Proc. Natl. Acad. Sci. USA
Vol. 73, No. 12, pp. 4521–4525, December 1976
Cell Biology

Proline and glycine uptake by renal brushborder membrane vesicles

(Na$^+$ gradient dependence)

PAMELA D. MCNAMARA, BOŽENA OŽEGOVIĆ*, LOUISE M. PEPE, AND STANTON SEGAL

Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia and the Departments of Pediatrics and Medicine, Medical School of the University of Pennsylvania, Philadelphia, Pa. 19104

Communicated by James B. Wyngaarden, August 30, 1976

ABSTRACT **Uptake of L-proline and glycine by rat renal brushborder membrane vesicles was seen to be osmotically sensitive, pH dependent, and occurred in the absence of proline and glycine metabolism. The uptake system for proline was Na$^+$ gradient dependent, and exhibited a dual system for entry, K_{m_1} = 0.067 mM and K_{m_2} = 5.26 mM. The uptake of glycine was also Na$^+$ gradient dependent, and exhibited a two K_m system, K_{m_1} = 0.22 mM and K_{m_2} = 4.00 mM. Studies of proline and glycine interactions indicate a shared site which has a lower affinity and higher capacity for glycine than for proline. The high affinity glycine site and low affinity proline site do not appear to be shared.**

The occurrence of inherited iminoglycinuria in man as well as a hyperexcretion of proline and glycine in the normal human neonate prompted numerous studies to delineate the mechanism of renal tubular reabsorption of proline and glycine. These employed rat renal cortical slices or isolated rabbit renal tubules *in vitro* and resulted in the concept of multiple systems for tubule cell uptake of glycine and proline (1–3). Inherent, however, in the use of such model systems is the difficulty of interpretation of transport data because of involvement of the two types of membranes of the proximal tubule cell, and the rapid metabolism of the substrates by these cells. Amino acid uptake by the tubule cells of the cortical slice or isolated tubule may take place either from the luminal brushborder or the basal smooth membrane, thereby making it difficult to discern the contribution of these membranes independently. In addition, proline taken into such cells was observed to be largely converted to intracellular glutamate, a process which may be a regulatory factor in the transport of proline across the membrane (4).

Recently, Kinne and his associates described the preparation of purified rat kidney brushborder membranes and reported the uptake of phenylalanine (5) by vesicles prepared from such membranes by the method of Hopfer *et al.* (6). Such a preparation obviates the objections for using intact cell preparations for determinations of the characteristics of renal brushborder transport properties.

MATERIALS AND METHODS

Membrane Preparations. Adult male Sprague-Dawley rats fed *ad libitum* on Purina rat chow and water were sacrificed by decapitation, the kidneys removed, decapsulated, and placed in saline on ice. All operations thereafter were performed at 4°. Kidney cortical slices were obtained with the Stadie-Riggs microtome and were homogenized (1:5, wt/vol) in a solution containing 0.25 M sucrose and 0.01 M triethanolamine hydrochloride at pH 7.4, using three strokes by hand in a Dounce homogenizer (A pestle) and one stroke in a tight fitting Potter-Elvehjem homogenizer with a Teflon pestle at 1000 rpm. The brushborder membranes were isolated by differential centrifugation by using the method of Pockrandt-Hemstedt *et al.* (7). The brushborder membranes were resuspended by homogenization (3 strokes in the Potter-Elvehjem homogenizer) in a volume of 1 mM Tris·*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid + 100 mM mannitol (THM buffer) at pH 7.4, equal to the original volume of cortical homogenate. The suspension was centrifuged for 20 min at 20,000 × *g*. The fluffy white upper ellet was resuspended in the same manner in THM buffer and centrifuged for 8 min at 30,000 × *g*, and the resultant pellet again resuspended in THM buffer and recentrifuged for 10 min at 30,000 × *g*. The final pellet was suspended in THM buffer to a concentration of 0.5 mg/ml as determined by the method of Lowry *et al.* (8). This vesicle preparation from brushborder membranes showed an alkaline phosphatase enrichment of 8-fold over the starting material while Na$^+$-K$^+$-ATPase was enriched only 2-fold.

