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Sodium Gradient- and Sodium Plus Potassium Gradient-Dependent L-Glutamate Uptake in Renal Basolateral Membrane Vesicles

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Summary. A membrane preparation enriched in the basolateral segment of the plasma membrane was isolated from the rat renal cortex by a procedure that included separation of particulates on a self-generating Percoll gradient. The uptake of L-glutamate by the basolateral membrane vesicles was studied. A Na^+ gradient ($[Na^+]_o > [Na^+]_i$) stimulated the uptake of L-glutamate and provided the driving force for the uphill transport of the acidic amino acid, suggesting a Na^+-L-glutamate cotransport system in the basolateral membrane. A K^+ gradient ($[K^+]_i > [K^+]_o$) increased the uptake additionally. This effect was specific for $K^+(Rb^+)$. The action of the K^+ gradient in enhancing the uptake of L-glutamate had an absolute requirement for Na^+. In the presence of Na^+, but in the absence of a Na^+ gradient. i.e., $[Na^+]_o = [Na^+]_i$, the K^+ gradient also energized the concentrative uptake of L-glutamate. This effect of the K^+ gradient was not attributable to an alteration in membrane potential. The finding of a concentrative uptake system for L-glutamate energized by both Na^+ ($[Na^+]_o > [Na^+]_i$ and K^+ ($[K^+]_i > [K^+]_o$) gradients in the basolateral membrane, combined with previous reports of an ion gradient-dependent uphill transport system for this amino acid in the brush border membrane, suggests a mechanism by which L-glutamate is accumulated intracellularly in the renal proximal tubule to extraordinarily high concentrations.

Renal proximal tubules accumulate L-glutamate (L-aspartate) to impressive levels, the intracellular concentrations of the dicarboxylic amino acids reaching more than 20 times their respective plasma concentrations [4, 5]. The presence in the kidney of a specific acidic amino acid transport system is indicated from physiological studies of renal clearance and uptakes of different classes of amino acids in cortical slices and isolated renal cells [11, 33, 34, 38, 41]. Strong genetic support for this suggestion has come from reports of two cases of human dicarboxylic aminoaciduria [18, 36]. Studies of transtubular rates of flux in microperfused tubules have demonstrated that dicarboxylic amino acid transport is Na^+ dependent [37].

The accumulation of L-glutamate by the polar tubular cell may be mediated by uptake across the luminal (brush border) membrane, the basolateral membrane, or both. Recently, the uptake of L-glutamate by isolated brush border membrane vesicles has been reported [26, 27, 31, 39]. The transport is specific for acidic amino acids, concentrative, and energized by a Na^+ gradient (extravesicular > intravesicular). It has also been found that the uptake of L-glutamate can be increased additionally by the presence of an intravesicular > extravesicular K^+ gradient [6, 28, 30, 32]. In this paper, we now report the uphill uptake of L-glutamate across the basolateral membrane. The transport is energized by both the Na^+ (extravesicular > intravesicular) and K^+ (intravesicular > extravesicular) gradients. Thus, the uptake of the acidic amino acid by basolateral membrane vesicles may be unique, in that previous studies of the uptake of amino acids (L-phenylalanine, L-proline) by this renal membrane have shown that the transports are neither concentrative nor Na^+ gradient dependent [8, 35]. The present finding of ion gradient-dependent L-glutamate systems at both the basolateral and luminal segments of the plasma membrane suggests a mechanism by which this important metabolite is accumulated intracellularly to extremely high levels.

Materials and Methods

Preparation of Basolateral Membranes

The kidneys from two male Sprague-Dawley rats (200–300 g) were used for each preparation of basolateral membranes. The excised

