Biochimica et Biophysica Acta, 599 (1980) 191—201

BBA 78743

THE EFFECTS OF POTASSIUM AND MEMBRANE POTENTIAL ON SODIUM-DEPENDENT GLUTAMIC ACID UPTAKE

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(Received September 25th, 1979)

Key words: Glutamic acid uptake; K^+ effect; Potential dependence; Na^+; (Proximal tubule membrane)

Summary

The uptake of L-glutamic acid into brush-border membrane vesicles isolated from rat renal proximal tubules is Na^+-dependent. In contrast to Na^+-dependent uptake of D-glucose, pre-equilibration of the vesicles with K^+ stimulates L-glutamic acid uptake. Imposition of a K^+ gradient ($[K_i^+] > [K_o^+]$) further enhances Na^+-dependent L-glutamic acid uptake, but leaves K^+-dependent glucose transport unchanged. If K^+ is present only at the outside of the vesicles, transport is inhibited. Intravesicular Rb^+ and, to a lesser extent, Cs^+ can replace intravesicular K^+ to stimulate L-glutamic acid uptake. Changes in membrane potential incurred by the imposition of an H^+-diffusion potential or anion replacement markedly affect Na^+-dependent glutamic acid uptake only in the presence of K^+. Experiments with a potential-sensitive cyanine dye also indicate that, in the presence of intravesicular K^+ a charge movement is involved in Na^+-dependent transport of L-glutamic acid.

The data indicate that Na^+-dependent L-glutamic acid transport can be additionally energized by a K^+ gradient. Furthermore, intravesicular K^+ renders Na^+-dependent L-glutamic acid transport sensitive to changes in the transmembrane electrical potential difference.

Introduction

L-Glutamic acid is reabsorbed in the proximal tubule and shares a common transport system with L-aspartic acid [1]. Histochemical experiments with

Abbreviations: DiS-C_2(5), 3,3′-diethylthiadicarbocyanine iodide; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-ethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid.

sections of frozen kidney cortex indicated a high accumulation ratio of L-glutamic acid in proximal tubule epithelium [2]. Electrophysiological measurements on the intact rat kidney proximal tubule confirmed that glutamic acid and aspartic acid are reabsorbed via the same system and showed that the addition of either amino acid to the luminal perfusate leads to a depolarization of the transmembrane electrical potential difference. This depolarization occurs only in the presence of Na^+ indicating an Na^+-dependent electrogenic transport of acidic amino acids in rat kidney [3]. By means of the same techniques, the existence of a second Na^+-dependent electrogenic transport system for glutamic acid at the basal (blood) side of the epithelial cell could be demonstrated [4].

The transport of L-glutamic acid into brush-border membrane vesicles isolated from rat renal cortex was first described by Weiss et al. [5]. They demonstrated that glutamic acid uptake is strictly Na^+-dependent and can be kinetically described as a two-component system. Na^+ stimulation of L-glutamic acid uptake into brush-border membrane vesicles isolated from rat or rabbit kidney cortex was also demonstrated by Seeger et al. [6] and Schneider et al. [7]. They found that changes in the membrane potential induced by anion replacement or valinomycin under K^+-gradient conditions do not influence the Na^+-dependent uptake of L-glutamic acid. Since the addition of L-glutamic acid also does not influence the fluorescence of a potential-sensitive dye, Schneider et al. [7] postulated an electroneutral Na^+/L-glutamic acid symport. These results are in apparent contradiction to the data obtained from electrophysiological methods which indicated a surplus of positive charges carried across the brush-border membrane during the Na^+-dependent transport of glutamic acid [3]. Furthermore, it is questionable whether an electroneutral Na^+/glutamic acid symport would be sufficient to account for the observed high accumulation ratios of acidic amino acids.

We therefore re-evaluated the question of driving forces of glutamic acid transport. Since preliminary experiments carried out by Schneider [8] as well as in our laboratory [9] suggested a role of K^+, special emphasis was placed on the involvement of K^+ in Na^+-dependent L-glutamic acid transport. It was found that Na^+-dependent uptake of L-glutamic acid additionally can be driven by an outward K^+ gradient. Furthermore, K^+, if present at the inner side of the vesicles, renders Na^+-dependent glutamic acid transport sensitive to changes in the transmembrane electrical potential difference.

