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Glutamine and Glutamic Acid Uptake by Rat Renal Brushborder Membrane Vesicles

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Summary. Glutamine uptake by rat renal brushborder vesicles occurred via two distinct saturable processes with K_m values of 0.145 and 8.5 mM which were stimulated by both ionic and sodium gradients with a pH optimum of 6.8–7.1. Glutamic acid uptake also occurred by a two-component system with K_m values of 0.016 and 3.60 mM. Both components were stimulated specifically by a sodium gradient. The low K_m system for glutamic acid had a pH optimum of 7.2–7.4. Glutamine entry at 0.06 mM was inhibited by a variety of amino acids at 3 mM, including dibasic amino acids, glycine, valine, and phenylalanine. Glutamic acid entry at 0.06 mM was inhibited 20–30% by 3 mM phenylalanine, valine, α-aminoisobutyric acid, and glutamine. No metabolic alteration of glutamic acid was observed on incubation with membrane vesicles, but glutamine was significantly hydrolyzed to glutamic acid upon prolonged incubation. Hydrolysis of glutamine was negligible at 15 sec incubation which was employed for measurement of initial rate of entry. These studies provide support for the existence of an uptake system in the brushborder of the renal proximal tubule cell capable of handling the reabsorption of glutamine normally present in glomerular filtrate.

An important aspect of the well-established role of the kidney in acid-base metabolism [7, 14, 16] involves the production of ammonia, which allows the kidney to compensate for acidosis. Glutamine is the major precursor of renal ammonia in man, dog, and rat, with more than 90% of the total ammonia produced being derived from this amino acid [2, 12, 15, 22]. The availability of glutamine for the production of ammonia in the mammalian kidney involves its transport into proximal tubule cells.

Essentially all of the glutamine filtered at the glomerulus is reabsorbed in the proximal tubules [13] and, since the renal extraction of glutamine is greater than the glomerular filtration rate during acidosis [18], both the basal-lateral and the luminal brushborder membranes must be

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involved in the intracellular accumulation of glutamine. The relative importance of each membrane has been implied in studies by a number of investigators. Oelert and Nagel [11] have reported a reduction in ammonia-genesis in normal dogs whose glomerular filtration rate (GFR) is reduced by ureteral clamping and concluded that renal ammoniagenesis is predominantly dependent on GFR and controlled by the availability of glutamine reabsorbed from the tubular urine to be used as substrate. Pilkington, on the other hand, studied mongrel dogs in chronic metabolic acidosis [13] and indicated that glutamine entering the tubular cell from both the basal-lateral and luminal brushborder is used for the generation of renal ammonia.

Controversy exists concerning the concentrative nature of glutamine uptake by kidney cortical cells. Welbourne [21] maintains that glutamine transport reflects rates of utilization by the two deamination enzymes present in the cell [2, 3] and postulates an uptake system by passive diffusion with the presence of several restricted intracellular glutamine pools to explain the apparent intracellular accumulation of glutamine. The prevalent view is that glutamine is transported against a renal concentration gradient by the tubule cell [4, 14, 18].

The present study was undertaken to characterize the normal uptake of glutamine and its metabolic derivative, glutamic acid, by isolated rat renal brushborder membrane vesicles. Use of the isolated membrane preparation allows for determination of luminal transport properties in the absence of extensive cellular metabolism.

Materials and Methods

All chemicals were of the highest purity available. Unlabeled amino acids were obtained from Mann Research Laboratories. The following labeled compounds were obtained from the New England Nuclear Corporation: L-[U-^{14}C] glutamine (251.4 mCi/mM); L-[U-^{14}C] glutamic acid (226 mCi/mM); and 3-0-[methyl-^{3}H] methyl-D-glucose (3.62 Ci/mM). Purity was determined by paper chromatography and found to be greater than 98% for all radioactive compounds. N-2-Hydroxyethyl-piperazine-N-2-ethanesulfonic acid (HEPES) buffer was purchased from Calbiochem.

Preparation

Adult male Sprague-Dawley rats fed *ad libitum* on Purina rat chow and water were sacrificed by decapitation. The kidneys were removed, decapsulated and placed in saline on ice. Membranes were isolated according to the method of Booth and Kenny [1] with minor modifications. Kidney cortical slices were obtained using a Stadie-Riggs microtome

and were homogenized (1:10, w/v) in MT buffer (10 mM mannitol + 2.0 mM Tris), pH 7.1, using three strokes by hand in a Dounce homogenizer and two strokes in a tight fitting Potter-Elvehjem homogenizer with a Teflon pestle at 1,000 rpm. Solid magnesium chloride was added to a final concentration of 10 mM, and the homogenate was stirred for 15 min on ice. The homogenate was then centrifuged at 0–2° for 12 min at $1,250 \times g$ in a Sorvall RC-2B refrigerated centrifuge. The supernatant was removed and centrifuged for 12 min at $16,000 \times g$. The fluffy white upper pellet was then resuspended in 1/2 original volume of MT buffer and again homogenized by using one stroke by hand in a tight fitting Potter-Elvehjem homogenizer with a Teflon pestle. Solid magnesium chloride was again added to a final concentration of 10 mM and the suspension was stirred for 15 min on ice. The suspension was then centrifuged at 0–2° for 12 min at $1,500 \times g$. The supernatant was removed and centrifuged for 12 min at $16,000 \times g$. The fluffy white membrane pellet was resuspended in MT buffer and recentrifuged for 12 min at $16,000 \times g$. The final pellet was suspended in Na^+ free buffer to a concentration of approximately 0.4–0.5 mg/ml as determined by the method of Lowry *et al.* [8]. The membrane vesicles showed a 12- to 14-fold increase in alkaline phosphatase activity over the homogenate, a demonstration of purity equal to brushborder vesicles obtained from free-flow electrophoresis [5].