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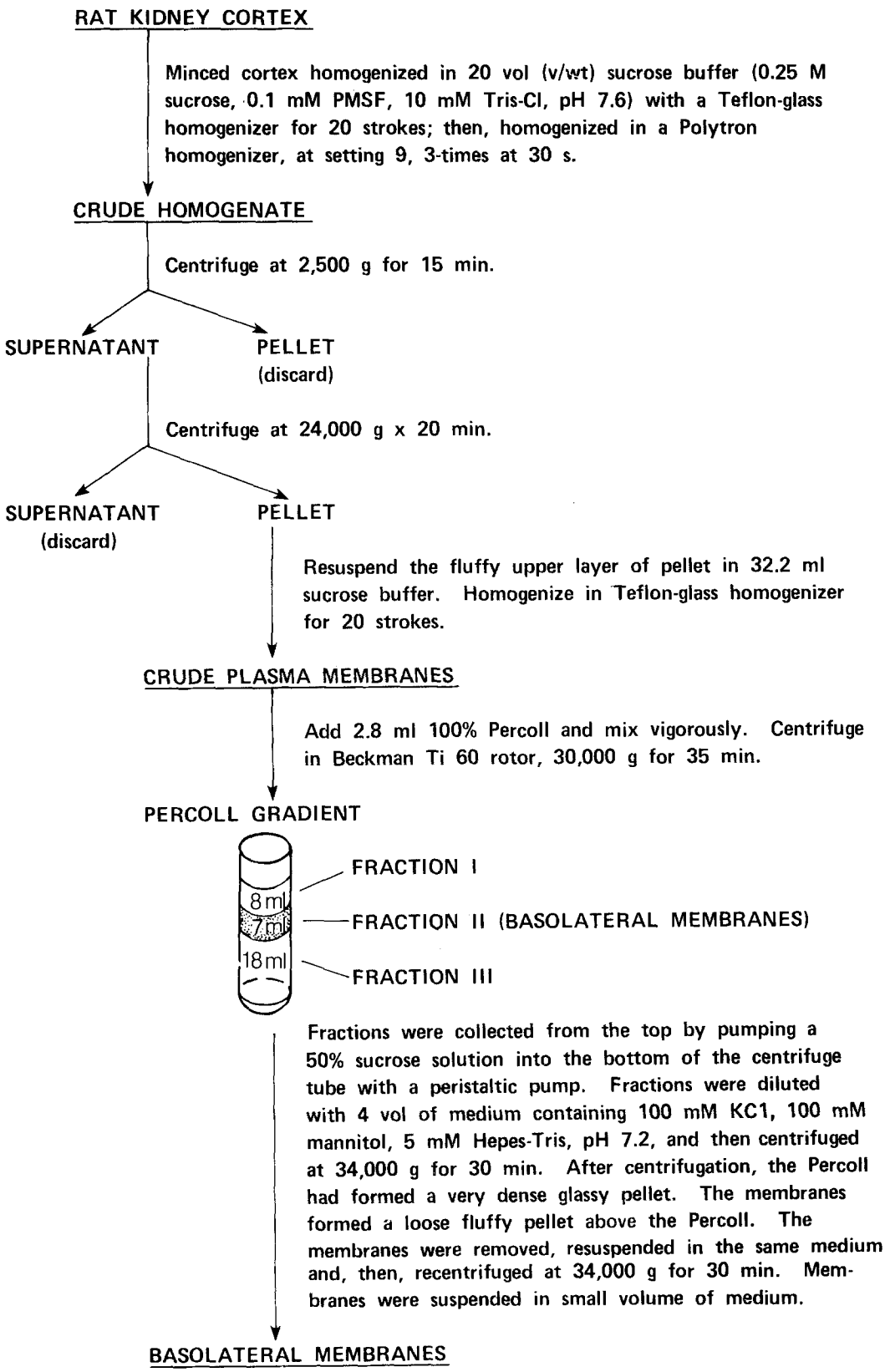

Fig. 1. Scheme for the preparation of renal basolateral membranes

kidneys were quickly chilled in ice-cold medium consisting of 0.25 M sucrose, 10 mM Tris-Cl buffer, pH 7.6, and 0.1 mM phenyl methyl sulfonyl fluoride (PMSF). All subsequent steps of the procedure were carried out in a coldroom (2–4°) or on ice. The kidneys were decapsulated; the cortices were dissected and then thoroughly minced. The subsequent steps in the isolation of the basolateral membranes are described in detail in Fig. 1. An important feature of this procedure was the use of a self-orienting Percoll (modified colloidal silica) gradient in the separation of the renal membranes. Recently, the use of Percoll gradients to isolate basolateral membranes from intestinal epithelial cells was reported [29]. The method described here for the separation of kidney membrane fractions represented a modification of this technique.[1] After centrifugation in the gradient, the Percoll was removed, and the basolateral membrane vesicles were washed and finally suspended in a medium containing 100 mM KCl, 100 mM mannitol, and 5 mM Hepes-Tris buffer, pH 7.2. In experiments in which the intravesicular medium was varied, the membrane vesicles were preloaded by diluting Fraction II and carrying out the entire washing procedure in the described medium.

Enzyme Assays

$(Na^+ + K^+)$ ATPase activity was measured as described [22], modified by the use of 75 mM imidazole-Cl, pH 7.5, as the buffer and 100 μM ouabain to inhibit the enzyme. Previously reported methods were used for the assays of maltase [3]; γ-glutamyltransferase [9]; lactate dehydrogenase [14]; cytochrome *c* oxidase [40]; and acid phosphatase [24], as modified [15]. Protein was determined by a standard procedure [16], with bovine serum albumin as the reference protein.

[1] We express our appreciation to Dr. H. Murer for the description of the Percoll gradient procedure as employed with intestinal cells prior to publication.

Transport Measurements

Uptakes of L-[^{3}H]glutamate and D-[^{14}C]glucose were measured by a Millipore filtration technique [1, 7] using 0.65 μm filters. Five microliters of the basolateral membrane suspension, 60 to 90 μg of protein, were incubated at 20 °C in a final volume of 50 μl. Uptakes measured at 15 sec and 90 min were linear with membrane protein, within this range. All incubations were carried out in triplicate with freshly prepared membranes. The results are expressed as the mean ± SEM.

Chemicals

L-[^{3}H]glutamate (22.7 Ci/mmol) was obtained from New England Nuclear. D-[^{14}C]glucose (240 Ci/mol) was purchased from Schwarz/Mann. 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 [21].

