Biochimica et Biophysica Acta, 685 (1982) 71–77
Elsevier Biomedical Press

BBA 71055

Na^+-INDEPENDENT L-ARGININE TRANSPORT IN RABBIT RENAL BRUSH BORDER MEMBRANE VESICLES

MARC R. HAMMERMAN *

Renal and Metabolism Divisions, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO 63110 and Laboratory of Molecular Aging, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore City Hospital, Baltimore, MD 21224 (U.S.A.)

(Received August 31st, 1981)

Key words: Brush border vesicle; Amino acid transport; L-Arginine; Na^+-independence; (Rabbit kidney)

Na^+-independent L-arginine uptake was studied in rabbit renal brush border membrane vesicles. The finding that steady-state uptake of L-arginine decreased with increasing extravesicular osmolality and the demonstration of accelerative exchange diffusion after preincubation of vesicles with L-arginine, but not D-arginine, indicated that the uptake of L-arginine in brush border vesicles was reflective of carrier-mediated transport into an intravesicular space. Accelerative exchange diffusion of L-arginine was demonstrated in vesicles preincubated with L-lysine and L-ornithine, but not L-alanine or L-proline, suggesting the presence of a dibasic amino acid transporter in the renal brush border membrane. Partial saturation of initial rates of L-arginine transport was found with extravesicular [arginine] varied from 0.005 to 1.0 mM. L-Arginine uptake was inhibited by extravesicular dibasic amino acids unlike the Na^+-independent uptake of L-alanine, L-glutamate, glycine or L-proline in the presence of extravesicular amino acids of similar structure. L-Arginine uptake was increased by the imposition of an H^+ gradient (intravesicular pH < extravesicular pH) and H^+ gradient stimulated uptake was further increased by FCCP. These findings demonstrate membrane-potential-sensitive, Na^+-independent transport of L-arginine in brush border membrane vesicles which differs from Na^+-independent uptake of neutral and acidic amino acids. Na^+-independent dibasic amino acid transport in membrane vesicles is likely reflective of Na^+-independent transport of dibasic amino acids across the renal brush border membrane.

Introduction

Studies of dibasic amino acid reabsorption performed using renal cortical slices established that active transport of dibasic amino acids, unlike transport of other amino acids, could occur despite a lack of Na^+ in the incubation medium [1,2]. In contrast, studies which utilized micropuncture techniques demonstrated definite Na^+-dependency for dibasic amino acid transport in the isolated renal tubule [3]. It has been suggested that the seeming incongruity of these observations was due to the fact that the studies of amino acid transport in renal cortical slices and isolated tubules measured transport across both the brush border and basal-lateral membranes of the renal tubular cell. Thus, differences in characteristics of dibasic amino acid transport across the two mem-

* Address for correspondence: Department of Internal Medicine, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, U.S.A.

Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid.

branes, such as a difference in requirement for Na^+, could result in variable findings when transport across both membranes was studied utilizing different techniques [4,5].

In order to characterize the transport of dibasic amino acids across the renal brush border membrane in the absence of the basal-lateral membrane, the transport of L-arginine was studied in membrane vesicles isolated from the renal brush border. The presence of both Na^+-independent and Na^+-dependent uptake of L-arginine was established in one study [5]. The specificity of L-arginine transport systems for dibasic amino acids was demonstrated in this study by experiments which showed selective inhibition of initial uptakes of extravesicular L-[^{14}C]arginine by extravesicular non-radioactively labelled dibasic amino acids. The interpretation of these findings was somewhat clouded by the acknowledged possibility that L-arginine bound to membrane vesicles at low substrate concentrations (0.1 mM). Experiments which might have provided evidence that L-arginine uptake at substrate concentrations below 0.1 mM consisted of transport of L-arginine into an intravesicular space and which might have provided a more definitive demonstration of L-arginine transporter specificity for dibasic amino acids, such as demonstration of accelerative exchange of L-[^{14}C]arginine for intravesicular L-arginine and other dibasic amino acids, were not reported.