Materials and Methods

Brush-border membrane vesicles were isolated from rat kidney cortex (male Wistar rats, 180–230 g body w.) by a calcium-precipitation method as described earlier in detail [10]. Alkaline phosphatase and aminopeptidase M, marker enzymes for the brush-border membrane, were enriched by a factor of 11—13 in the isolated brush-border membranes as compared to the initial homogenate.

After the second calcium precipitation the membranes were loaded with different buffers in the following manner. The brush-border membrane pellet obtained after the centrifugation (31 000 × *g*, 12 min) was suspended in 10 mM mannitol/2 mM Tris-HCl buffer used during the membrane isolation. 1 ml

aliquots were then added to 60-ml of different buffers and homogenized in a glass-Teflon homogenizer (1200 rev./min, 15 strokes). The membrane suspensions were kept on ice for 20 min and membranes were collected by centrifugation at 48 000 × *g* for 20 min. Membranes were then resuspended in small volumes of the buffers and used for transport studies. The protein concentration of these suspensions was 3—6 mg/ml. The composition of the different buffers used for loading of the membranes as well as that of the incubation media will be given in the legends to the figures. All media used for membrane isolation and transport were filtered through a Sartorius 0.2 μm pore-diameter filter immediately before use to avoid bacterial contamination.

Solute uptake was measured using radioactively labelled substrates (D-[^{3}H(n)]glucose, 18 Ci/mmol; D-[U-^{14}C]glucose, 310 mCi/mmol, and L-[2,3-^{3}H]glutamic acid, 22.6 Ci/mmol). The incubation media contained 100 mM mannitol and 20 mM Hepes-Tris, pH 7.4. The concentration of L-glutamic acid and D-glucose was 0.1 mM. Exceptions as well as further additions are given in the legends to the figures. The incubation was started by adding 20 μl of membrane suspension to 100 μl of incubation medium at 25°C. The uptake was stopped by adding an aliquot of 20 μl of the reaction mixture to 1 ml of an ice-cold stop solution containing 100 mM mannitol, 20 mM Hepes-Tris, pH 7.4 and 150 mM NaCl. The stop solution containing the aliquot was immediately transferred with a Pasteur pipette onto a membrane filter (cellulose nitrate, 0.6 μm pore-size, Sartorius, Göttingen) kept under suction. The filter was immediately washed with 5-ml ice-cold stop solution. The radioactivity remaining on the filters was analyzed by standard liquid scintillation counting techniques.

Fluorescence measurements for detection of membrane potential changes were performed with a Shimadzu fluorometer RF 510. The fluorescence of 3 μM DiS-C_2(5) in 2 ml buffer was excited at 645 nm and measured at 675 nm. Addition of 20 μl vesicle suspension (3—6 mg protein/ml) caused a decrease in fluorescence. After a steady level of fluorescence was reached, L-glutamic acid and D-glucose were added by a syringe to the cuvette which was continuously stirred by a magnetic flea. Fluorescence changes were recorded on a chart recorder.

Hepes and Mes were obtained from Serva, Heidelberg. Valinomycin and FCCP from Boehringer-Mannheim and DiS-C_2(5) from Eastman-Kodak, Rochester, NY. Isotopes were purchased from NEN (Boston, MA). All other chemicals used throughout this study were obtained from Merck, Darmstadt, and were of at least analytical grade purity.

Results

Earlier experiments documented that L-glutamic acid uptake by vesicles is specifically stimulated by an Na^+ gradient [5—7]. In the intact cell, besides an inwardly-directed Na^+ gradient, a K^+ gradient in the opposite direction also exists. We therefore tested the Na^+-dependent uptake of L-glutamic acid into brush-border membrane vesicles in the presence or absence of K^+. The results given in Fig. 1 (left) show the influence of K^+ on L-glutamic acid uptake under conditions of an Na^+ gradient, i.e., Na^+ is initially only present outside the