Transport Studies. Membrane vesicles, suspended in Na$^+$-free THM buffer at pH 7.4, were used for the transport studies. The standard uptake experiment, which is under conditions of a Na$^+$ gradient unless otherwise stated, consisted of 0.5 ml of freshly prepared membrane vesicles in THM buffer at 22°, which was added at the starting time to a disposable 10 × 75 mm test tube containing 0.1 μCi of ^{14}C-labeled amino acid, 0.1 μCi of 3-*O*-[*methyl*-^{3}H]methyl-D-glucose, 50 μmol of NaCl, and unlabeled amino acid to bring the incubation mixture to the desired final concentration of amino acid. The mixture was stirred on a Vortex genie for 6 sec. Membrane vesicles in THM buffer were preincubated with 100 mM NaCl for 40 min in experiments where uptake in the absence of Na$^+$ gradient was to be studied.

At various times ranging from 15 sec to 20 min, the incubation mixture was transferred by pasteur pipette to a Millipore filter apparatus. Uptake was stopped by rapid filtration of the mixture through a Millipore filter (HAWP, 0.45 nm) and washed once with 5 ml of 154 mM NaCl in 1 mM Tris·*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, at pH 7.4. Filtration of the incubation mixture took less than 2 sec and the entire filtration and washing procedure was completed in 15 sec. The filter was air dried and counted in a Packard Tricarb Scintillation Spectrometer in 10 ml of dilute Liquifluor (New England Nuclear Corp.). Counting efficiency and crossover values were determined using the automatic external standard. Medium samples from appropriate incubation mixtures were counted in each experiment in 10 ml of dilute Liquifluor plus 0.5 ml of Soluene 100 (Packard).

In each experiment, background values for retention of radioactivity by the Millipore filters were determined by filtration of incubation mixtures containing buffer only (no membranes) or incubation mixtures with membranes denatured by boiling for 1.5 min. Background values varied with incubation conditions and were subtracted from the experimental values.

Abbreviation: THM buffer, 1 mM Tris·*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 100 mM mannitol at pH 7.4.

* Visiting investigator. Present address: Laboratory of Experimental Medicine, University of Zagreb, Zagreb, Yugoslavia.

Analysis of Metabolic Activity. Aliquots (1 ml) of freshly prepared membrane vesicle suspension were incubated with 0.06 mM [^{14}C]proline or 0.06 mM [^{14}C]glycine in THM buffer + 100 mM NaCl for 30 min at 22° in sealed incubation flasks containing glass centerwells (9). After 30 min, 1 ml of Hyamine (Packard) was injected into the centerwell and 0.3 ml 3 M H_2SO_4 was injected into the incubation medium. $^{14}CO_2$ formed after uptake was released and absorbed by the Hyamine over a 45-min period of shaking at 22°. The Hyamine was then counted in 14 ml of dilute Liquifluor. Controls run in parallel contained ^{14}C-labeled amino acid either in buffer only or incubated with freshly prepared kidney tubules (10).

Mixtures containing brushborder vesicles which had been incubated under standard conditions for 30 min were treated with trichloroacetic acid (final concentration 10%) and the components of the supernatants separated by descending paper chromatography on Whatman 3MM in butanol:acetic acid: water (24:6:10, vol/vol). The chromatograms were cut into 1 cm sections and assayed for radioactivity. Amino acids in the incubations and in known controls were localized on the chromatogram by ninhydrin reaction as well as by determination of radioactivity.

Materials. All chemicals were of the highest purity available. Unlabeled amino acids were obtained from Mann Research Laboratories. The following labeled compounds were obtained from New England Nuclear Corp.: L-[*U*-^{14}C]arginine (226 mCi/mM); [*U*-^{14}C]glycine (102 mCi/mM); L-[*U*-^{14}C]lysine (306 mCi/mM); L-[*U*-^{14}C]proline (251 mCi/mM); and 3-*O*-[*methyl*-^{3}H]methyl-D-glucose (3.62 Ci/mM). Triethanolamine hydrochloride was purchased from Boehringer-Mannheim and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer came from Calbiochem.