A second study of L-arginine uptake into renal brush border membrane vesicles confirmed the presence of Na^+-independent and Na^+-dependent uptake of L-arginine. Significant binding of L-arginine to membrane vesicles was demonstrated in the absence of Na^+, and consequently only Na^+-dependent L-arginine transport in membrane vesicles was characterized in detail [6].

Our initial report of Na^+-independent L-arginine uptake in renal brush border membrane vesicles reported an equilibrated uptake of 0.1 mM L-arginine which was exactly what would have been expected for the intravesicular accumulation of non-bound solute [7–9]. The present studies are reported, therefore, to readdress the issue of L-arginine binding to brush border membrane vesicles in the absence of Na^+. In addition they further characterize Na^+-independent L-arginine transport in brush border vesicles and distinguish it from the uptakes of other types of amino acids in the absence of Na^+.

Materials and Methods

Preparation of brush border membrane vesicles. Rabbit renal brush border membrane vesicles were isolated as described previously [8,10–13]. Following each of the last three centrifugations the membranes were suspended in a solution consisting of 300 mM mannitol, 1 mM Hepes-Tris (1 mM Hepes adjusted with Tris hydroxide) or other solutions as indicated in the text. The quality of the preparations was randomly evaluated by specific enzyme markers [9]. Enrichment of purified membrane vesicles compared to renal cortex homogenate was 12–18-fold for trehalase and γ-glutamyltransferase.

Measurement of amino-acid transport. Uptake of amino acids was measured by a Millipore filtration technique which has been previously described in detail [8,11–13]. In the present study 10 μl of membrane suspension were warmed for 1 min at 20°C and incubation at 20°C was initiated by the addition of 40 μl of 300 mM mannitol, 1 mM Hepes-Tris (pH 7.5) or other solutions as indicated in the text, which contained L-[^{3}H]arginine or other radioactively labelled amino acid and additional constituents as indicated. The additions replaced mannitol isosmotically. Incubations were terminated and the filters washed with 154 mM NaCl/1 mM Hepes-Tris (pH 7.5). Values for nonspecific retention of radioactivity on the filters (less than 0.04% of the total radioactivity in the incubation mixture) were subtracted from the values of the incubated samples. All incubations were carried out in triplicate with freshly prepared brush border membrane vesicles. Each experiment was performed on at least three separate occasions with different membrane preparations.

Protein was assayed by the method of Lowry et al. [14] using bovine serum albumin as the standard.

Initial rates of amino acid uptake were measured after 30 s of incubation and varied linearly with brush border membrane protein concentrations over the range used in experiments.

Differences between means in Table I and II were analyzed using Dunnett's multiple comparison procedure [15].

Identification and recovery of amino acids transported in brush border vesicles. It has been previously demonstrated that L-alanine, L-glutamate and L-proline are not metabolized by brush border membrane vesicles [12,13,16]. Following incubation for 30 s and 60 min with L-[^{3}H]arginine or [^{3}H]glycine, membrane vesicles were retained on Millipore filters and amino acids were extracted as described elsewhere [13]. The extracts were chromatographed on thin-layer cellulose plates with solvent systems consisting of isopropanol/formic acid/water (40:2:10, v/v). Greater than 90% of the radioactivities taken up by membrane vesicles had R_F values identical with authentic L-arginine or glycine after both 30 s and 60 min of incubation. No other discreet radioactive spots were detected. These findings indicate that neither L-arginine nor glycine was metabolized by the membrane vesicles.

Materials. L-[^{3}H]Alanine (13.2 Ci/mmol), L-[^{3}H]arginine (21.6 Ci/mmol), L-[^{3}H]glutamate (24.1 Ci/mmol), [^{3}H]glycine (9.39 Ci/mmol), L-[^{3}H]proline (29.6 Ci/mmol) and D-[^{3}H]glucose (18.1 Ci/mmol) were obtained from New England Nuclear. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was obtained from Pierce Chemical Co. Valinomycin was obtained from Sigma Chemical Co. Other chemicals were of the highest purity available from commercial sources. All solutions were filtered through 0.45 μm Millipore filters prior to use [17].