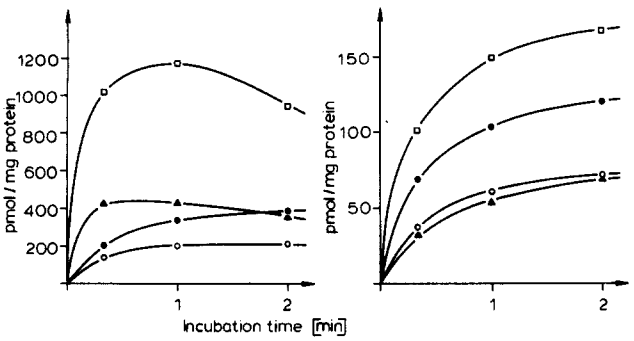

Fig. 1. Effect of K^+ on the uptake of L-glutamic acid into renal brush-border membrane vesicles in the presence (left) and absence (right) of an Na^+ gradient. The uptake was determined under four different conditions: (•) no K^+ present inside or outside the vesicles ($[K_i^+] = [K_o^+] = 0$); (▲) K^+-equilibrated ($[K_i] = [K_o] = 50$ mM); (○) K^+ gradient ($[K_i] = 0$, $[K_o] = 50$ mM); (□) K^+ gradient ($[K_i] = 50$ mM, $[K_o] = 8.3$ mM). Vesicles for the experiment shown in the left diagram were loaded with 200 mM mannitol; 20 mM Hepes-Tris, pH 7.4; 50 mM choline chloride or 50 mM KCl. For experiments under Na^+-equilibrated conditions, mannitol was partially replaced by 50 mM NaCl (right). The incubation media contained 100 mM mannitol, 50 mM NaCl, 50 mM choline chloride or 50 mM KCl and 20 mM Hepes, titrated to pH 7.4. The equilibrium values (glutamic acid uptake determined after 60 min) are 153.8 ± 22.5 pmol/mg protein (Na^+-gradient conditions) and 129.3 ± 23.1 pmol/mg protein (Na^+-equilibrated conditions) [4].

vesicles. Labelled glutamic acid was added at zero time and uptake was stopped at the times indicated.

In the absence of K^+, intravesicular L-glutamic acid concentration exceeds the equilibrium values taken 60 min after the addition of the amino acid. If K^+ is present at the outside L-glutamic acid uptake is inhibited. If, however, K^+ is present at equal concentrations at both sides of the membrane, a stimulation of L-glutamic acid uptake is observed. This stimulation is even higher when a K^+ concentration difference exists ($[K_i^+] > [K_o^+]$). Na^+-dependent uptake of D-glucose is not influenced by the presence of K^+ (not shown, see also Fig. 3).

The data shown in Fig. 1 (right) were obtained with Na^+-equilibrated vesicles. In comparison to Fig. 1 (left), the uptake of L-glutamic acid is slower than that in the presence of an Na^+ gradient. Pre-equilibration with K^+, as well as the presence of K^+ only in the incubation medium, inhibits L-glutamic acid uptake. In the presence of an outwardly-directed K^+ gradient, however, L-glutamic acid uptake is stimulated. This shows that a K^+ concentration difference ($[K_i^+] > [K_o^+]$) provides a driving force for glutamic acid uptake under conditions of an Na^+ gradient as well as in Na^+-equilibrated vesicles.

In the following experiment it was tested as to whether cations other than K^+ show a similar transtimulatory effect on L-glutamic acid uptake. Renal brush-border membrane vesicles were preloaded with various cations as indicated in Fig. 2. Na^+ was present initially only in the incubation medium. Except for Na^+-loaded vesicles, glutamic acid transport was therefore measured under Na^+-gradient conditions. It can be seen that Rb^+, at least at the concentration used in this experiment, can substitute K^+ completely, whereas Cs^+ stimulates L-glutamic acid less than K^+ and Rb^+ in incubations of short duration. For 2 min incubation, Cs^+ stimulation exceeds the K^+ stimulation, probably because the Cs^+ gradient across the membrane dissipates more slowly than the K^+ gradient.