RESULTS AND DISCUSSION

General Characteristics of Amino Acid Uptake by Brushborder Vesicles. The vesicle preparation we have employed is osmotically active and is similar to that of Evers *et al.* (5). The amount of amino acid taken up by the vesicles after 5 min of incubation decreases linearly as the osmolarity of the solution is raised by the addition of sucrose, thus indicating transport into an osmotically reactive intravesicular space. The data do, however, indicate by extrapolation that, at an infinitely high osmolarity when intravesicular space would be negligible, there exists a degree of association of amino acid with the brushborder components which may reflect simple binding.

In our experiments we have also used 3-*O*-methyl-D-glucose to measure trapped and diffused space within the vesicles. The choice of 3-*O*-methyl-D-glucose as a control substance was made after careful analysis of its transport properties into cortical slices as well as into the membrane vesicles. We found no evidence for active or facilitated transport of this compound into cortical slices, and found no effect of 3-*O*-methyl-D-glucose on the uptake of any amino acid or of α-methyl-D-glucoside. In the vesicle preparations, 3-*O*-methyl-D-glucose reacted linearly to osmotic perturbations and extrapolation of the data to an infinitely high osmolarity showed the line to pass through the origin of the graph, which gave no indication of any binding to the membranes. No stimulation of uptake of 3*O*-methyl-D-glucose was observed in the presence or absence of a Na^+ gradient or in the complete absence of Na^+. Our data for amino acid uptake are expressed as nmol of amino acid taken up in excess of that of 3-*O*-methyl-D-glucose. Therefore, we are emphasizing the carrier-mediated uptake of the amino acids while correcting for "leakiness" of any vesicle preparation used.

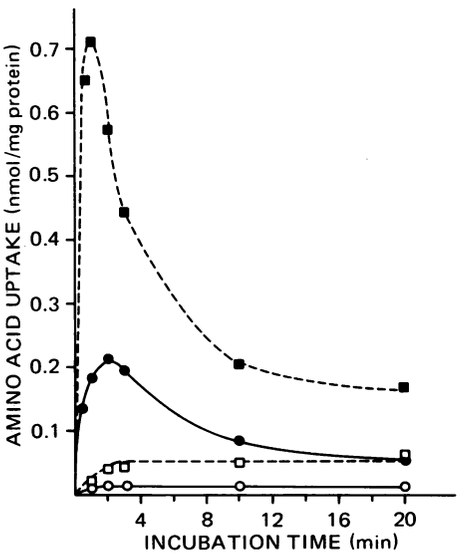

FIG. 1. Uptake of 0.06 mM L-proline (■, □), and 0.06 mM glycine (●, ○) by isolated rat renal brushborder vesicles in the presence (■, ●) and absence (□, ○) of a Na^+ gradient. The vesicles were suspended in buffer containing THM and were incubated with 0.06 mM ^{14}C-labeled amino acid, 3-*O*-[*methyl*-^{3}H]methyl-D-glucose, and 100 mM NaCl as stated in the *text*. For conditions of Na^+ equilibration (□, ○), the vesicles were preincubated with 100 mM NaCl for 40 min before incubation with ^{14}C-labeled amino acid and 3-*O*-[*methyl*-^{3}H]methyl-D-glucose. Values shown are the means of eight determinations.

Chromatographic analysis of the intravesicular contents after incubation for 30 min with 0.06 mM proline or 0.06 mM glycine showed no breakdown of labeled proline or glycine during that time. No $^{14}CO_2$ was produced by brushborder vesicles incubated with either [^{14}C]proline or [^{14}C]glycine while, under the same conditions, isolated tubules metabolized [^{14}C]proline to $^{14}CO_2$ at a rate of 1 nmol/mg of protein in 30 min assuming total conversion of proline to CO_2.