Results

Na^+-independent transport of L-arginine in brush border membrane vesicles

The uptake of 0.1 mM L-[^{3}H]arginine in brush border membrane vesicles was determined as a function of time (Fig. 1). A steady state of L-arginine uptake was reached by 60 min of incubation. The uptake at steady state was 107 pmol L-[^{3}H]arginine per mg membrane protein. The Na^+-independent steady-state uptake of 0.1 mM D-[^{3}H]glucose in brush border membrane vesicles was 98 pmol D-glucose per mg membrane protein (not illustrated) consistent with the findings of others [8]. Thus, the uptake of 0.1 mM L-[^{3}H]arginine at steady-state approximated the uptake of 0.1 mM D-[^{3}H]glucose, a solute believed not to bind to membrane vesicles [8,9].

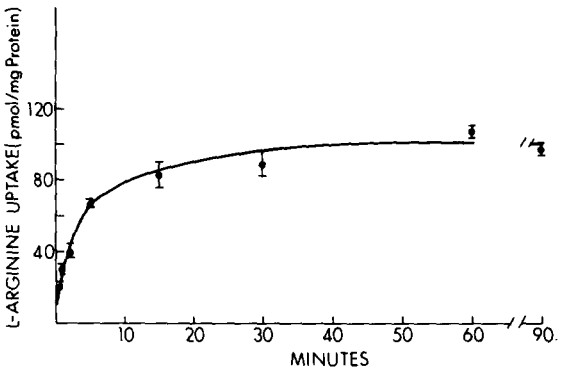

Fig. 1. The uptake of 0.1 mM L-arginine in renal brush border membrane vesicles as a function of time. Uptake was measured in the absence of Na^+. Data are expressed as mean ± S.E.

The effect of extravesicular osmolality on L-[^{3}H]arginine uptake at steady-state (60 min) was determined by incubating brush border membrane vesicles in sucrose solutions of varying osmolalities so as to decrease intravesicular space (Fig. 2) [9]. The uptake of L-[^{3}H]arginine was inversely proportional to extravesicular osmolality from 300 to 860 mM. Extrapolation to infinite extravesicular osmolality (zero intravesicular space) estimated 10% residual L-[^{3}H]arginine uptake. Thus, 90% of L-[^{3}H]arginine uptake at steady state could be

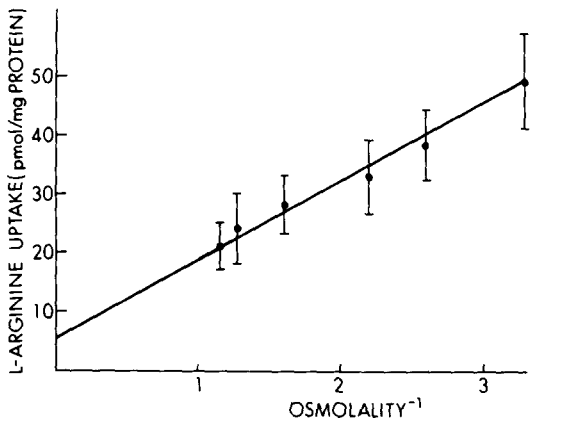

Fig. 2. The effect of increasing extravesicular osmolality on the steady state uptake of 0.05 mM L-[^{3}H]arginine in brush border membrane vesicles. The extravesicular osmolality was adjusted with sucrose. Data are expressed as mean ± S.E. The solid line was calculated from all experimental points using the least-squares method (regression coefficient = 0.93).

TABLE I

THE EFFECT OF INTRAVESICULAR AMINO ACIDS ON THE UPTAKE OF L-[^{3}H]ARGININE IN BRUSH BORDER MEMBRANE VESICLES

Renal brush border membranes were suspended in 300 mM mannitol, 1 mM Hepes-Tris with 0.02 mM non-radioactive amino acid, so as to allow transport on non-radioactive amino acid into the vesicles. Initial rates of 0.01 mM L-[^{3}H]arginine transport and steady-state (60 min) L-[^{3}H]arginine uptakes were measured. The initial rate of L-[^{3}H]arginine uptake in the absence of intravesicular amino acid was considered the control value (3.9±0.7 pmol L-arginine per mg protein per 30 s). Data are expressed as mean ± S.E. Differences between means were analyzed using Dunnett's multiple comparison procedure.