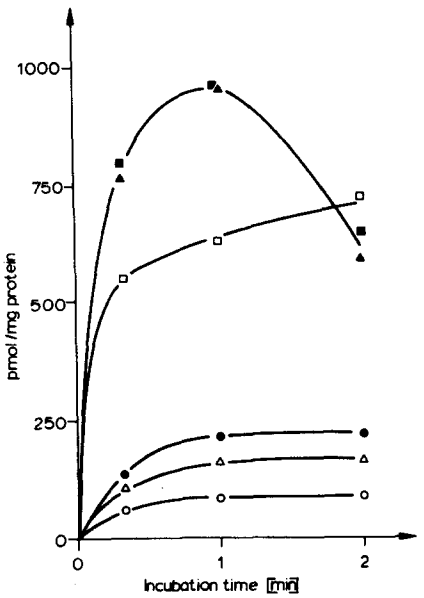

Fig. 2. Specificity of the cation trans effect on Na^+-dependent L-glutamic acid uptake into renal brush-border membrane vesicles. Vesicles were preloaded with 100 mM mannitol, 50 mM choline chloride, 20 mM Hepes-Tris, pH 7.4 and in addition choline chloride (●), NaCl (○), KCl (■), RbCl (▲), LiCl (△) and CsCl (□) (each at 50 mM). The incubation medium contained 100 mM mannitol; 50 mM choline chloride; 50 mM NaCl; 20 mM Hepes-Tris, pH 7.4. The equilibrium value of L-glutamic acid uptake is 148.5 ± 15.9 pmol/mg protein [6].

When Li^+, choline or Na^+ is present inside the vesicles, uptake is much smaller. The equilibrium values for glutamic acid uptake are not affected by the different cations.

Fig. 3 (left) shows how the stimulation of L-glutamic acid uptake depends

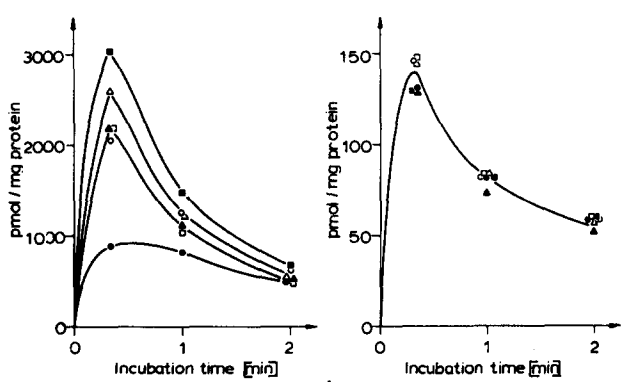

Fig. 3. The K^+-concentration dependency of the stimulation of L-glutamic acid uptake (left) and D-glucose uptake (right). K^+ was present at equal concentrations inside and outside the vesicles. (●) 0, (○) 2, (▲) 5, (△) 10, (■) 20, (□) 50 (in mM K^+, respectively). The vesicles were preloaded with 100 mM mannitol, 20 mM Hepes-Tris (pH 7.4) and the indicated KCl concentrations, KCl being varied against choline chloride. The incubation media had identical composition but contained in addition 50 mM NaCl. The equilibrium value for L-glutamic acid uptake is 72.2 ± 17.1 and for D-glucose uptake 58.2 ± 6.9 pmol/mg protein [6].

also on the K^+ concentration. L-Glutamic acid transport was determined under Na^+-gradient conditions. However, there was no K^+ gradient, i.e., vesicles were equilibrated with different K^+ concentrations. K^+ concentrations as low as 2 mM are able to stimulate the uptake. The maximal effect occurs at 20 mM K^+ whereas at 50 mM K^+ the stimulation decreases again.

Fig. 3 (right) shows for comparison the uptake of D-glucose which was determined in the same experiment. In contrast to L-glutamic acid, D-glucose transport is unaffected by variations in the K^+ concentration.

In the following, the sensitivity of Na^+- and K^+-dependent L-glutamic acid transport to changes in the transmembrane electrical potential difference was analyzed. The electrical potential difference can be varied by the addition of valinomycin when a K^+ gradient is present, or by addition of an uncoupler when an H^+ gradient exists.