Na^+-Gradient Dependence of Proline and Glycine Uptake. Fig. 1 shows the typical pattern of uptake of 0.06 mM proline and 0.06 mM glycine by brushborder vesicles in the presence of Na^+ gradient and under conditions of Na^+ equilibration inside and outside the vesicles. Both amino acids show the "overshoot" phenomenon of stimulated uptake in the presence of a Na^+ gradient, but no overshoot after Na^+ equilibration. Maximal proline uptake is seen at 1 min while maximal glycine accumulation is seen after 2 min of uptake in the presence of a Na^+ gradient. After 40 min, the same level of uptake is reached by vesicles incubated under the conditions of Na^+ gradient and by vesicles equilibrated with Na^+ before incubation.

When choline chloride is substituted for NaCl in the standard assay, no overshoot phenomenon is observed (Table 1). Under conditions of Na^+ gradient, the rate of uptake of glycine during the first min of incubation is about 13 times faster than in the absence of a Na^+ gradient. For proline, the Na^+ gradient stimulates uptake about 39 times the value in the absence of the gradient. No stimulation of initial uptake occurs in the presence of a choline chloride gradient for either proline or glycine.

Fig. 2 shows the effect of stepwise substitution of Na^+ by choline$^+$ on the initial rate of uptake, defined as uptake in 15 sec, of 0.06 mM proline (Fig. 2A) and of 0.06 mM glycine (Fig. 2B). For both these amino acids, the initial rate of uptake increases as a function of Na^+ concentration. The Lineweaver-Burk plots indicate the apparent K_m for Na^+ is 38 mM in the

Table 1. Uptake of L-proline and glycine by brushborder membrane vesicles

Substrate	Time (min)	Uptake (nmol/mg of protein) Gradient conditions NaCl	Gradient conditions CholineCl	Nongradient conditions NaCl	Nongradient conditions CholineCl
Glycine (0.06 mM)	1	0.1818	0.0191	0.0136	0.0168
	2	0.2119	0.0198	0.0120	0.0071
	3	0.1957	0.0218	0.0144	0.0196
	10	0.0859	0.0568	0.0094	0.0103
	20	0.0561	0.0664	0.0159	0.0336
Proline (0.06 mM)	1	0.7128	0.0467	0.0181	0.0350
	2	0.5765	0.0648	0.0400	0.0395
	3	0.4464	0.0427	0.0414	0.0336
	10	0.2054	0.0797	0.0477	0.0588
	20	0.1167	0.0561	0.0662	0.0752

The vesicles were preincubated either in a medium containing THM buffer or in the same solution containing 100 mM NaCl or 100 mM choline chloride in addition. Uptake was studied as described in Fig. 1. Values given are the means of four to eight determinations.

0.06 mM proline system and is 20 mM in the 0.06 mM glycine system.

Effect of pH on Proline and Glycine Uptake. Because transport of amino acids into cortical slices has been shown to be influenced by pH (11, 12) the effect of extravesicular pH was studied by using membrane vesicles prepared in pH 7.4 buffer, but resuspended finally in THM buffers of varying pH values from 6.0 to 8.0. Fig. 3. shows the patterns of initial uptake of 0.06 mM proline and .06 mM glycine with increasing pH of the medium. Proline reaches a maximum value by pH 7.0 which remains constant between pH 7.0 and 8.0. Glycine uptake reaches its maximum value by pH 7.4 and remains constant through pH 8.0.

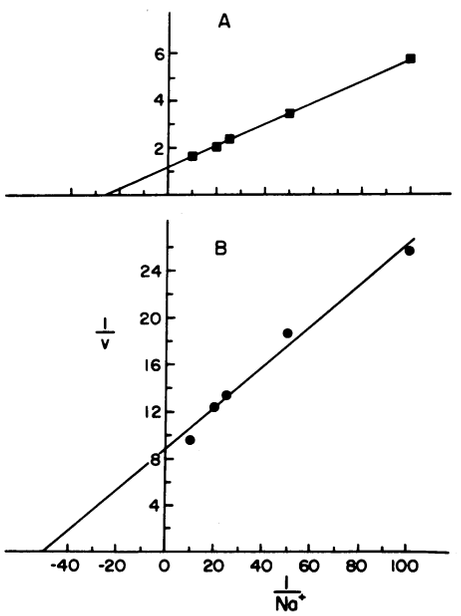

FIG. 2. Effect of $[Na^+]$ on initial rate of 0.06 mM L-proline (A) and 0.06 mM glycine (B) uptake by brushborder vesicles. The vesicles were incubated with ^{14}C-labeled amino acid and 3-*O*-[*methyl*-^3H] methyl-D-glucose for 15 sec under gradient conditions where sodium was replaced stepwise by choline in the standard incubation medium. Values shown are means of eight determinations.

