are established and the possibility of interconversion of the two molecules in vivo is explored. Oxidation of L-ascorbic acid does not appear to occur in intact surviving renal slices or isolated tubules but reduction of dehydro-L-ascorbic acid is brought about in the kidneys of rat and guinea pig (unpublished observations). Dehydro-L-ascorbic acid is transported at the renal brush-border by a facilitated diffusional process that is Na^+-independent [21]. It has been previously established that L-ascorbic acid is co-transported with sodium at the brush-border [1].

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