Transport Studies

Glutamine and glutamic acid uptake was studied by the techniques of McNamara *et al.* [10]. Because of the acidity of higher concentration of glutamic acid, the buffer system employed by Segal *et al.* [17] was used for examining the kinetics of uptake of glutamic acid.

Analysis of Metabolic Activity

Analysis of metabolic activity was performed as described previously [10]. Aliquots (1 ml) of freshly prepared membrane vesicle suspension were incubated with 0.06 mM [^{14}C]glutamine or 0.06 mM [^{14}C]glutamic acid in the appropriate THM buffer (2 mM Tris-N-2-hydroxyethylpiperazine-N′-2 ethanesulfonic acid + 100 mM mannitol), pH 7.4, plus 100 mM NaCl for varying time intervals at 22 °C. At the end of each time interval, trichloroacetic (TCA) was added to the mixture to a final concentration of 10%, placed on ice for 10 min, and then centrifuged at 10,000 rpm for 10 min at 0–2 °C. The components of the supernatants were separated by descending paper chromatography on Whatman 3 MM cellulose in Butanol/acetic acid/water (24:6:10, v/v/v). The chromatograms were cut into 1/2-cm sections and assayed for radioactivity. Amino acids in the incubation and in known controls were localized on the chromatogram by ninhydrin reaction and by determination of radioactivity. The evolution of $^{14}CO_2$ from [^{14}C]glutamine and [^{14}C]glutamic acid was measured by the method of Weinstein and Segal [20].

Results

Time Dependence of Uptake

Glutamine and glutamic acid uptake by brushborder vesicles are concentration dependent and saturable. Our data for amino acid uptake