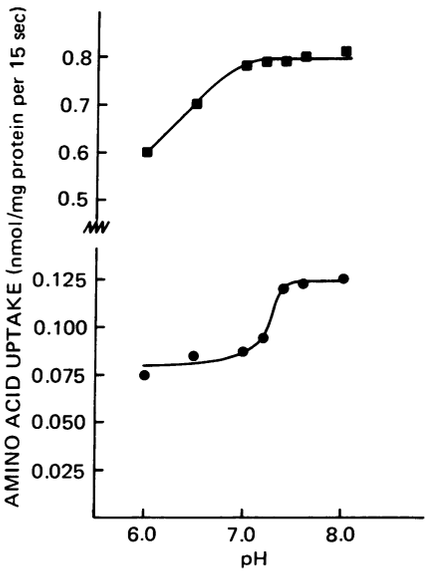

FIG. 3. Effect of pH on initial rate of uptake of 0.06 mM L-proline (■) and 0.06 mM glycine (●). Vesicles were suspended in THM buffers at varying pH values as shown. Incubations were performed as in Fig. 1 under gradient conditions for 15 sec. Values given are the means of four determinations.

Dependence of Proline and Glycine Uptake on Substrate Concentration. The uptake of proline and glycine is concentration dependent and saturable. As shown in Fig. 4, the double reciprocal plot of proline uptake shows a two-limbed curve that indicates the presence of multiple transport systems (13). At low substrate concentrations (0.0184–0.3058 mM proline) an apparent K_m of 0.067 mM and V_{max} of about 1.763 nmol/mg of protein per 15 sec was determined from the Lineweaver-Burk plot. Over a range of higher substrate concentrations, 0.5270–3.9137 mM proline, values for apparent K_m and V_{max} are 1.62 ± 0.30 mM and 4.67 ± 0.64 nmol/mg per 15 sec (mean ± standard error of four separate experiments using a total of 16 determinations per substrate concentration).

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

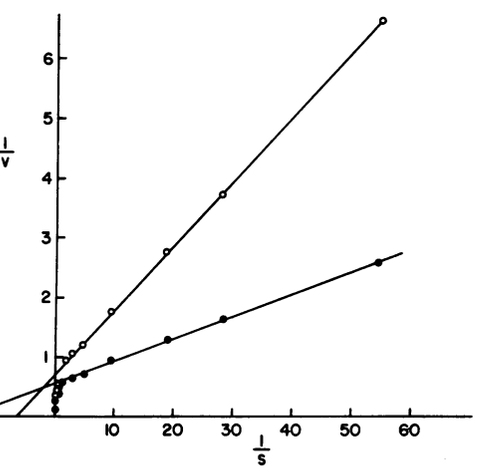

FIG. 4. Influence of L-proline concentration and of 20 mM glycine on the uptake of L-proline by brushborder vesicles. Uptake was studied as described in the *text* using proline concentration of 0.0184–3.9137 mM with (○) and without (●) the addition of 20 mM glycine. Values for 15 sec uptake are given and represent the mean of 10 to 16 determinations for each substrate concentration.