Results

Isolation of the Basolateral Membranes and Evaluation of the Preparation

Table 1 shows that the crude plasma membrane fraction, prepared as described in Materials and Methods and Fig. 1, comprised about 10% of the total protein of the renal cortex homogenate and had approximately 60% of the total $(Na^+ + K^+)$-ATPase activity, the "marker" enzyme for the basolateral membrane [22]. The specific activity of the enzyme increased from

Table 1. Specific activities, recoveries, and enrichment factors for marker enzymes in different fractions obtained during the isolation of the basolateral membranes

	(Na+K) ATPase	Maltase	γ-Glutamyl transferase	Lactate dehydrogenase	Acid phosphatase	Cytochrome *c* oxidase	Protein
Homogenate	0.053 ± 0.008 (100)	0.194 ± 0.010 (100)	0.66 ± 0.08 (100)	0.112 ± 0.005 (100)	0.070 ± 0.009 (100)	0.452 ± 0.036 (100)	453 ± 6 (100)
Crude plasma membranes	0.222 ± 0.036 (60 ± 8)	0.629 ± 0.037 (55 ± 2)	2.31 ± 0.28 (58 ± 1)	0.036 ± 0.012 (2.7 ± 1.1)	0.107 ± 0.016 (25 ± 2)	0.500 ± 0.002 (10.4 ± 1.3)	49 ± 2 (10.7 ± 0.5)
Fraction I	0.368 ± 0.086 (5.5 ± 3.2)	0.132 ± 0.045 (0.7 ± 0.3)	0.60 ± 0.12 (1.0 ± 0.4)	0.014 ± 0.004 (0.08 ± 0.01)	0.110 ± 0.029 (1.0 ± 0.2)	0.099 ± 0.007 (0.053 ± 0.003)	2.4 ± 0.7 (0.53 ± 0.15)
Fraction II	0.603 ± 0.080 (16.1 ± 1.7) 11.4*	0.204 ± 0.049 (1.7 ± 0.3) 1.05*	0.92 ± 0.05 (3.0 ± 0.3) 1.39*	0.022 ± 0.005 (0.24 ± 0.06) 0.20*	0.113 ± 0.019 (2.3 ± 0.1) 1.61*	0.101 ± 0.012 (0.27 ± 0.06) 0.22*	5.0 ± 0.5 (1.10 ± 0.12)
Fraction III	0.208 ± 0.009 (41.2 ± 10.6)	0.795 ± 0.046 (34 ± 2)	3.02 ± 0.45 (35 ± 1)	0.028 ± 0.011 (1.0 ± 0.2)	0.100 ± 0.012 (12.3 ± 1.5)	0.358 ± 0.041 (2.6 ± 0.1)	35.4 ± 1.0 (8.2 ± 0.6)

The homogenate, crude plasma membranes, and the Percoll gradient fractions were obtained as described in Fig. 1 and the text. Enzyme specific activity values are reported as μmol/min·mg protein. Protein values are given as mg. The values in parentheses represent the total units of activity in the fraction relative to the total activity for that enzyme in the homogenate and are reported as the percentage recovery. The values designated with an asterisk represent the specific activity in Fraction II (basolateral membranes) relative to the specific activity in the homogenate and are indicative of the enrichment factor. The specific activity values represent the mean ± SEM for 5 preparations. The recovery values represent the mean ± SEM for 3 preparations.

Table 2. D-Glucose uptake by the membranes (basolateral) localized in Fraction II of the Percoll gradient

Incubation	D-Glucose uptake (pmol/mg protein)	
	15 sec	90 min
Na^+ gradient present	16.2±1.6	37.0±4.7
Na^+ gradient absent	15.2±0.8	41.5±5.4

Membrane vesicles representing Fraction II were obtained as described in Fig. 1. The vesicles were preloaded with 100 mM KCl, 100 mM mannitol, and 5 mM Hepes-Tris buffer, pH 7.5. The incubation medium contained 100 mM mannitol, 5 mM Hepes-Tris, pH 7.5, 50 μM D-[^{14}C]glucose, and either 100 mM NaCl or 100 mM KCl. Each datum represents the mean ±SEM for 6 experiments.

0.053 to 0.222 μmol/min·mg protein, an enrichment of fourfold. The brush border segment of the plasma membrane was also enriched in the crude plasma membrane fraction, as indicated by similar fold increases in specific activities of maltase and γ-glutamyltransferase, marker enzymes for the microvillar membrane [25]. When then crude plasma membrane preparation was centrifuged on a Percoll gradient, the basolateral and brush border enzymes distributed differently. The bulk of the maltase and γ-glutamyltransferase activites was localized in the centrifuge tube in the bottom 18 ml, designated Fraction III. In contrast, ($Na^+ + K^+$)ATPase activity was concentrated in the overlying 7 ml, designated Fraction II. Relative to the specific activities in the crude plasma membrane preparation, ($Na^+ + K^+$)ATPase in Fraction II was increased additionally, three-times, whereas maltase and γ-glutamyltransferase were decreased to about a third of their values. The specific activity of ($Na^+ + K^+$)ATPase in Fraction II was 0.60 μmol/min·mg protein, and this value indicated that the particulates in Fraction II were enriched 11-fold with basolateral membranes relative to the cortex homogenate. The biochemical purity of Fraction II was also evaluated by assays of other enzymes. The enrichment factors for lactate dehydrogenase and cytochrome *c* oxidase were only 0.2, suggesting little contamination by the cytosol or mitochondria. Some contamination by the lysosomal enzyme, acid phosphatase, was found, but the enrichment factor was only 1.6 and the total recovery was 2%, relative to the crude homogenate. In contradistinction, 16% of the total ($Na^+ + K^+$)ATPase activity was recovered in Fraction II. Table 1 also shows that the protein yield of this fraction was approximately 5 mg from the kidneys of two rats.