In Fig. 4 the influence of a K^+-diffusion potential on L-glutamic acid and D-glucose uptake is shown. Under all conditions Na^+ was present only at the outside of the vesicles. If K^+ is also present only in the incubation medium, the addition of valinomycin should create an inside positive membrane potential difference and transport processes carrying a surplus of positive charges like Na^+-dependent D-glucose transport should be inhibited. This is seen in Fig. 4 (right). L-Glutamic acid uptake, however, is not influenced by the addition of valinomycin when K^+ is at the outside only, suggesting an electroneutral transport under these conditions. With K^+ present at higher concentrations in the intravesicular space than in the incubation medium, valinomycin will create an inside negative electrical potential difference, which stimulates 20- and 60-s D-glucose uptake as seen in Fig. 4 (right); L-glutamic acid uptake shows a small, but reproducible stimulation at the 20-s values. Vesicles, which have been

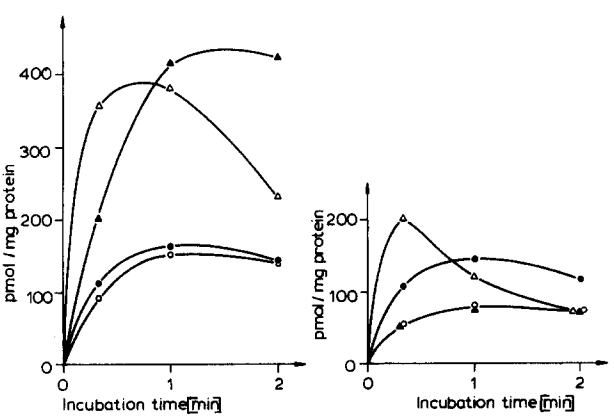

Fig. 4. The effect of K^+-diffusion potentials on the uptake of L-glutamic acid (left) and D-glucose (right) uptake into renal brush-border membrane vesicles. The uptake was studied under an inwardly-directed K^+ gradient ($[K_o]$ = 50 mM, $[K_i]$ = 0) without (●) and with (○) 83 µg/ml valinomycin, or under an outwardly-directed K^+ gradient ($[K_o]$ = 8.3, $[K_i]$ = 50 mM), again without (▲) or with (△) valinomycin. Vesicles were preloaded with either 200 mM mannitol or 100 mM mannitol/50 mM potassium cyclamate. 20 mM Hepes-Tris was used as buffer. The incubation medium contained 50 mM sodium cyclamate with or without 50 mM potassium cyclamate. Osmolarity was kept constant by the addition of mannitol. 60-min uptake of glutamic acid is 102.3 ± 19.6 pmol/mg protein and the equilibrium value for glucose uptake is 78.1 ± 15.3 pmol/mg protein [7].

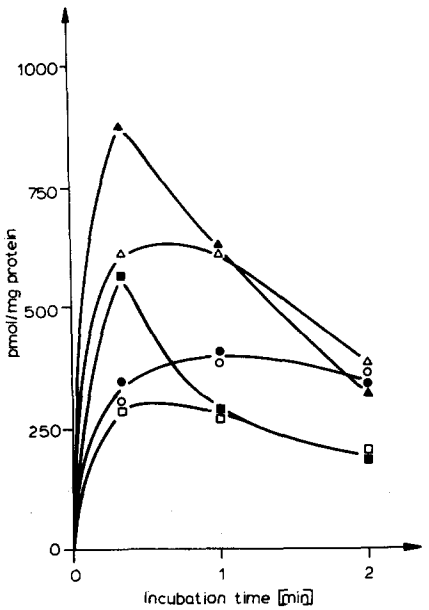

Fig. 5. The influence of H^+-diffusion potentials on the Na^+-dependent uptake of L-glutamic acid into renal brush-border membrane vesicles. A pH gradient ($pH_i < pH_o$) was created in the absence of K^+ (○,●) or under K^+-equilibrated (□,■) and K^+-gradient (△,▲) conditions. Controls (○,□,△) contained no FCCP, a parallel series 83 μg/ml FCCP (●,■,▲). Vesicles were preloaded with 100 mM mannitol, 50 mM Mes-Tris, pH 6.25 and either 50 mM KCl/50 mM choline chloride or 100 mM choline chloride. The incubation media contained in addition NaCl and were buffered with Hepes-Tris to pH 7.4. The equilibrium uptake of L-glutamic acid is 88 ± 10 pmol/mg protein [9].

incubated with L-glutamic acid for 1 min or longer, however, show a decrease in the amino acid uptake when valinomycin is present.