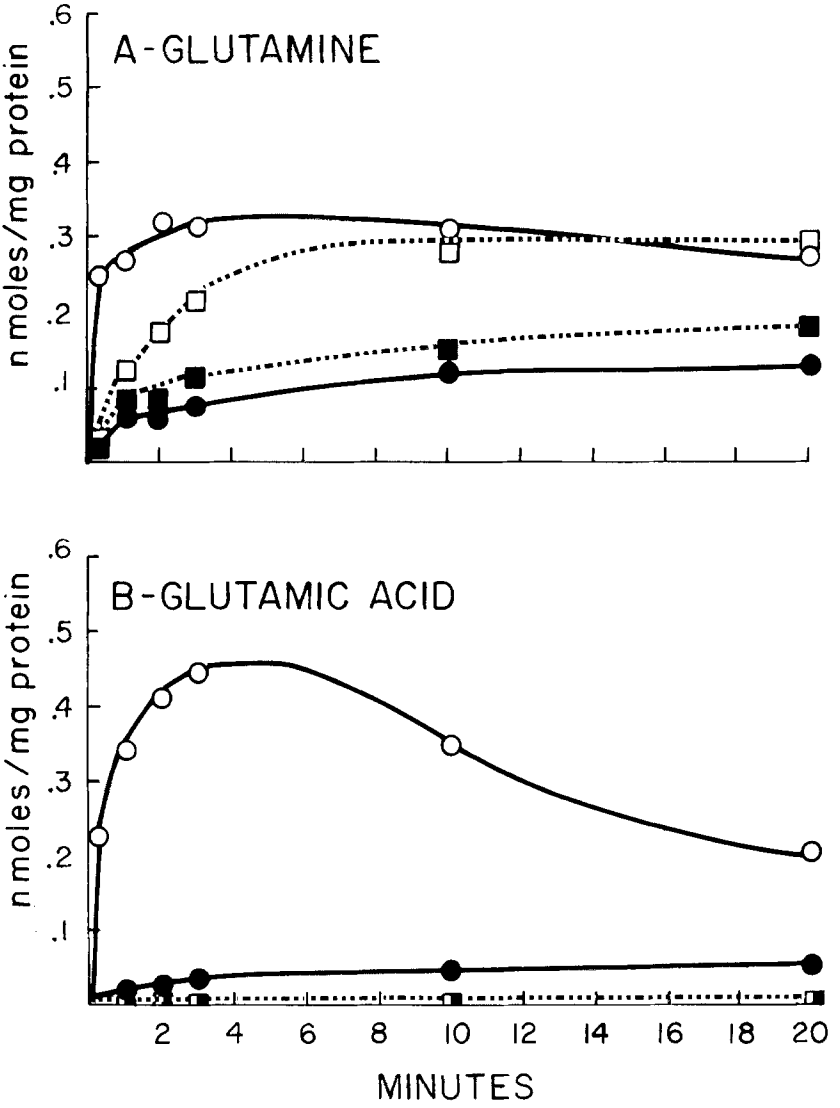

Fig. 1. Uptake of 0.06 mM glutamine (*A*) and 0.06 mM glutamic acid (*B*) by brushborder vesicles in the presence of NaCl (○) or choline Cl (□) gradient and under conditions of equilibration of NaCl (●) or choline Cl (■) between extravesicular and intravesicular spaces. For glutamine uptake studies membranes were suspended in THM buffer (2 mM Tris-HEPES + 100 mM mannitol), pH 7.4, at 22 °C, and 0.5 ml of membrane (0.4–0.5 mg protein/ml) was incubated with 0.1 μCi ^{14}C-glutamine, 0.1 μCi 3-0-[methyl-^{3}H] methyl-D-glucose and 100 mM NaCl (○) or choline Cl (□). Unlabeled glutamine was added to make a final concentration of 0.06 mM glutamine. Membranes were incubated for time intervals from 15 sec to 20 min in a disposable tube. After the incubation interval the mixture was transferred by Pasteur pipette to a Millipore filter apparatus. Uptake was stopped by rapid filtration through a Millipore filter (HAWP 0.45 nm) and washed once with 5 ml of buffered saline, pH 7.4. Filters were dried and counted in a Packard Tricarb Scintillation Spectrometer as previously described [10]. For conditions of Na^+ equilibration (●) or

are expressed as nmol of amino acid taken up per mg protein in excess of 3-0-methyl-D-glucose, thus emphasizing the carrier-mediated uptake of amino acids while correcting for diffused or trapped space. We have previously reported on the use of 3-0-methyl-D-glucose to measure trapped and diffused space [10], and Silverman *et al.* [19] have shown it does not interact with the brushborder surface. In all our studies, 3-0-methyl-D-glucose behaved the same as L-glucose.

Figure 1 *A* shows the typical pattern of uptake of 0.06 mM glutamine, pH 7.4, by brushborder vesicles in the presence of a NaCl or choline Cl gradient and under conditions of equilibration of NaCl or choline Cl between extravesicular and intravesicular space. The uptake of glutamine appears to be stimulated by both NaCl and choline Cl gradients with stimulation in the presence of Na^+ being greater than in the presence of choline$^+$. The initial rate of 0.06 mM glutamine uptake in the presence of a Na^+ gradient is 6 times that seen when Na^+ is equilibrated. In contrast, the initial rate of 0.06 mM glutamine uptake in the presence of a choline gradient is 1.4 to 2.0 times greater than the initial uptake seen when choline is equilibrated. The typical "overshoot" for Na^+-stimulated amino acid uptake seen with glycine and proline [10] is not observed although Na^+ dependence is clearly demonstrated.

Figure 1 *B* shows the typical pattern of uptake of 0.06 mM glutamic acid, pH 7.4, by brushborder vesicles in the presence of a NaCl or choline Cl gradient and under conditions of equilibration of NaCl or choline Cl between extravesicular and intravesicular space. Glutamic acid uptake exhibits a Na^+ dependent "overshoot" phenomenon of stimulated uptake similar to that seen with glycine and proline. Maximal uptake due to the Na^+ gradient occurs at 3 min and at that time is 11-fold that

choline equilibration (■), the vesicles were preincubated with 100 mM NaCl or 100 mM choline Cl for 40 min before incubation with labeled substrate. Membranes were suspended in 20 mM THM buffer (20 mM Tris + 20 mM HEPES + 60 mM mannitol) for glutamic acid (*B*) uptake studies. Unlabeled glutamic acid of the desired concentration in 5 µl of 0.5 N HCl was added to the incubation mixture which contained 0.1 µCi ^{14}C-glutamic acid, and 0.1 µCi 3-0-[methyl-^{3}H]methyl-D-glucose and 100 mM NaCl or choline chloride. The incubation mixture had a final concentration of 0.06 mM glutamic acid and a final pH of 7.4. Membrane vesicles were incubated, filtered, washed and counted as described for glutamine uptake studies. Values for uptake are means of 16 determinations. A Student's *t*-test between time points demonstrates that, for glutamine, the Na^+ gradient curve is statistically different from all other glutamine curves at a level of $P<0.01$. The differences between the Na^+-equilibration curve, choline-equilibration curve, and choline-gradient curve are statistically significant at the $P<0.01$ level. For glutamic acid, the Na^+-gradient curves differ significantly ($P<0.01$) from all other curves which do not differ from each other

under conditions of Na^+ equilibration. Little or no uptake in excess of diffusion occurs under conditions of choline gradient or equilibration.

The hydrolysis of 0.06 mM glutamine to glutamic acid and ammonia in the presence of brushborder vesicles is negligible at pH 7.4 for short time periods (15 sec). Glutamic acid accounts for only 1.6% of the total label present in the incubation at 15 sec as determined by descending paper chromatography. All subsequent studies of initial uptake (15 sec), therefore, reflect glutamine entry prior to hydrolysis. After 30 min, the metabolism of glutamine by brushborder membranes is significant, glutamic acid accounting for 31.8% of the label present. The uptake curves in Fig. 1*A* reflect total radioactivity only. Chromatographic analysis of brushborder vesicles incubated with 0.06 mM glutamic acid at pH 7.4 shows no significant ($<1\%$) alteration of glutamic acid at any time point up to 30 min. No $^{14}CO_2$ is produced when brushborder membrane vesicles are incubated with 0.06 mM [^{14}C]glutamine or with 0.06 mM [^{14}C]glutamic acid.