The membrane preparation, designated Fraction II, was also evaluated functionally to demonstrate that the preparation was largely free of brush border membrane contamination. If microvillar membrane vesicles were a significant contaminant, then appreciable Na^+ gradient-dependent D-glucose uptake would be found [1]. Table 2 shows that the initial (15 sec) rate of uptake of the sugar was virtually the same in the presence and absence of a Na^+ gradient. Moreover, the uptake at 15 sec was considerably less than the uptake at equilibrium (90 min), indicating no overshoot, as would be expected from brush border membrane vesicles. Further, D-glucose uptake, in the presence or absence of the Na^+ gradient, was inhibited about 50% by 0.5 mM cytochalasin B (data not shown), a known inhibitor of sugar transport across the basolateral membrane [12]. On the other hand, when D-glucose uptake was examined with the membranes in Fraction III, the region of the Percoll gradient that contained substantial brush border membrane enzyme activity (Table 1), the Na^+ gradient-dependent rate of uptake was seven times the Na^+-independent rate and Na^+ gradient-energized uphill transport of D-glucose was observed; the initial (15 sec) uptake in the presence and absence of the gradient was 173±8 and 23± pmol/mg protein, respectively. This finding indicated that functional brush border membrane vesicles could be obtained after membrane separation on a Percoll gradient and that the failure to find Na^+ gradient-dependent sugar transport in Fraction II was not due to possible inhibition of the uptake system by Percoll. Rather, it suggested that Fraction II was substantially free of transport activity characteristic of brush border membrane vesicles. These results, together with the enzyme studies in Table 1, provided strong evidence that the predominant membrane in Fraction II was the basolateral membrane. Therefore, this Fraction was used to study the L-glutamate transport system in basolateral membrane vesicles, as reported below.

Ion Gradient-Dependent Uptake of L-*glutamate*

The uptake of 25 μM L-[^{3}H]glutamate by renal basolateral membrane vesicles as a function of time is illustrated in Fig. 2. When both the intravesicular and extravesicular media were 100 mM KCl in 100 mM buffered mannitol, the initial (15 sec) rate of uptake was low and steady-state levels were reached by 90 min. When the intravesicular medium was the same but the extravesicular medium was changed to 100 mM NaCl in 100 mM buffered mannitol, the uptake of L-glutamate was markedly stimulated. Initial rates were increased approximately 10 times. Accumulation of L-glutamate in the membrane vesicles was maximal in about 3 min. Thereafter, the amount of amino acid decreased, indicating net efflux. After

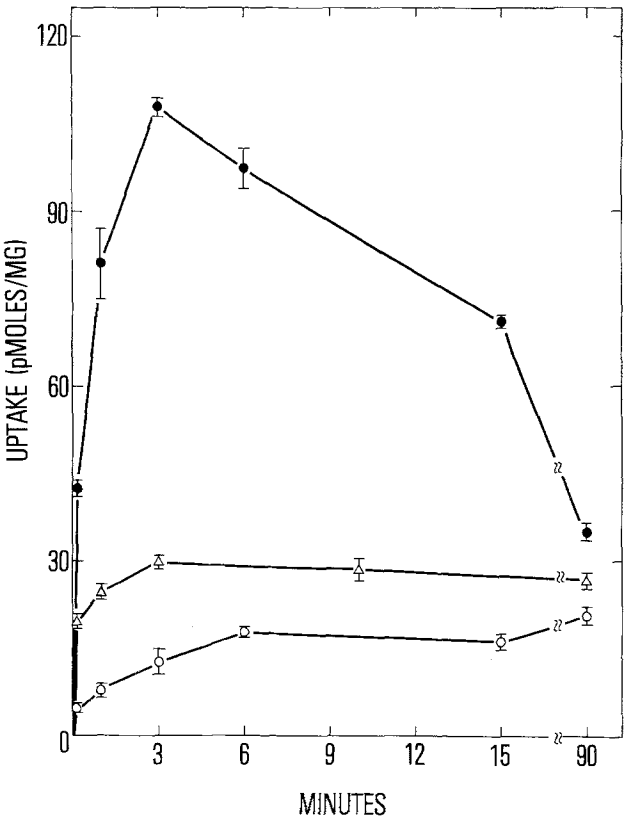

Fig. 2. The time course of L-glutamate uptake by basolateral membrane vesicles in the presence of a Na^+ gradient (extravesicular > intravesicular) and a K^+ gradient (intravesicular > extravesicular). Membrane vesicles were preloaded with a medium containing 100 mM KCl, 100 mM mannitol, and 5 mM Hepes-Tris, pH 7.5, and were incubated in a medium containing 25 μM L-[^{3}H]glutamate, 100 mM buffered mannitol, and, and 100 mM KCl (○), or 100 mM NaCl (●), or 50 μM D-[^{3}H]glucose, 100 mM buffered mannitol, and 100 mM NaCl (△)