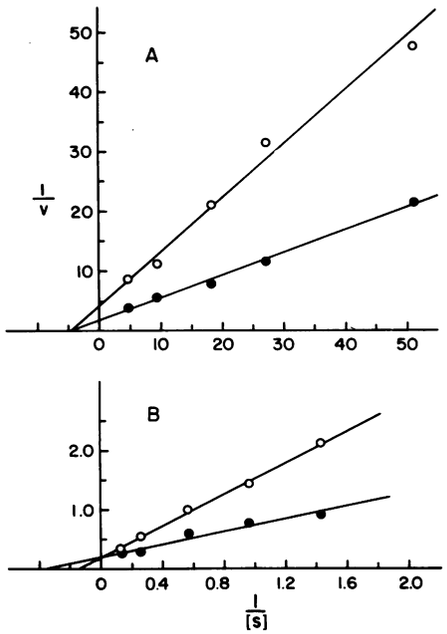

FIG. 5. Influence of glycine concentration and of 20 mM L-proline on the initial rate of uptake of glycine by brushborder vesicles. (A) Uptake after 15 sec of incubation under Na^+ gradient conditions over a range of 0.0195–0.209 mM glycine in the presence (○) and absence (●) of 20 mM L-proline. (B) Uptake after 15 sec of incubation under Na^+ gradient conditions over a range of 0.697–7.629 mM glycine in the presence (○) and absence (●) of 20 mM L-proline. Values given are the mean of 10 to 16 determinations for each substrate concentration.

By using the kinetic parameters determined from the Lineweaver-Burk plots as an initial estimate, we calculated parameters with computer methods from Eq. 1 to give the best fit to the observed total transport data as has previously been described (1). These calculated parameters are $K_{m_1} = 0.067$ mM, $V_{max_1} = 1.76$ nmol/mg per 15 sec, $K_{m_2} = 5.26$ mM, $V_{max_2} = 4.55$ nmol/mg per 15 sec. Nonlinear regression analysis of the original data by a Digital PDP 10 computer substantiated the existence of the two component system for uptake.

Fig. 5 shows the double reciprocal plots of glycine uptake which also exhibited a two-limbed curve. In the low concentration range of 0.0195–0.209 mM glycine (Fig. 5A), the apparent K_{m_1} and V_{max_1} determined from the Lineweaver-Burk plot are 0.20 mM and 0.53 nmol/mg per 15 sec. Fig. 5B shows the data for glycine uptake which was measured over a concentration range of 0.697–7.629 mM glycine. Kinetic parameters derived from the Lineweaver-Burk plot are $K_{m_2} = 2.54$ mM and $V_{max_2} = 4.77$ nmol/mg per 15 sec.

By using the Lineweaver-Burk parameters as initial estimates, we calculated kinetic parameters from Eq. 1 to give the best fit to observed total uptake. These calculated values are $K_{m_1} = 0.22$ mM, $V_{max_1} = 0.18$ nmol/mg per 15 sec, $K_{m_2} = 4.0$ mM, and $V_{max_2} = 6.6$ nmol/mg per 15 sec.

Transport Interactions of Amino Acids with Brushborder Membranes. The uptake of 0.06 mM labeled L-proline, glycine, L-arginine, and L-lysine was measured after 3 min of incubation in the presence of a Na^+ gradient. The effect of 7.5 mM unlabeled amino acids on each of the above ^{14}C-labeled amino acids was also determined. The results are presented in Table 2. Uptake of 0.06 mM proline is inhibited by proline itself and by glycine and phenylalanine. Glycine uptake is also inhibited by these amino acids—glycine itself, proline, and phenylalanine. The dibasic amino acids, arginine and lysine, inhibit themselves and one another but have no effect on proline or glycine. Lysine uptake is slightly inhibited by phenylalanine. These data show a specificity of amino acid interaction with brushborder membrane vesicles which reflects that seen in transport studies with cortical slices (1, 12, 14). The effect of phenylalanine on lysine as well as on proline and glycine has not yet been examined.

Table 2. Interaction of amino acids with brushborder membranes

Additions (7.5 mM unlabeled amino acid)	Uptake of ^{14}C-labeled amino acid (nmol/mg of protein per 3 min)			
	L-Proline (0.06 mM)	Glycine (0.06 mM)	L-Arginine (0.06 mM)	L-Lysine (0.06 mM)
None	0.4429	0.1499	0.0903	0.1753
L-Arginine	0.4579	0.1528	0*	0.0079*
Glycine	0.2164*	0.0368*	0.0918	0.1739
L-Lysine	0.4129	0.1440	0.0087*	0.0019*
L-Proline	0.0437*	0.0894*	0.0903	0.1886
L-Phenylalanine	0.0901*	0.0345*	0.0906	0.1048*

Incubation conditions are described in Fig. 1. Values given are the means of six to eight determinations.
* Differs significantly ($P < 0.001$) from the uptake value when no additions were made.