Intravesicular amino acid	Initial rate of L-[^{3}H]arginine uptake (% change from control)	Steady-state L-[^{3}H]arginine uptake (pmol L-arginine per mg protein)
None	0	14.7±2.0
L-Arginine	45.2±6.3 [a]	12.2±4.2
D-Arginine	2.1±4.5	15.6±3.4
L-Lysine	39.1±6.7 [a]	11.2±5.6
L-Ornithine	36.7±6.0 [a]	16.7±2.4
L-Alanine	1.8±5.6	13.6±3.2
L-Proline	3.4±5.2	16.6±4.5

[a] Different from control, $P<0.01$

accounted for by intravesicular accumulation of L-arginine.

Additional evidence that L-arginine uptake in renal brush border membrane vesicles represented transport into an intravesicular space rather than binding to membranes was obtained from experiments measuring accelerative exchange diffusion (Table I). Brush border membrane vesicles were suspended during the final three centrifugations of the purification procedure in 300 mM mannitol 1 mM Hepes-Tris solutions which contained 0.02 mM concentrations of non-radioactively labelled L-arginine D-arginine, L-lysine, L-ornithine, L-alanine, L-proline or no addition (preincubation). In this manner the non-radiolabelled amino acids were transported into the intravesicular space. The initial rates of uptake of 0.01 mM L-[^{3}H]arginine were then measured in the membrane vesicles which had been preincubated with non-radioactive amino acids and in vesicles which were preincubated in mannitol solution alone. There was a significant stimulation of the initial rate of L-[^{3}H]arginine uptake over that measured in the absence of preincubation with amino acid when the brush border membrane vesicles were preincubated with L-arginine, L-lysine, or L-ornithine, but not D-arginine or L-alanine or L-proline. This observation, consistent with a demonstration of accelerative exchange diffusion, provides evidence that at least the difference between the L-[^{3}H]arginine uptake measured in the absence of a preincubation and that measured following a L-arginine preincubation is representative of L-arginine transport into an intravesicular space [18]. In addition, the finding that the accelerative exchange of L-[^{3}H]arginine was stimulated by intravesicular L-lysine and L-ornithine, but not L-alanine or L-proline, is consistent with the pres-

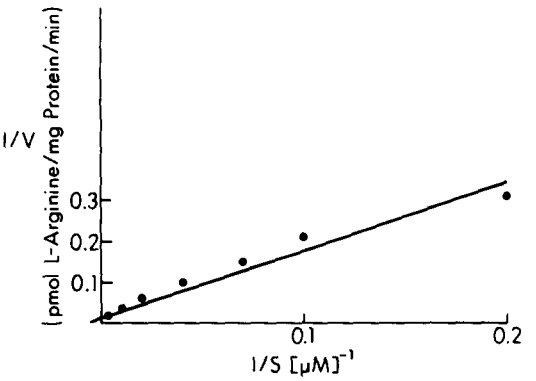

Fig. 3. The relationship between L-arginine concentration and initial rates of uptake. Data are the means of five separate experiments. The solid line was calculated from all experimental points using the least-squares method (regression coefficient =0.97).

ence of a transport system specific for dibasic amino acids in the renal brush border membrane vesicles [19].

The relationship between L-arginine concentration and initial rate of L-arginine uptake was non-linear over the range of 0.005 mM to 1.0 mM L-arginine, providing evidence for saturability of transport. A Lineweaver-Burk analysis of the concentration dependence (Fig. 3) revealed an apparent K_m of 161 μM and apparent V of 78 pmol L-arginine per mg protein per min.