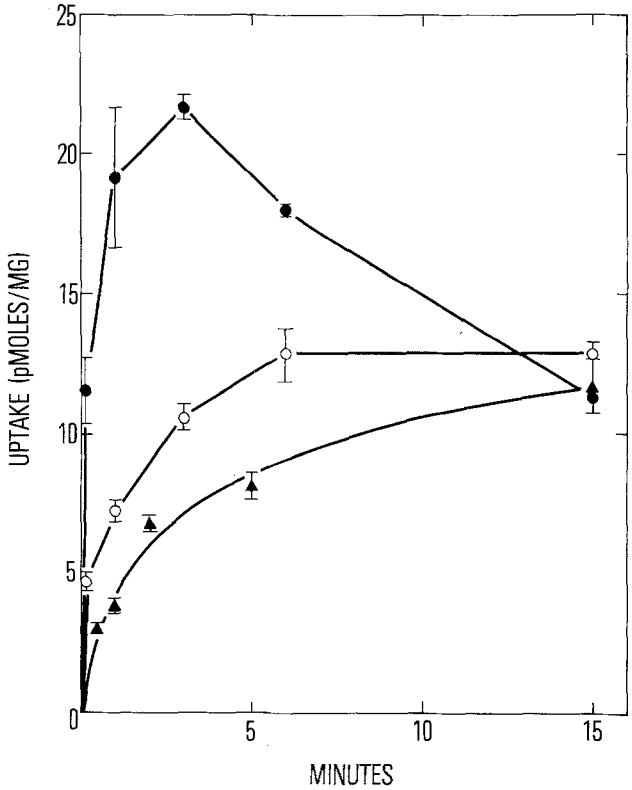

Fig. 3. Demonstration that a K^+ gradient could energize the uphill transport of L-glutamate and the requirement for Na^+. Membrane vesicles were preloaded with 75 mM K_2SO_4, 25 mM Na_2SO_4, and 5 mM Hepes-Tris, pH 7.5, and were incubated in a medium containing 25 mM Na_2SO_4, 5 mM Tris-Hepes, pH 7.5, and either 75 mM tetraethylammonium sulfate (●) or 75 mM K_2SO_4 (○). Other membrane vesicles were preloaded with 75 mM K_2SO_4, 75 mM mannitol, and 5 mM Hepes-Tris, pH 7.5, and incubated in a medium containing 75 mM tetraethylammonium sulfate, 75 mM mannitol, and 5 mM Hepes-Tris, pH 7.5 (▲). The concentration of L-[^{3}H]glutamate was 25 μM

90 min, the level of uptake in the presence of the Na^+ gradient ($[Na^+]_o > [Na^+]_i$) and K^+ gradient ($[K^+]_i > [K^+]_o$) had fallen almost to the level attained in the absence of gradients, suggesting that equilibrium was being approached. At the peak of the "overshoot", the accumulation of L-glutamate reached three to four times the 90-min value. This finding suggested that the imposition of large Na^+ and/or K^+ gradients provided the driving force to effect the transient movement of L-glutamate into renal basolateral membrane vesicles against its concentration gradient. This view was supported by the finding that when the membrane vesicles were preloaded with NaCl and KCl and the vesicles incubated in a medium containing the same concentrations of NaCl and KCl so that ($[Na^+]_o = [Na^+]_i$) and ($[K^+]_i = [K^+]_o$), i.e., there were no ion gradients, the overshoot or uphill transport was abolished (Fig. 3). This result indicated that it was not the concentrations of Na^+ and K^+ *per se*, but the gradients that were crucial in energizing uphill transport of the amino acid into the membrane vesicle.

In a parallel experiment, Fig. 2 also shows that although a Na^+ gradient was present, the uptake of D-glucose did not exhibit a transient overshoot. This finding provided further evidence that the concentrative uptake of L-glutamate by the membrane preparation could not be attributed to contamination by brush border membrane vesicles. From the amount of the 50 μM D-glucose taken up by the basolateral membrane vesicles at equilibrium, it could be estimated that the average intravesicular space of the vesicles, as prepared, was 0.6 μl/mg of membrane protein. The uptake of L-glutamate after 90 min of incubation, assuming equilibrium was established, was a little greater than expected from this intravesicular