The nature of the proline–glycine interaction has been examined in more detail in order to determine the underlying mechanism. Fig. 4 shows the effect of 20 mM glycine on proline uptake. In the low proline concentration range, glycine acts as a competitive inhibitor with an apparent K_{m_i} of 0.15 mM for proline in the presence of 20 mM glycine, derived from the double reciprocal plot. In the high proline concentration range, glycine did not significantly affect proline uptake. Fig. 5A shows 20 mM proline to be a noncompetitive inhibitor of glycine uptake in the low glycine concentration range with no alteration in the apparent K_m. In Fig. 5B, on the other hand, 20 mM proline acts as a competitive inhibitor of glycine uptake over the higher glycine concentration range. The apparent K_{m_i} for glycine in this range and in the presence of 20 mM proline is 6.24 mM.

Our studies indicate that glycine and L-proline are transported by isolated rat renal brushborder membrane vesicles by sodium- and pH dependent- saturable transport systems. The data are consistent with a mechanism that catalyzes the cotransport of sodium ion and amino acids such as has been demonstrated in isolated intestinal membranes for L-alanine (15), and isolated renal brushborder membranes for L-phenylalanine (5).

The role of Na^+ in renal brushborder membrane vesicle transport of glycine and proline is, however, 2-fold. The first is in the dependence of initial velocity of uptake on Na^+ concentration which appears to be a saturable phenomenon with a K_m for Na^+ at low amino acid concentrations of 38 mM and 20 mM for proline and glycine, respectively. This is a direct interaction of Na^+ with the amino acid transport system. The second is the influence of the ion gradient which leads to the overshoot phenomenon shown in Fig. 1. As shown in Table 1, the overshoot is not seen if the vesicles are preincubated in Na^+ solution so that there is no difference in Na^+ across the brushborder membrane. Sigrist-Nelson *et al.* (15) and Evers *et al.* (5) have postulated that the electrochemical potential difference of Na^+ across the membrane provides the driving force for

amino acid accumulation and have formulated the events leading to the overshoot (5).

The analysis of the dependence of uptake on concentration of amino acids suggests that there are two systems mediating the transport of glycine and proline, a high K_m (high capacity system) and a low K_m (lower capacity) system. The calculated low K_m of 0.22 mM and 0.067 mM for glycine and proline uptake, respectively, and high K_m of 4.00 mM and 1.76 mM for glycine and proline, respectively, are not too dissimilar from that observed in kidney cortex slices from both Sprague-Dawley (16) and Long-Evans (1) rats. The findings of the dual mechanisms for amino acid uptake by brushborder membrane vesicles suggests that the entry of amino acids into tubule cells of the cortical slice *in vitro* may occur via the luminal brushborder and that the two K_m's do not represent different entry systems of the luminal and basolateral cell membranes. Indeed, Evers *et al.* (5) work with basolateral membrane vesicles of rat tubules indicated that there is an absence of Na^+ dependent phenylalanine transport by these membranes. It should be pointed out that the two systems with differing K_m values may represent two populations of vesicles. Microperfusion studies indicate that a lower K_m system resides in luminal membranes of proximal tubule cells adjacent to the glomerulus while the higher K_m entry system is present in cells farther down in the proximal tubule (17). The two systems for glycine and proline that we observed in our vesicle preparation are not believed to be due to contamination with basolateral membranes since the enrichment of alkaline phosphatase and Na^+-K^+-dependent ATPase reported here is comparable to that of pure brushborder membranes isolated by free flow electrophoresis by Evers *et al.* (5).