Concentration Dependence

Dependence of the initial uptake on substrate concentration was examined in the presence of a Na^+ gradient over the concentration range 0.0177 to 8.82 mM for glutamine and 0.0265 to 2.66 mM for glutamic acid. The Lineweaver-Burk plots for both glutamine and glutamic acids are shown in Fig. 2. The two-limbed nature of the plots for both glutamine and glutamic acid indicates two uptake systems for each amino acid. The observed K_{m_1} for glutamine (Fig. 2*A*) is 0.21 mM with a $V_{\max_1}$ of 1.33 nmol/mg/15 sec over a substrate concentration range of 0.0177 to 0.3051 mM and an observed K_{m_2} of 3.37 with a $V_{\max_2}$ of 10.76 for the substrate concentrations of 0.5281 to 8.823 mM. The observed K_{m_1} for glutamic acid (Fig. 2*B*) is 0.023 with a $V_{\max_1}$ of 0.291 over a substrate concentration range of 0.0265 to 0.0885 mM and an observed K_{m_2} of 3.69 with a $V_{\max_2}$ of 4.37 over the substrate concentrations of 0.4425 to 2.655 mM.

Calculated kinetic parameters were determined by using observed data as initial estimates and finding the best fit to total observed values for initial uptake by nonlinear regression analysis as previously described [10]. The calculated values for initial glutamine uptake are $K_{m_1}=0.145$, $V_{\max_1}=0.62$, $K_{m_2}=8.50$, and $V_{\max_2}=16.90$. The calculated values for