Effect of extravesicular amino acids on the initial rate of radioactively labelled amino acid uptake

Table II shows the effects of different amino acids (5.0 mM) added extravesicularly to brush border vesicles, on the initial rates (30 s) of 0.025 mM L-[^{3}H]alanine, L-[^{3}H]arginine, L-[^{3}H]-glutamate, [^{3}H]glycine, and L-[^{3}H]proline uptake. All uptakes were measured in mannitol media as described in Materials and Methods. When amino-acid uptake was measured in the presence of an initial Na^+ gradient (extravesicular Na^+ > intravesicular Na^+) it was possible to demonstrate maximal inhibition of initial rates of radioactively labelled amino acid uptake by amino acids of similar structure [12,13,16]. For example, the uptake of 0.025 mM L-[^{3}H]proline was inhibited most by 5.0 mM L-proline and L-hydroxyproline [13]. In the present experiments, uptake of L-[^{3}H]arginine was clearly more selectively inhibited by amino acids of a similar structure (dibasic amino acids) than was the uptake of other amino acids.

Effect of a transient H^+ gradient on L-arginine uptake

Brush border membrane vesicles were suspended in solutions containing 225 mM mannitol/50 mM Mes-Tris (pH 5.5) or 200 Mm mannitol/50 mM Hepes-Tris (pH 7.5) during the final three centrifugations of the purification procedure. The uptake of 0.025 mM L-[^{3}H]arginine in 200 mM mannitol/50 mM Hepes-Tris (pH 7.5) (vesicles suspended in pH 7.5 solution; no H^+ gradient) or 205 mM mannitol/12.5 mM Mes/50 mM Hepes-Tris (pH 7.3) (vesicles suspended in pH 5.5 solution; H^+ gradient) was measured in the presence

TABLE II

THE EFFECT OF EXTRAVESICULAR AMINO ACIDS ON THE INITIAL RATE OF RADIOACTIVELY LABELLED AMINO ACID UPTAKE

The effect of 5.0 mM extravesicular non-radioactively labelled amino acid on the initial rate of uptake of 0.025 mM labelled amino acids was determined in the absence of Na^+. The uptake of 0.025 mM labelled amino acid in the absence of extravesicular non-labelled amino acid was designated 100% uptake. Data are expressed as mean ± S.E.

	Relative rate of labelled amino acid uptake (%)				
Extravesicular amino acid	L-[^{3}H]Alanine	L-[^{3}H]Arginine	L-[^{3}H]Glutamate	[^{3}H]Glycine	L-[^{3}H]Proline
None	100	100	100	100	100
L-Alanine	81 ± 4	92 ± 6	87 ± 20	84 ± 11	84 ± 12
L-Leucine	86 ± 6	95 ± 8	88 ± 6	99 ± 8	95 ± 3
L-Arginine	98 ± 3	39 ± 2	130 ± 7	96 ± 5	81 ± 10
L-Lysine	99 ± 3	41 ± 8	162 ± 8	99 ± 9	82 ± 2
L-Ornithine		36 ± 7			
L-Glutamate	106 ± 9	91 ± 2	100 ± 11	73 ± 2	107 ± 18
L-Aspartate	98 ± 8	96 ± 8	74 ± 12	67 ± 3	109 ± 12
Glycine	100 ± 3	92 ± 6	92 ± 6	74 ± 3	94 ± 15
L-Proline	106 ± 1	102 ± 7	113 ± 3	82 ± 11	115 ± 11
L-Hydroxyproline	101 ± 10	107 ± 8	95 ± 6	107 ± 11	104 ± 9

TABLE III

THE EFFECT OF A H^+ GRADIENT ON L-ARGININE UPTAKE

Brush border membrane vesicles were suspended in solution containing 225 mM mannitol/50 mM Mes-Tris (pH 5.5) or 200 mM mannitol/50 mM Hepes-Tris (pH 7.5) during the final three centrifugations of the purification procedure and 0.025 L-[^{3}H]arginine uptake was measured in the presence and absence of an H^+ gradient ±FCCP as described in the text. FCCP was added to brush border membrane vesicles in 95% ethanol. Ethanol alone was added to vesicles in experiments where FCCP was not utilized. Data are expressed as mean ±S.E. Differences between means were analyzed using Dunnett's multiple comparison procedure.