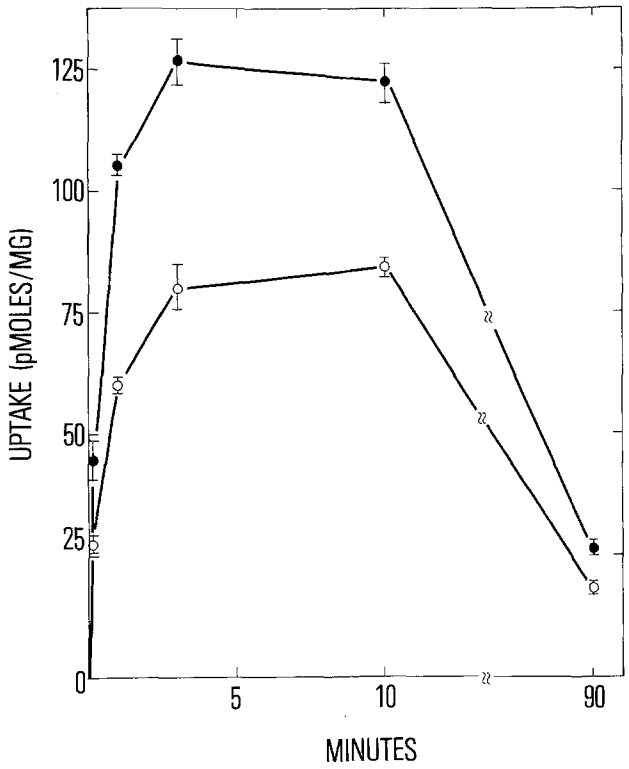

Fig. 4. Demonstration that a Na^+ gradient could energize the uphill transport of L-glutamate and the enhancement of the uptake by the presence of intravesicular K^+. Membrane vesicles were preloaded with 100 mM mannitol, 5 mM Hepes-Tris, pH 7.5, and 100 mM KCl (●), or 100 mM choline chloride (○), and were incubated in a medium containing 100 mM NaCl, 100 mM buffered mannitol, and 25 μM L-[^{3}H]glutamate

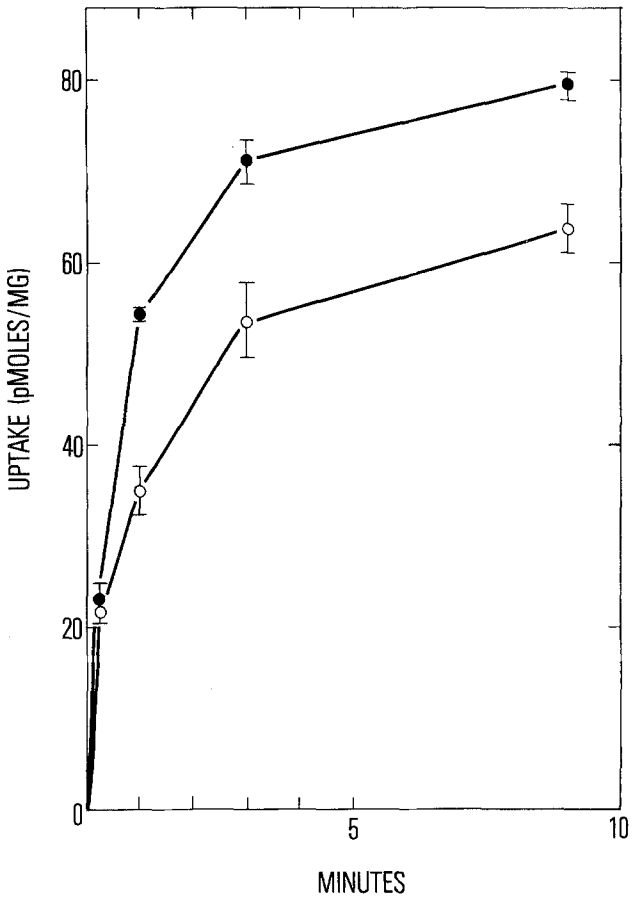

Fig. 5. The requirement of a K^+ gradient (intravesicular > extravesicular) for stimulating the Na^+ gradient-dependent L-glutamate uptake. Membrane vesicles were preloaded with 75 mM KCl, 75 mM choline chloride, and 5 mM Hepes-Tris, pH 7.5. Uptakes were initiated by diluting the membranes 10-fold into a medium containing 25 μM L-[^{3}H]glutamate, 75 mM NaCl, 5 mM Hepes-Tris, pH 7.5, and either 75 mM choline chloride to give a $[K^+]_i/[K^+]_o = 10$ (●), or 75 mM KCl, to give a $[K^+]_i/[K^+]_o = 1$ (○)

volume, suggesting slight binding of the electrically charged L-glutamate to the membrane, as was seen for the uptake of the amino acid by the brush border membrane [31]. The possible metabolism of L-glutamate by the membranes was ruled out.

The finding of the concentrative uptake of L-glutamate in the presence of both a Na^+ gradient ($[Na^+]_o > [Na^+]_i$) and a K^+ gradient ($[K^+]_i > [K^+]_o$) prompted the question whether either one or the other gradient, or both, contributed to the driving force. Figure 3 describes an experiment in which the basolateral membrane vesicles were preloaded with K^+, and L-glutamate uptake was measured either in the presence of Na^+, but without a Na^+ gradient ($[Na^+]_o = [Na^+]_i$), or in the complete absence of Na^+. It was found that the K^+ gradient effected the transient accumulation of the amino acid to a level greater than the final equilibrium value. Therefore, the K^+ gradient ($[K^+]_i > [K^+]_o$), by itself, could energize the uphill transport of L-glutamate. Importantly, Fig. 3 also shows that only in the presence of Na^+ was there a marked enhancement in uptake. Thus, the effect of the K^+ gradient in stimulating the uptake of L-glutamate had an absolute requirement for Na^+.