The influence of the membrane potential on L-glutamic acid uptake has also been examined under conditions of an outwardly-directed H^+ gradient (Fig. 5). The membranes were preloaded with buffers at pH 6.25 with or without K^+. At zero time the vesicles were suspended in a medium of pH 7.4. Na^+ was present only in the incubation medium (Na^+-gradient condition). In the complete absence of K^+ the addition of the uncoupler, FCCP, which will create an inside negative H^+-diffusion potential, changes L-glutamic acid transport only slightly. When K^+ is present in the intravesicular space, however, FCCP addition markedly stimulates 20-s uptake of L-glutamic acid.

The data given in Figs. 4 and 5 indicate that a clear potential-sensitivity of L-glutamic acid transport can only be detected in the presence of intravesicular K^+. In order to confirm these results we tried to vary the membrane potential difference by adding different sodium salts to the incubation medium, keeping the Na^+ concentration constant. The anions applied were the relatively impermeable gluconate and SO_4^{2-}, and more permeable ones like Cl^-, SCN^- and NO_3^-. Under conditions of a sodium salt gradient the membrane potential will be influenced by the relative permeabilities of Na^+ and the anions. Fig. 6 (left) shows that in the absence of K^+ only a small stimulation of L-glutamic acid is observed when gluconate or SO_4^{2-} is replaced by more permeable anions. In K^+-equilibrated vesicles, however, a 2.7-fold stimulation of the 20-s uptake of

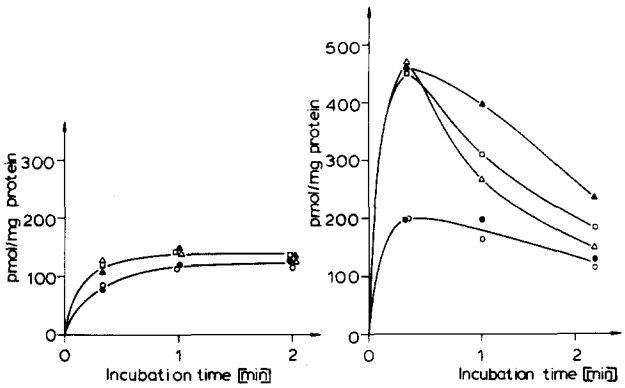

Fig. 6. The effect of an anion replacement on L-glutamic acid uptake into renal brush-border membrane vesicles in the absence (left) or presence (right) of 20 mM K^+. Each incubation medium contained one of the following anions: gluconate (•), SO_4^{2-} (○) Cl^- (▲), SCN^- (△), NO_3^- (□). The cations were Na^+ (50 mM) and K^+ (0 or 20 mM), the osmolarity being kept constant with mannitol. The vesicles were preloaded with 100 mM mannitol; 20 mM Hepes-Tris, pH 7.4; 20 mM potassium gluconate. For the experiment in the absence of K^+, potassium gluconate was replaced by mannitol. 60-min uptake of glutamic acid is 164.8 ± 8.3 pmol/mg protein [4] in the presence and 227.2 ± 7.9 pmol/mg protein [5] in the absence of K^+.

L-glutamic acid is observed by replacing less permeable anions by more permeable ones. This again indicates a potential-sensitive transport process which is predominantly seen in the presence of K^+.

The influence of K^+ on the potential sensitivity of L-glutamic acid transport was also investigated by the use of a potential-sensitive cyanine dye, DiS-C_2(5) [11]. This dye was shown to monitor changes in membrane potential which occur during the Na^+-dependent, potential-sensitive transport of D-glucose or L-phenylalanine [12,13]. Fig. 7 shows redrawn traces of fluorescence changes