Experimental conditions	Initial rate of L-arginine uptake (pmol L-arginine per mg protein per 30 s)	Steady-state L-arginine uptake (pmol L-arginine per mg protein)
No H^+ gradient	4.3±0.3	33.0±4.5
No H^+ gradient + FCCP	4.8±0.5	34.3±6.1
H^+ gradient	6.4±0.5 [a]	43.3±3.8
H^+ gradient + FCCP	8.7±0.9 [b]	39.3±2.3

[a] H^+ gradient > no H^+ gradient, $P<0.05$.
[b] H^+ gradient + FCCP > H^+ gradient, $P<0.05$.

and absence of 0.01 mM FCCP. FCCP is known to facilitate the movement of H^+ across membranes [20,21]. The initial rate of L-[^{3}H]arginine uptake was significantly increased by the imposition of an H^+ gradient (intravesicular pH < extravesicular pH) and further increased by the addition of FCCP (Table III).

Discussion

The present studies demonstrate Na^+-independent transport of L-arginine in rabbit renal brush border membrane vesicles. Though some binding of L-arginine to membrane vesicles may occur, the data strongly suggest that the bulk of L-arginine uptake represents transport into an intravesicular space. Thus: (a) the steady-state uptake of L-arginine was comparable to that of D-glucose, a solute which does not bind to brush border membrane vesicles; (b) the steady-state uptake of L-arginine decreased with increasing extravesicular osmolality; and (c) accelerative exchange diffusion was demonstrated after preincubation of membrane vesicles with L-arginine, but not D-arginine.

These studies have confirmed that finding that Na^+-independent L-arginine transport can be accelerated by induction of a lumen-negative membrane potential. The lumen-negative potential was induced by H^+ efflux in the face of an H^+ gradient (intravesicular pH < extravesicular pH) and luminal electronegativity (H^+ efflux) was increased by FCCP [5,6]. L-arginine uptake was enhanced by the H^+ gradient and further enhanced by FCCP.

Sodium inhibition of L-arginine transport in brush border membrane vesicles has been reported in the absence of experimental conditions which increase luminal electronegativity [5–7]. The present findings are consistent with the proposal that such inhibition is due to the induction of a lumen-positive membrane potential by Na^+ rather than to inhibition of L-arginine binding [5].

Previous studies of Na^+-independent and Na^+-dependent L-arginine transport in brush border vesicles utilized inhibition of radioactively labelled L-arginine transport by extravesicular nonlabelled amino acids to argue for dibasic amino acid specificity of the L-arginine transport system. Although selective inhibition of L-arginine transport by extravesicular dibasic amino acids provides a strong argument for commonality of transport systems, such inhibition could result from selective dissipation of the membrane potential (inside negative) by positively charged dibasic amino acids or by interference with L-arginine interaction with the membrane transport system by a mechanism other than competition for a common transporter. Thus inhibition of L-arginine uptake by extravesicular dibasic amino acids need not imply that the L-arginine transport system is shared by other dibasic amino acids.

The present studies demonstrate that L-lysine

and L-ornithine, but not L-alanine or L-proline, elicit accelerative exchange of L-[^{3}H]arginine. These findings provide strong evidence that the Na^+-independent L-arginine transport system in the brush border membrane is shared by other dibasic amino acids.

Na^+-independent uptake of L-arginine in brush border membrane vesicles differs from Na^+-independent uptake of L-alanine, L-glutamate, and L-proline in that: (a) initial rates of L-arginine uptake measured over a wide range of L-arginine concentrations exhibit partial saturability; and (b) the uptake of L-[^{3}H]arginine is inhibited by extravesicular non-labelled dibasic amino acids. Thus Na^+-independent L-arginine transport shares characteristics with Na^+-dependent neutral and acidic amino acid and imino acid transport [12,13,16].