The experiment illustrated in Fig. 4 demonstrates that a Na^+ gradient ($[Na^+]_o > [Na^+]_i$), by itself, in the complete absence of K^+ could provide the driving force for the uphill transport of L-glutamate by the membrane vesicles. However, when a K^+ gradient ($[K^+]_i > [K^+]_o$) was present in addition to the Na^+ gradient ($[Na^+]_o > [Na^+]_i$), uptake was augmented. This augmentation of the Na^+ gradient-dependent uptake of L-glutamate in membrane vesicles preloaded with K^+ required a K^+ gradient ($[K^+]_i > [K^+]_o$). As demonstrated in Fig. 5, membrane vesicles preloaded with the same concentration of K^+, but uptake measured

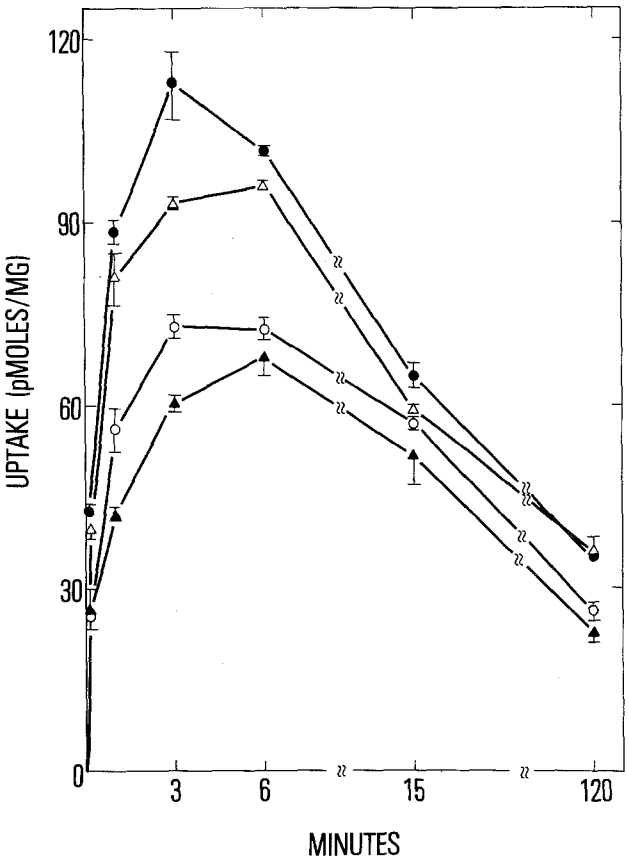

Fig. 6. The specificity of intravesicular K^+ in stimulating the Na^+ gradient-dependent uptake of L-glutamate. Membrane vesicles were preloaded with 100 mM KCl (●), choline chloride (○), tetraethylammonium chloride (▲) or RbCl (△) in 100 mM mannitol and 5 mM Hepes-Tris, pH 7.5. The incubation medium contained 100 mM NaCl, 100 mM buffered mannitol, and 25 μM L-[^{3}H]glutamate

Table 3. The effect of a valinomycin-induced membrane potential on the K^+ gradient ($[K^+]_i > [K^+]_o$) plus Na^+ gradient ($[Na^+]_o > [Na^+]_i$)-dependent uptake of L-glutamate by basolateral membrane vesicles

Valinomycin	L-Glutamate uptake (pmol/30 s · mg protein)
(−)	31.7 ± 1.0
(+)	34.8 ± 1.5

Membrane vesicles were preloaded with 75 mM K_2SO_4, 75 mM mannitol, and 5 mM Hepes-Tris, pH 7.5, and pretreated with or without valinomycin (5 μg) in 2% ethanol. The vesicles were incubated in a medium containing 25 μM L-[^{3}H]glutamate, 75 mM Na_2SO_4, 75 mM mannitol, and 5 mM Hepes-Tris, pH 7.5

in the absence of a gradient, i.e., $[K^+]_i = [K^+]_o$, did not elicit the enhancement seen with the K^+ gradient. These results indicated, therefore, that the concentrative uptake of L-glutamate by the basolateral membranes was energized by a Na^+ gradient ($[Na^+]_o > [Na^+]_i$), or a K^+ gradient ($[K^+]_i > [K^+]_o$), in the presence of Na^+. Moreover, uphill uptake was amplified by the presence of both gradients.

The specificity for K^+ in increasing the Na^+ gradient-dependent uptake of L-glutamate is shown in Fig. 6. The effect of the cation gradient (intravesicular > extravesicular) was relatively specific for K^+, only Rb^+ could substitute. Membrane vesicles preloaded with choline$^+$ or tetraethylammonium$^+$ did not stimulate transport relative to uptake due to the Na^+ gradient alone.

The experiment described in Table 3 shows the effect of a valinomycin-generated membrane potential, interior negative, on the K^+ gradient ($[K^+]_i > [K^+]_o$) plus Na^+ gradient ($[Na^+]_o > [Na^+]_i$) uptake of L-glutamate by the basolateral membrane vesicles. Valinomycin had no measurable effect. It was important to note that in this experiment the sulfate anion was used. In the presence of this relatively electrophoretically impermeant anion, valinomycin would induce the inside negative membrane potential without appreciably dissipating the K^+ chemical gradient, at least for the first 30 sec of uptake [32]. This finding is consistent with the view that the ion gradient-dependent transport system for L-glutamate in the basolateral membrane was an electroneutral process.