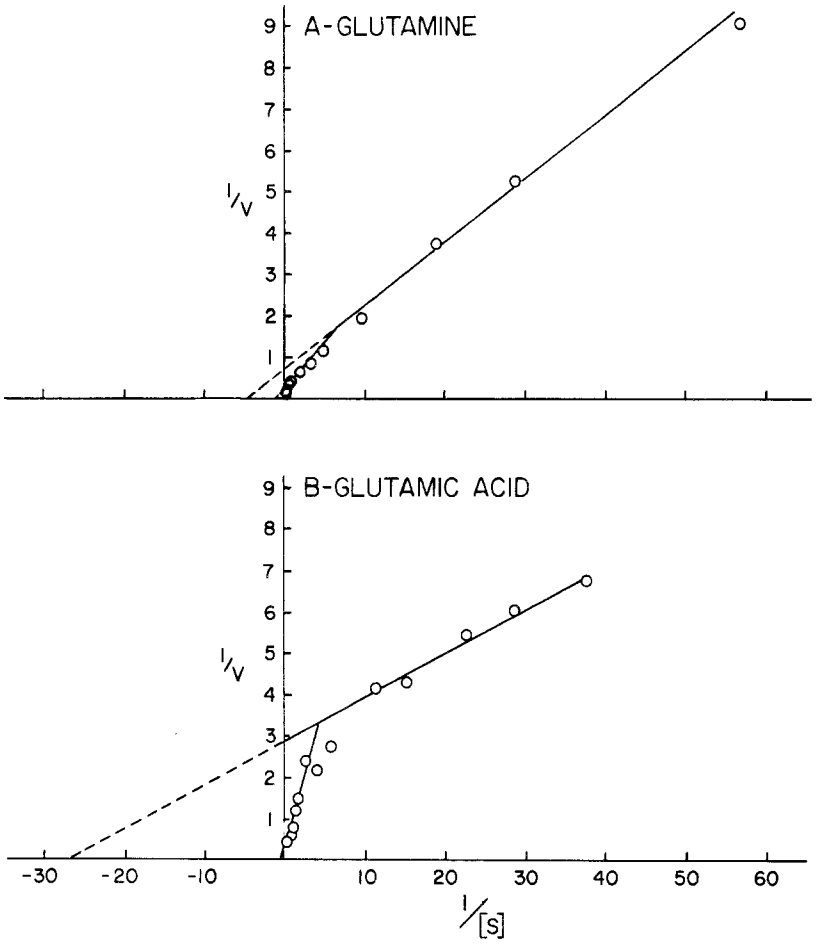

Fig. 2. Dependence of the initial rate of uptake on substrate concentration for glutamine (*A*) and glutamic acid (*B*) by brushborder vesicles in the presence of NaCl gradient. Glutamine uptake (*A*) was studied over a substrate concentration of 0.0177 to 8.8230 mM. Unlabelled glutamine was added to the incubation mixture (0.1 μCi ^{14}C-glutamine, 0.1 μCi-3-0-[methyl-^{3}H] methyl D-glucose, 100 mM NaCl, 0.5 ml membranes in THM buffer, pH 7.4) to achieve desired concentrations. Uptake at 15 sec was studied as described in Fig. 1 for glutamine studies. Glutamic acid uptake (*B*) was studied over a substrate concentration of 0.0177 to 2.655 mM. Unlabeled glutamic acid was added to the incubation mixture (0.1 μCi ^{14}C-glutamic acid, 0.1 μCi 3-0-[methyl-^{3}H]-methyl-D-glucose, 100 mM NaCl, 0.5 ml membrane vesicles in 20 mM THM buffer) to achieve desired concentration. The final pH was 7.4 at all glutamic acid concentrations. Uptake at 15 sec was studied as described in Figure 1 for glutamic acid uptake studies. Values are shown on a double-reciprocal plot and represent the means of 12 to 16 determinations. The K_m's for glutamine are observed to be 0.2068 ± 0.0098 and 3.3663 ± 0.4041, which differ significantly at the $P < 0.001$ level as do the V_{max} values of 1.3337 ± 0.0750 and 10.758 ± 0.8063. The K_m's for glutamic acid are observed to be 0.02261 ± 0.00466 and 3.693 ± 0.237, which differ significantly at $P < 0.001$ as do the V_{max} values of 0.29115 ± 0.01049 and 4.36483 ± 0.31097 for 5 separate experiments

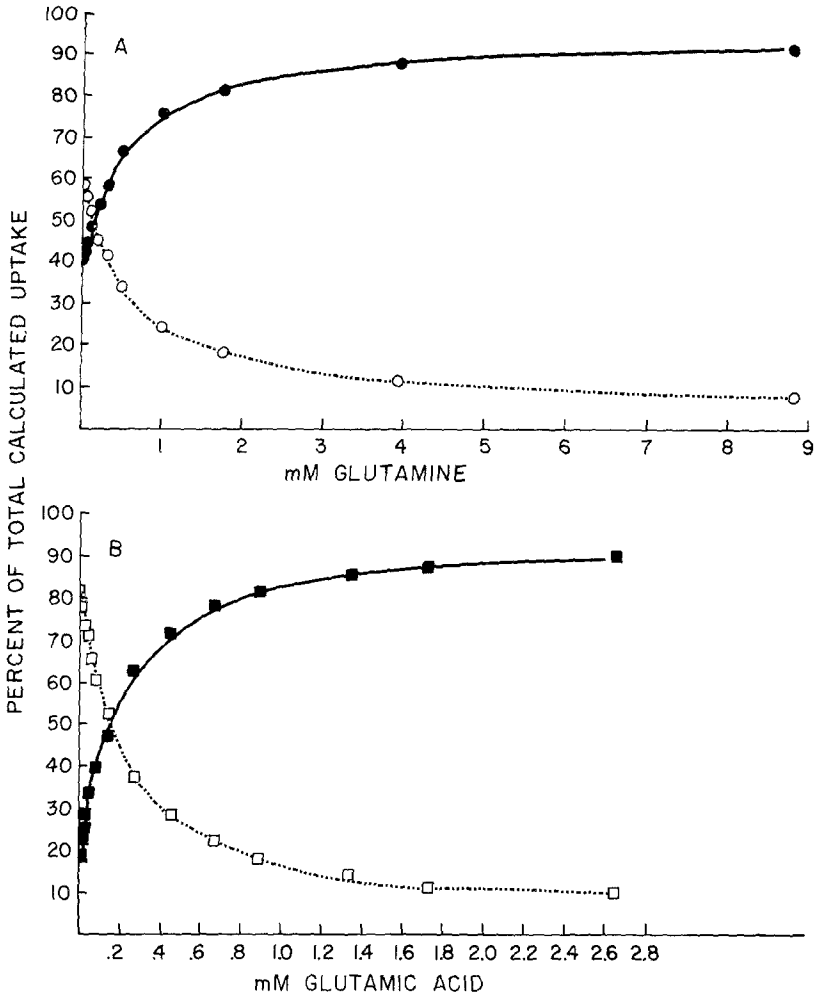

Fig. 3. The relative contribution to total uptake of the low K_m (high affinity) system and the high K_m (low affinity) system calculated for glutamine (*A*) and glutamic acid (*B*) uptake. By using observed kinetic parameters determined from the Lineweaver-Burk plots as an initial estimate, the kinetic parameters for a two-component system for uptake of glutamine (*A*) and glutamic acid (*B*) by brushborder vesicles were calculated as previously described [10]. The relative contribution of each system was determined from Eq. (1):

$$V_{\text{total}} = \frac{V_{\max_1}[S]}{[S]+K_{m_1}} + \frac{V_{\max_2}[S]}{[S]+K_{m_2}} \tag{1}$$

initial glutamic acid uptake are $K_{m_1}=0.016$, $V_{\max_1}=0.19$, $K_{m_2}=3.60$, and $V_{\max_2}=4.00$. The relative contributions of the two transport systems to total uptake of both glutamine and glutamic acid at different substrate concentrations are shown in Figure 3.