Na^+-independent transport of L-arginine in brush border vesicles is likely reflective of Na^+-independent transport of L-arginine across the luminal membrane of the renal tubular cell. The finding of a characteristic Na^+-independent transport of dibasic amino acids in preparations such as renal cortical slices probably reflects, at least in part, a characteristic Na^+-independent transport of dibasic amino acids across the brush border membrane.

Schultz et al. have suggested a model for dibasic amino acid transport across the luminal surface of the rabbit ileum in which the positively charged terminal amino group of a cationic amino acid substituted for Na^+ in the binding of the amino acid to its cell surface receptor prior to transport [22]. Thus, as first suggested by Fox et al. [1] the terminal cationic moiety of the dibasic amino acid was proposed to satisfy the requirement for transport that Na^+ satisfied for neutral and acidic amino acids. It is possible that the similarities between Na^+-dependent transport of neutral and acidic amino acids in rabbit renal brush border vesicles and Na^+-independent transport of the dibasic amino acid L-arginine can be explained by this model. This being the case, Na^+-dependent and Na^+-independent transports of L-arginine in membrane vesicles would be accomplished by a single transport system for dibasic amino acids. Na^+-independent transport of L-arginine in isolated membrane vesicles and across the luminal membrane of the renal tubular cell in vivo may, therefore, reflect the interaction of a positively charged amino acid with a membrane transport system the requirements of which for a positively charged moiety can be satisfied in this manner or by Na^+.

Acknowledgements

The technical assistance of Mrs. Naomi Jones and the typing skills of Ms. Lynn Wesselmann are gratefully acknowledged. These studies were supported in part by USPHS Grant 1 R01 AM27600 from the National Institute of Arthritis, Metabolism and Digestive Diseases.

References

1 Fox, M., Thier, S., Rosenberg, L. and Segal, S. (1964) Biochim. Biophys. Acta 79, 167–176
2 Segal, S., Schwartzman, L., Blair, A. and Bertoli, D. (1967) Biochim. Biophys. Acta 135, 127–135
3 Ullrich, K.J., Rumrich, G. and Kloss, S. (1974) Pflugers Arch. 351, 49–60
4 Segal, S., McNamara, P.D. and Pepe, L.M. (1977) Science 197, 169–171
5 Busse, D. (1978) Arch. Biochem. Biophys. 191, 551–560
6 Hilden, S.A. and Sacktor, B. (1981) Arch. Biochem. Biophys. 210, 289–297
7 Hammerman, M.R. (1975) Fed. Proc. 4, 309
8 Aronson, P.S. and Sacktor, B. (1975) J. Biol. Chem. 250, 6031–6039
9 Sacktor, B. (1977) Curr. Topics Bioenerg. 6, 39–81
10 Berger, S.J. and Sacktor, B. (1970) J. Cell. Biol. 47, 637–645
11 Hammerman, M. and Sacktor, B. (1978) Biochim. Biophys. Acta 509, 338–347
12 Fass, S.J., Hammerman, M.R. and Sacktor, B. (1977) J. Biol. Chem. 252, 583–590
13 Hammerman, M.R. and Sacktor, B. (1977) J. Biol. Chem. 252, 591–595
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
15 Dunnett, C.W. (1964) Biometrics 20, 482–491
16 Schneider, E.G., Hammerman, M.R. and Sacktor, B. (1980) J. Biol. Chem. 255, 7650–7655
17 Mitchell, M.E., Aronson, P.S. and Sacktor, B. 91974) J. Biol. Chem. 249, 6971–6975
18 Rosenberg, T. and Wilbrandt, W. (1957) J. Gen. Physiol. 41, 289–296
19 Schwartzman, L., Blair, A. and Segal, S. (1967) Biochim. Biophys. Acta 135, 120–126
20 Henderson, P.J.F., McGiven, J.D. and Chappell, J.B. (1969) Biochem. J. 111, 521
21 Hopfer, U., Lehninger, A.L. and Thompson, T.E. (1968) Proc. Natl. Acad. Sci. USA 59, 484–490
22 Schultz, S.G., Yu-tu, L., Alvarez, O.O. and Curran, P.F. (1970) J. Gen. Physiol. 56, 621–639