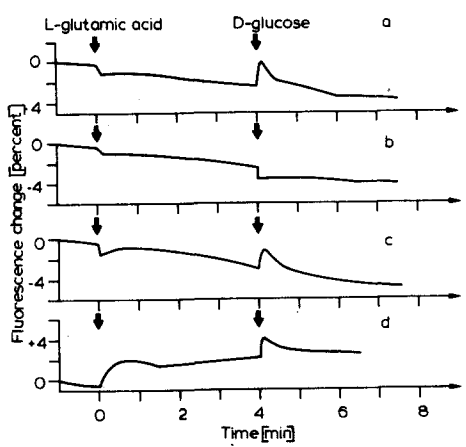

Fig. 7. Effects of L-glutamic acid and D-glucose on the fluorescence of a potential-sensitive cyanine dye in brush-border membrane vesicles. Fluorescence measurements were performed under the following conditions: 75 mM Na_2SO_4 inside and outside (trace a); 75 mM K_2SO_4 present on both sides (trace b); 65 mM Na_2SO_4 plus 10 mM K_2SO_4, present on both sides (trace c); and 75 mM Na_2SO_4 in the incubation medium, 75 mM K_2SO_4 in the vesicles (trace d). To 2-ml buffer containing 20 mM Hepes-Tris (pH 7.4), 3 μM DiS-C_2(5) and the indicated salts, 20 μl of vesicles (5 mg/ml) protein were added. The arrows indicate the addition of 1.8 mM L-glutamic acid and 2.47 mM D-glucose.

of DiS-$C_2(5)$ in a suspension of rat renal brush-border membrane vesicles. The addition of D-glucose to 75 mM Na_2SO_4-equilibrated vesicles causes a transient fluorescence increase, indicating a transient inside positive potential difference (Fig. 7, trace a). Since this fluorescence change cannot be seen in K_2SO_4-equilibrated vesicles (Fig. 7, trace b), it must be due to Na^+-dependent uptake of D-glucose into the vesicles. However, the addition of L-glutamic acid to Na^+-equilibrated vesicles shows no change other than a dilution-dependent fluorescence decrease, which indicates that a potential-sensitive transport is not present (Fig. 7, trace a). Even in the presence of 20 mM K^+ on both sides of the membrane, Na^+-equilibrated vesicles show no fluorescence increase when L-glutamic acid is added to the medium (Fig. 7, trace c). If, however, Na^+ is present only on the outside and K^+ only on the inside of the vesicles, a clear and reproducible fluorescence increase can be seen which indicates a potential-sensitive transport of L-glutamic acid under these conditions (Fig. 7, trace d).

Discussion

The experimental results presented above demonstrate that Na^+-gradient-driven L-glutamic acid uptake is stimulated by K^+ present at the inner membrane surface, but not at the outer membrane surface. K^+ stimulation of L-glutamic acid uptake is greater under conditions of an outward K^+ gradient ($[K_i^+] > [K_o^+]$). Furthermore, Na^+-dependent L-glutamic acid transport becomes sensitive to changes in the transmembrane electrical potential difference when K^+ is present inside the vesicles. In the absence of K^+ only a small fraction of L-glutamic acid transport responds to changes in the electrical potential difference.

Since brush-border membrane vesicles isolated from rat kidney cortex are predominantly right-side-out oriented [14], this study demonstrates a strong sidedness of the K^+ effect. K^+ stimulates only at the cytoplasmic membrane surface. Since K^+ stimulation of Na^+-gradient-driven glutamic acid uptake already occurs under conditions where no K^+ gradient exists, it cannot be due to K^+-diffusion potential effects. This is supported by the fact that D-glucose uptake known to be potential-sensitive (e.g., Ref. 12) is not altered by pre-equilibrating the vesicles with K^+. Na^+-dependent transport of L-phenylalanine and L-ornithine is also not affected by K^+ (Leopolder, A., Burckhardt, G. and Murer, H., unpublished results). Therefore, we have to consider a direct effect of K^+ on the Na^+-dependent L-glutamic acid transport system. A possible mode of K^+ action is via a modifier site. However, since K^+ stimulation of L-glutamic acid uptake is higher in the presence of a K^+ concentration difference ($[K_i^+] > [K_o^+]$), i.e., under conditions where the K^+ gradient could provide an additional energy source, we consider K^+ efflux to be involved in this stimulatory effect. In line with this assumption is the stimulation of glutamic acid uptake by an outward K^+ gradient ($[K_i^+] > [K_o^+]$) even in the absence of an inward Na^+ gradient. The inhibition of L-glutamic acid uptake by an inwardly-directed K^+ gradient ($[K_i^+] < [K_o^+]$) also fits the model. The observation that K^+ pre-equilibration stimulates glutamic acid uptake under Na^+-gradient conditions, but inhibits the uptake in the absence of an Na^+ gradient, is more difficult to understand. A likely explanation could be that intravesicular Na^+ competes with K^+ at the

internal surface. As, under Na^+-gradient conditions, initial intravesicular Na^+ concentrations are low, the stimulatory effect of K^+ can be more clearly observed than in Na^+-equilibrated vesicles. Competition between Na^+ and K^+ at the internal surface may also be the reason why, in Na^+- and K^+-equilibrated vesicles, no fluorescence change is observed after the addition of L-glutamic acid.