Sodium and pH Dependence

The dependence of initial uptake (15 sec) of glutamine and glutamic acid on Na^+ concentration was studied by stepwise replacement of NaCl with choline Cl under conditions of ionic gradients. The results are shown in Figure 4. Figure 4*A* shows the initial uptake of 0.02 and 2.1 mM glutamine. At both substrate concentrations the initial rate of uptake increases with Na^+ concentration. The observed K_m for Na^+ is 13.8 for 0.02 mM glutamine transport where 65% of uptake is calculated to come from the low K_m system. The observed K_m for Na^+ is 10.0 for 2.1 mM glutamine uptake (83% high K_m system). Thus, the high K_m (low affinity) system for glutamine uptake appears to have about the same Na^+ affinity as the low K_m (high affinity) system.

The effect of Na^+ concentration on the initial uptake of 0.03 mM glutamic acid (75% low K_m system) and on 0.60 mM glutamic acid (76% high K_m system) are shown in Figure 3*B*. The observed K_m for Na^+ is 52.1 for 0.03 mM glutamic acid and 76.9 for 0.6 mM glutamic acid. Both high and low K_m systems for glutamic acid uptake express definite affinities for Na^+.

The effect of extravesicular pH on the initial uptake of glutamine and glutamic acid in the presence of a Na^+-gradient was studied. Membrane vesicles prepared in pH 7.1 MT buffer were resuspended in THM buffers of pH values varying from 6.0 to 8.0. The initial uptake of 0.06 mM [^{14}C]glutamine is shown in Figure 5*A* over a pH range of 6.0 to 8.0. A pH optimum for uptake of 6.8–7.1 is observed. Figure 5*B* shows the pattern of initial uptake of 0.06 mM [^{14}C]glutamic acid with increasing extravesicular pH. A pH optimum of 7.2–7.4 is observed. Initial uptake rapidly decreases above and below the pH optimum.

Transport Interaction with Other Amino Acids

Table 1 shows the interactions of various amino acids with the uptake processes for both glutamine and glutamic acid. At the 0.06 mM substrate concentrations used, glutamine uptake occurs via both high and low K_m systems about equally, while about 75% of glutamic acid uptake takes place via the low K_m system. Three mM concentrations of all the amino acids tested significantly inhibits the uptake of glutamine. Glutamic and aspartic acids are among the least inhibitory (about 25%),