The interaction of proline with the glycine uptake system we have observed, correlates well with the observations of Hillman *et al.* (2) using rabbit tubules, and of Mohyuddin and Scriver (1) using Long-Evans rat cortical slices that the glycine high K_m site is common to proline and glycine with proline acting as a competitive inhibitor. The noncompetitive inhibition of glycine uptake in the vesicles by proline over the lower glycine concentration range may reflect the large contribution of the glycine high K_m system to total uptake. At 0.107 mM glycine, a substrate concentration below the low K_m value, almost 70% of total uptake is contributed by the high K_m system according to Eq. 1; thus, the effect of 20 mM proline on uptake in the low glycine concentration range reflects the inhibition of the glycine high K_m system. Our glycine uptake data lend further credence to the existence of a high affinity but low capacity unshared glycine system and a low affinity with high capacity glycine-proline shared system in human kidney (18).

The effects of 20 mM glycine on proline uptake in the vesicles are in agreement with those of Hillman and Rosenberg (3) and are not inconsistent with the observed data using human kidney (4). The low K_m system for proline is shared with glycine-glycine acting as a competitive inhibitor of this system. The shared proline–glycine site exhibits a higher affinity for proline (K_m = 0.067 mM) than glycine (K_m = 4.00 mM) and a lower capacity for proline (V_{max} = 1.76 nmol/mg per 15 sec) than for glycine (V_{max} = 6.60 nmol/mg per 15 sec). No effect of 20 mM glycine was seen on the proline high K_m system. The use of higher glycine concentrations is contraindicated by the evidence of osmotic sensitivity of transport in the vesicle preparation. However, Hillman and Rosenberg (3) have found no inhibition of the high K_m system for proline by glycine concentrations as high as 100 mM using a rabbit tubule preparation. These data disagree with the conclusions of Mohyuddin and Scriver (1) using Long-Evans rat cortical slices.

This work was supported by Grant AM 10894 from the National Institutes of Health, Bethesda, Md.

1. Mohyuddin, F. & Scriver, C. R. (1970) *Am. J. Physiol.* **219,** 1–8.
2. Hillman, R. E., Albrecht, I. & Rosenberg, L. E. (1968) *J. Biol. Chem.* **243,** 5566–5571.
3. Hillman, R. E. & Rosenberg, L. E. (1969) *J. Biol. Chem.* **244,** 4494–4498.
4. Holtzapple, P., Genel, M., Rea, C. & Segal, S. (1973) *Pediat. Res.* **7,** 818–825.
5. Evers, J., Murer, H. & Kinne, R. (1976) *Biochim. Biophys. Acta* **426,** 598–615.
6. Hopfer, U., Nelson, K., Perrotto, J. & Isselbacher, K. R. (1973) *J. Biol. Chem.* **248,** 25–32.
7. Pockrandt-Hemstedt, H., Schmitz, J. E., Kinne-Saffran, E. & Kinne, R. (1972) *Pflügers Arch.* **333,** 297–313.
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
9. Weinstein, A. N. & Segal, S. (1968) *Biochim. Biophys. Acta* **156,** 9–16.
10. Roth, K. S., Hwang, S. M. & Segal, S. (1976) *Biochim. Biophys. Acta* **426,** 675–687.
11. Baerlocher, K. E., Scriver, C. R. & Mohyuddin, F. (1971) *Biochim. Biophys. Acta* **249,** 353–363.
12. Segal, S., Schwartzman, L., Blair, A. & Bertoli, D. (1967) *Biochim. Biophys. Acta* **135,** 127–135.
13. Neame, K. D. & Richards, T. G. (1972) *Elementary Kinetics of Membrane Carrier Transport* (John Wiley & Sons, New York), pp. 41–94.
14. Wilson, O. H. & Scriver, C. R. (1967) *Am. J. Physiol.* **213,** 185–190.
15. Sigrist-Nelson, K., Murer, H. & Hopfer, U. (1975) *J. Biol. Chem.* **250,** 5674–5680.
16. Segal, S., Rea, C. & Smith, I. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 372–376.
17. Lingard, J., Rumrich, G. & Young, J. A. (1973) *Pflügers Arch.* **342,** 13–28.
18. Scriver, C. R. (1968) *J. Clin. Invest.* **47,** 823–835.