From our experiments it cannot be decided whether Na^+-dependent glutamic acid transport proceeds via two distinct systems or one system. A model would be feasible in which intravesicular K^+ modifies the transport system so that Na^+-dependent L-glutamic acid uptake will be coupled to K^+ efflux. Intravesicular Na^+ could then inhibit K^+-induced modification of the transport system. An Na^+-and K^+-stimulated transport system for glutamic acid has thus far been described only for synaptosomal membrane vesicles [15].

Unfortunately, the direct demonstration that a K^+ efflux occurs during the Na^+-dependent uptake of L-glutamic acid is lacking. Experiments using radioactively labelled Rb^+ failed thus far. A possible reason for this failure could be a high K^+/Rb^+ conductivity of the brush-border membrane obliterating the small differences in the Rb^+ distribution caused by Na^+-dependent uptake of L-glutamic acid.

When K^+ is present in the intravesicular space, L-glutamic acid transport becomes sensitive to changes in the electrical potential difference across the vesicular membrane. This has been tested by examining the effects of valinomycin in the presence of a K^+ gradient and FCCP in the presence of an H^+ gradient. Furthermore, this is documented by the anion-replacement experiments in the presence of K^+. Seeger et al. [6] and Schneider et al. [7] could not find an effect of valinomycin in the presence of K^+ gradients. As seen in Fig. 4 (left) the valinomycin-induced potential change stimulates L-glutamic acid uptake only during short incubation periods. If incubation times exceed 1 min, valinomycin decreases rather than increases L-glutamic acid uptake. This may be due to a partial dissipation of the chemical K^+ gradient, which additionally drives glutamic acid uptake. So, with valinomycin, two effects are competing: the inside negative membrane potential stimulates L-glutamic acid uptake, whereas the dissipation of the K^+ gradient decreases it. This may be the reason why so far, a potential sensitivity of glutamic acid transport in experiments with valinomycin-induced K^+-diffusion potentials was not detected.

Experiments with H^+-diffusion potentials or anion replacement, where changes in a K^+ gradient are ruled out by pre-equilibration of the vesicles with K^+, however, clearly demonstrate a potential-sensitive transport of L-glutamic acid. These tracer-flux experiments providing evidence for a potential sensitivity in the presence of K^+ are supported by the fluorescence experiments using a potential-sensitive dye. Our findings are in agreement with the electrophysiological data, which were of course obtained in the presence of intracellular K^+.

As the uptake of L-glutamic acid in the presence of K^+ can be enhanced by an inside negative electrical potential difference, one has to conclude that the uptake of L-glutamic acid occurs with a surplus of positive charges. If we further consider that the uptake of the negatively charged glutamic acid is Na^+-dependent, i.e., that it occurs as an Na^+ symport which is additionally driven by K^+, a symport of one glutamic acid molecule with three Na^+ and an antiport

with one K^+ have to be postulated. In the absence of K^+ an electroneutral symport of one Na^+ with one molecule of glutamic acid seems to be predominant. This assumption is based on the observation that alterations of the membrane potential leave Na^+-dependent L-glutamic acid uptake nearly unchanged.

A transport system, where two or more Na^+ and K^+ are involved at the same time, should lead to very high intracellular accumulation ratios of L-glutamic acid. This 'speciality' in the driving forces for L-glutamic acid transport could help to explain the high accumulation ratios observed in intact renal cortical tissue.

Acknowledgements

We thank Professors Ullrich and Frömter for their interest and stimulating discussions which enabled us to carry out the present work. The artwork of the figures by Mrs. Rentel and Miss Silz is gratefully acknowledged.

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