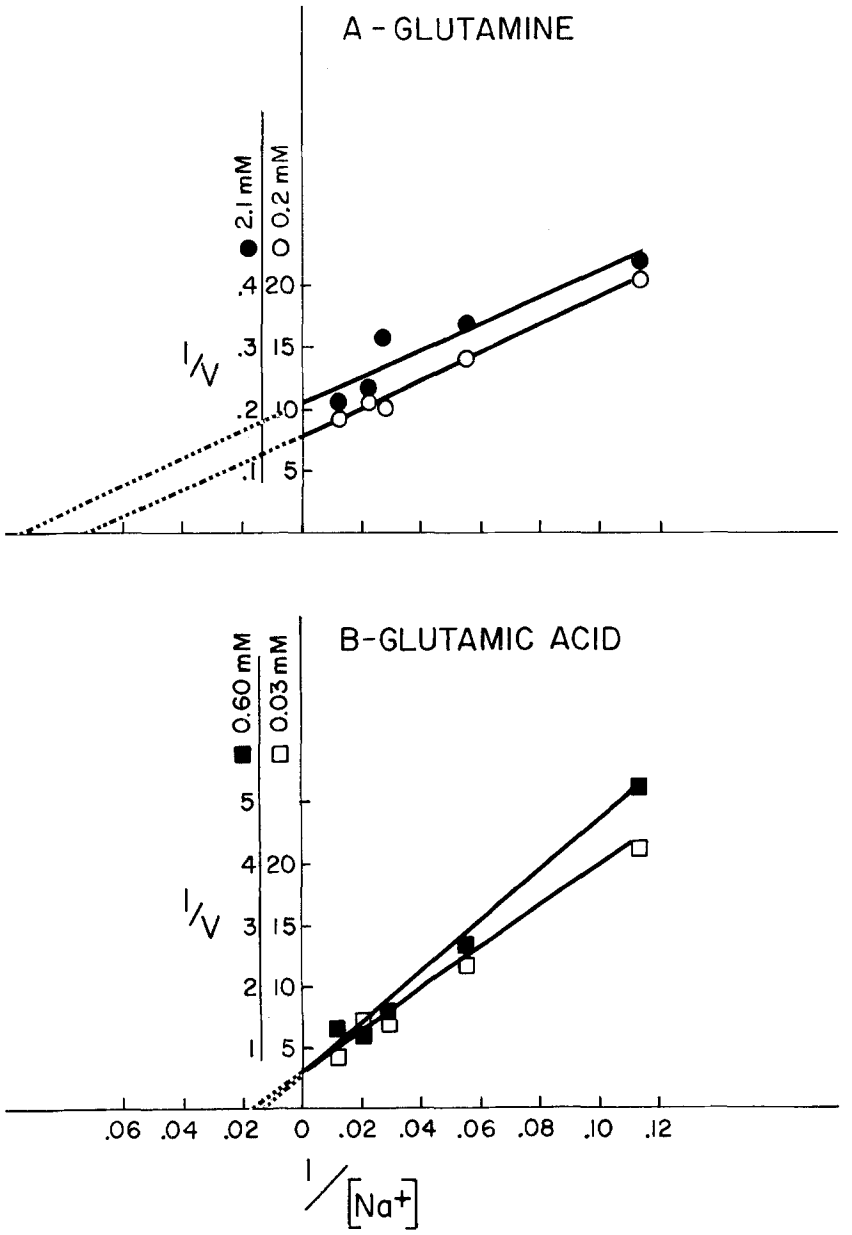

Fig. 4. Effects of NaCl gradient on initial rate of glutamine (*A*) and glutamic acid (*B*) uptake. Membrane vesicles were incubated with 0.02 mM glutamine (○) or 2.1 mM glutamine (●) as described in Figure 1 for 15 sec under gradient conditions where sodium was replaced stepwise by choline, maintaining a total ionic gradient of 200 mosmoles from extravesicular to intravesicular space. Membrane vesicles were also incubated with 0.03 mM glutamic acid (□) and 0.60 mM glutamic acid (■) in standard incubation mixture maintained at pH 7.4 under NaCl gradient conditions described above. All incubation mixtures were filtered, washed, and counted as described in Figure 1. Values are means of 4 determinations

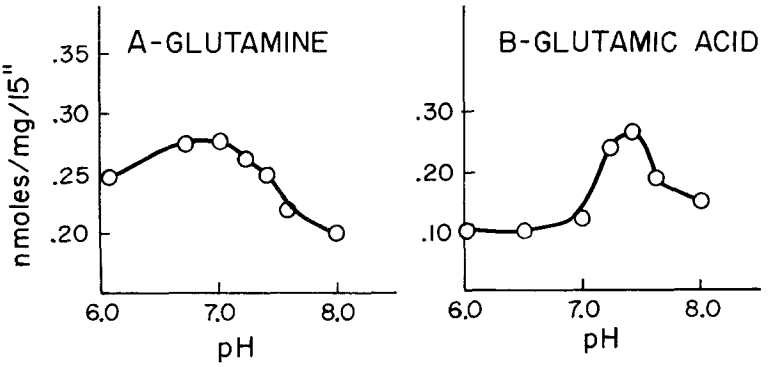

Fig. 5. Effect of extravesicular pH on the initial rate of uptake of 0.06 mM glutamine (*A*) and 0.06 mM glutamic acid (*B*) by brushborder vesicles. Membrane vesicles were suspended in THM buffers at pH values varying from 6.0 to 8.0. Incubations were performed as described in Figure 1 for a 15-sec interval in the presence of a NaCl gradient. Filters were washed once with 5 ml of buffered saline at the same pH as the incubation mixture. Values given are means of 8 determinations. The difference between uptake at pH 6.0 and 7.0 for glutamine is significant to the $P<0.05$ level and between pH 7.0 and 8.0 the difference is significant at the $P<0.01$ level. For glutamic acid the difference between uptake at pH 7.0 and 7.4 is significant ($P<0.01$) as is the difference between pH 7.4 and 8.0 ($P<0.001$)

Table 1. Influence of unlabeled amino acids on initial uptake of 0.06 mM glutamine and 0.06 mM glutamic acid

	% initial uptake remaining	
3 mM unlabeled	^{14}C glutamine	^{14}C glutamic acid
None	100.0±1.96	100.0±3.54
Glutamine	–	77.5±4.10[b]
L-arg	50.6±3.92[a]	93.0±4.30
L-lys	55.0±2.04[a]	107.3±6.60
L-orn	48.8±1.86[a]	90.3±2.63
gly	56.8±2.92[a]	94.7±6.07
L-val	35.3±4.54[a]	79.4±5.93[c]
AIB[d]	77.8±2.92[a]	71.0±8.60[c]
L-pro	70.3±2.88[a]	95.0±4.98
L-phe	30.2±1.07[a]	65.9±4.60[a]
L-glutamic acid	76.4±2.67[a]	–
L-asp	78.2±5.33[b]	–

Influence of unlabeled amino acids on initial uptake (15 sec) of 0.06 mM glutamine and 0.06 mM glutamic acid. Vesicles were incubated with 0.06 mM glutamine or 0.06 mM glutamic acid as described in Figure 1 in the presence or absence of unlabeled 3-mM amino acids for 15 sec. The pH was maintained at 7.4 for all incubations. After 15 sec the incubation mixture was filtered, washed and counted. Values are the mean ± SE of 8 to 12 determinations. [a] $p<0.001$, [b] $p<0.01$. [c] $p<0.02$. [d] AIB = α-aminoisobutyric acid.

while valine and phenylalanine inhibit the most (about 75%). Glutamic acid uptake, on the other hand, is only significantly inhibited by phenylalanine, valine, AIB, or glutamine by about 30%.

Discussion

In this report, we have demonstrated that glutamine is transported by isolated rat renal brushborder membranes by a sodium and pH dependent, saturable system. A sodium and pH dependent, saturable system for glutamic acid was also observed. The dissimilarities in kinetic parameters, pH sensitivity, patterns of uptake in the presence or absence of Na^+ or an electrochemical gradient, affinity for Na^+, and the ability of other amino acids to inhibit uptake between glutamine and glutamic acid seem to indicate that these systems are not shared (Table 2), although the two amino acids do have some inhibitory effect on each other. Glutamine and glutamic acid were studied in parallel to determine if glutamine deamination at the brushborder by membrane bound, phosphate independent, maleate-stimulated glutaminase was an integral part of glutamine transport. Our data suggest that the uptake of glutamine may be independent of hydrolysis, especially since the parameters of glutamic acid uptake are different.