Discussion

The results reported in this paper described a scheme in which a self-generating Percoll gradient was used for the preparation of renal membranes enriched in the basolateral segment of the plasma membrane. The method represented a modification of a procedure recently developed for the isolation of the basolateral membrane from intestinal cells [6]. Moreover, we showed, for the first time, that the Percoll gradient-prepared membranes could be utilized for transport studies. The present procedure offered several advantages compared to other methods [10, 15, 19, 20, 23]. It was relatively rapid and simple, i.e., neither a free-flow electrophoresis apparatus nor a zonal centrifuge was required. Additionally, membrane fractions from the same gradient could be used to examine transport systems in both the basolateral membrane (Fraction II) and the brush border membrane (Fraction III). Recently, the separation of two species of brush border membranes on a Percoll gradient was reported [17].

Although the Na^+ gradient-dependent uphill transport of amino acids by renal brush border membrane vesicle was previously established [27], earlier studies indicated that amino acid (L-proline and L-phenylalanine) uptake by the basolateral membrane

vesicle was neither Na^+ gradient-stimulated nor concentrative [8, 35]. The present study of L-glutamate transport by the renal basolateral membrane demonstrated that a Na^+ gradient could energize the uphill transport of the acidic amino acid (Fig. 4). Indeed, recent studies suggest that the stimulation of amino acid uptake across the contraluminal membrane by a Na^+ gradient might be more prevalent than previously supposed. It was reported that external Na^+ could increase the rate of L-alanine uptake by intestinal basolateral membranes [19]. Investigations in our own laboratory confirmed this observation, and we also found that a Na^+ gradient stimulated the uptake of L-glutamate by these intestinal membrane vesicles.[2] With renal preparations, bath-to-lumen glycine flux in the perfused segment of isolated proximal tubules was recently shown to be enhanced by the presence of Na^+ in the perfusate [2]. More recently (after the present study was completed), it was reported that a Na^+ gradient increased the uptake of L-glutamine, although an overshoot was not demonstrated [23], and the stimulation of L-glutamate uptake by Na^+ was noted in a preliminary communication [13].

Importantly, we now demonstrated that a K^+ gradient (intravesicular > extravesicular) (Fig. 2) in addition to a Na^+ gradient (extravesicular > intravesicular) could provide the driving force for the uphill transport of L-glutamate into the basolateral membrane vesicle. Moreover, from the results that the K^+ gradient energized the concentrative uptake of L-glutamate in the absence of membrane electrical potential changes, and in the absence of Na^+ and anion gradients, it can be hypothesized that the transport of L-glutamate was coupled to the transmembrane flux of K^+. It should be emphasized, however, that the efflux of K^+ from the membrane vesicle coupled to the uptakes of L-glutamate and Na^+ was not demonstrated directly in this paper. Also, although not examined with basolateral membranes, with brush border membranes the ionic effects on glutamate transport were reversible, in that glutamate efflux from vesicles was stimulated by a K^+ gradient ($[K^+]_o > [K^+]_i$) [28].

The findings that an inwardly directed Na^+ gradient and an outwardly directed K^+ gradient energized the uptake of L-glutamate across the basolateral membrane as well as across the brush border membrane [6, 28, 32] suggested a model mechanism whereby the acidic amino acid could be accumulated to high levels in the renal tubular cell. This hypothesis is illustrated schematically in Fig. 7. In the cell, *in situ*, it would be the ouabain-sensitive ATPase localized in the basolateral membrane [22] and catalyzing

[2] B. Sacktor, I.L. Rosenbloom, C.T. Liang, and L. Cheng *(unpublished observations)*.

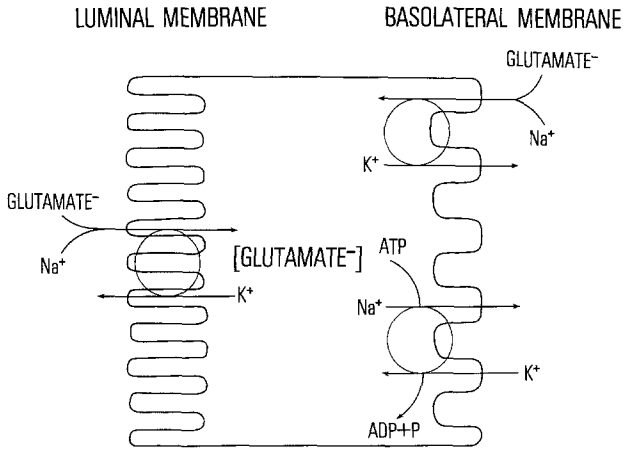

Fig. 7. Schematic model for L-glutamate uptake by the renal tubular cell

the extrusion of Na^+ coupled to the intake of K^+ that maintains the Na^+ electrochemical gradient (extracellular > intracellular) and the K^+ electrochemical gradient (intracellular > extracellular). L-Glutamate in the glomerular filtrate would be taken up at the luminal membrane against its concentration gradient by a brush border membrane co-transport system that is coupled to the influx of Na^+ and efflux of K^+ moving down their respective electrochemical gradients [32]. An analogous system exists at the basolateral membrane in which L-glutamate in the peritubular fluid would be transported uphill into the cell, presumably likewise co-transported with the movements of Na^+ and K^+ down their respective electrochemical gradients. Thus, a unique mechanism, the concentrative uptakes of L-glutamate energized by a Na^+ gradient, augmented by a K^+ gradient, at both sides of the polar tubular cell, could account for the exceedingly high intracellular concentration of this metabolically important amino acid in the renal tubule.

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