Table 2. Uptake by renal brushborder membrane vesicles

	Glutamine	Glutamic acid
Uptake	Stimulated by choline chloride gradient	No $choline^+$ gradient stimulation
	Na^+ gradient stimulation $>$ $choline^+$ gradient stimulation	Stimulated by Na^+ gradient
Substrate dependence	Two component system	Two component system
	$K_{m_1}=0.145$; $V_{max_1}=0.62$	$K_{m_1}=0.016$; $V_{max_1}=0.19$
	$K_{m_2}=8.50$; $V_{max_2}=16.90$	$K_{m_2}=3.60$; $V_{max_2}=4.00$
Dependence on Na^+	K_m for $Na^+=13.8$ mM for 0.02 mM glutamine	K_m for $Na^+=52.1$ mM for 0.03 mM glutamic acid
	K_m for $Na^+=10.0$ mM for 2.1 mM glutamine	K_m for $Na^+=76.9$ mM for 0.60 mM glutamic acid
Dependence on extravesicular pH	Optimum 6.8–7.1	Optimum 7.2–7.4

For both glutamine and glutamic acid, the dependence of initial velocity of uptake on Na^+ concentration appears to be an example of contransport of Na^+ and amino acid. The direct interactions of Na^+ with the transport systems appear to be saturable phenomena with K_m values for Na^+ in the glutamine system at 10 to 13 mM, while those for Na^+ in the glutamic acid system range from 51 to 77 mM.

A second effect of Na^+ is the influence of an ionic gradient which leads to stimulation of initial uptake. Glutamine uptake is generally enhanced by an ionic gradient and specifically stimulated by a sodium ion gradient. The uptake curve for glutamine in the presence of a choline chloride gradient (Fig. 1*A*) is increased over the value for uptake under conditions of choline chloride equilibrium. The curve for glutamine uptake in the presence of a NaCl gradient demonstrates a greater total stimulation by Na^+ gradient. By subtracting the chloride gradient-stimulated curve from the NaCl gradient-stimulated curve, a rapid, transient overshoot due to Na^+ gradient alone can clearly be seen. Glutamic acid uptake, on the other hand, shows only specific stimulation of uptake by a Na^+ gradient. No enhancement of uptake by a choline chloride gradient is observed.

The results of the studies of inhibition of glutamine and glutamic acid uptake by other amino acids suggest that the processes involved are different. Glutamine is not a potent inhibitor of glutamic acid entry nor is glutamic acid a strong inhibitor of glutamine uptake. The glutamine uptake is inhibited by many amino acids and glutamine may share a transport system with them. Apparently, the addition of the amide nitrogen group provides a structural change which makes glutamine similar to both neutral and dibasic amino acids for transport. The exact interrelationships must await more detailed kinetic analyses varying substrate-inhibitor ratios in order to discern the nature of the interactions.

The importance of the renal metabolism of glutamine has long been established. Van Slyke *et al.* [22] demonstrated that glutamine is the major precursor of urinary ammonia in the dog. This also has been shown to be true in man [12] and rat [15]. Mammalian kidney has two pathways for the utilization of glutamine to produce urinary ammonia [2, 3]. The major pathway involves glutaminase I [3.5.1.2]. The minor pathway involves glutamine transaminase [2.6.1.15]. Both of these enzymes are located in the proximal tubule cell and are dependent on an intracellular accumulation of glutamine. Therefore, transport is an important step leading to the deamination of glutamine and the production of urinary ammonia. Luminal transport is extremely effective in

reabsorbing glutamine and avoiding excessive losses of glutamine in the urine. Under normal conditions less than 1% of the filtered glutamine appears in the urine. The plasma concentration and therefore the glomerular filtrate concentration of glutamine and glutamic acid in rats average 0.78 and 0.15 mM, respectively [6]. Using kinetic parameters calculated from these experiments, 33% of the reabsorption of glutamine would be mediated by the low K_m system and 67% by the high K_m system, while reabsorption of glutamic acid would be mediated by both the low K_m and high K_m systems equally.

Intracellular concentrations in excess of extracellular concentrations can be achieved by either an active transport system or passive diffusion in the presence of several restricted intracellular pools. It has been shown that tubular urine glutamine concentration can be reduced to almost zero, while renal tissue concentrations range from 0.344 μmol/g to 0.631 μmol/g [9]. The prevalent view is that glutamine is actively transported into the proximal tubule cell from the glomerular filtrate, leading to the intracellular accumulation of glutamine. On the other hand, Welbourne [21] has postulated that glutamine transport involves passive diffusion and reflects the rate of utilization by the pathways of glutamine deamination present in the cell. Our study provides support for the existence of a sodium-dependent, saturable uptake system in the brush-border of the renal proximal tubule cell capable of handling the reabsorption of glutamine normally present in the glomerular filtrate.

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