Biochimica et Biophysica Acta, 511 (1978) 274—284

BBA 78090

ON THE DEVELOPMENT OF GLYCINE TRANSPORT SYSTEMS BY RAT RENAL CORTEX

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(Received December 13th, 1977)

Summary

The initial uptake of glycine by renal cortical slices from newborn Sprague-Dawley and Long-Evans rats is the same as that observed in adult tissues. Both newborn and adult tissue possess similar high and low affinity glycine transport systems which require an examination of velocity measurements over a wide range of concentration (0.02—50.0 mM) for their discernment. Initial rates of glycine uptake by isolated renal tubule fragments from newborn and adults are similar at a physiological substrate concentration but at high glycine levels there appears to be a decrease in velocity of uptake (V) associated with the high K_m system in the young. Whatever preparation of renal cortex is studied, there is a consistent finding that immature tissue is able to accumulate much higher intracellular levels of glycine than the adult, a finding consistent with slower efflux from the cell. An interpretation of the etiology of physiologic aminoaciduria in young animals should take this into account.

Introduction

For a number of years rat renal cortical slices have served as a model system in which to study the characteristics of sugar and amino acid transport mechanisms in the proximal tubule [1—3]. Using this model, we examined developmental transport changes and reported identical rates of uptake of glycine by newborn and adult Sprague-Dawley rat kidney slices. This observation of similar initial uptake of glycine has been confirmed using isolated rat renal cortical tubules from both the adult and newborn Sprague-Dawley animals [4].

On the other hand, Weber and Cairns [5] reported impaired uptake of glycine by newborn as compared to adult cortical slices in the Wistar rat strain, a pattern of initial uptake also found by Baerlocher et al. [6,7] in the Long-Evans strain. The latter investigators explained their results by finding that the immature Long-Evans rat kidney slice was deficient in a high-affinity glycine-

specific transport system present in the adult animal. Differences in preparative technique, buffer and rat strains have been invoked to explain our own observations and that of Weber and Cairns [5] and Baerlocher et al. [6,7].

Because of the importance of the implication of a single glycine transport system as an explanation for physiological aminoaciduria of the newborn and the discordant results of several previous studies, we have examined glycine uptake and characterized its transport system using various buffers in both the Long-Evans and Sprague-Dawley rat strains. The results of this work form the basis of this report.

Materials and Methods

Adult male (150—200 g) and pregnant females of Sprague-Dawley and Long-Evans strain (Quebec Breed) were obtained from Charles River Breeding Laboratories and fed ad libitum on Purina rat chow. After parturition, pups were used either within 65 h of birth (newborn) or at 7 days of age (immature). No discrimination was made with regard to sex in either newborn or immature animals.

All animals were killed by decapitation. Kidney cortex slices from adult male animals were prepared with a Stadie-Riggs microtome and handled as described in previous reports [1—3]. Newborn and immature kidney cortex was sliced by either of two methods. The first described by Segal and Smith [8] involves making free-hand slices, weighing 1—2 mg each, from the surface with a sharp blade. The second consisted of making a longitudinal single 6—7 mg slice with a chilled Stadie-Riggs microtome according to Baerlocher et al. [6]. Isolated renal tubule fragments were made from adult and newborn animals by the method of Burg and Orloff [9] as modified by Roth et al. [10,11].

Three buffers were employed in these experiments: (1) Krebs-Ringer bicarbonate buffer (Buffer A) whose composition is: 118.5 mM NaCl/4.75 mM KCl/2.53 mM $CaCl_2$/1.2 mM K_2HPO_4/1.2 mM $MgSO_4$/25 mM $NaHCO_3$; (2) Tris/glucose buffer (Buffer B) utilized by Baerlocher et al. [6,7,12] whose composition is: 120 mM NaCl/5.0 mM KCl/5.00 mM $CaCl_2$/1.0 mM $MgSO_4$/15 mM Tris/5.00 mM glucose; (3) Buffer A with 10 mM sodium acetate and 5% fetal calf serum for incubation of isolated tubules (Buffer C).

The technique for determining the in vitro uptake and intracellular content of ^{14}C-labeled amino acids in kidney cortex slices from newborn and adult animals has been described [1,4,13]. When newborn or 7-day-old tissue was studied either three slices made by the razor blade or one slice made by Stadie-Riggs microtome were placed into 30-ml plastic bottles containing 2 ml buffer, unlabeled, and uniformly labeled [^{14}C]glycine, to the stated concentrations. The vessels were gassed with O_2/CO_2 (95 : 5, v/v) sealed and incubated at 37°C in a Dubnoff metabolic shaker for the times indicated. Three adult slices were incubated per flask except where buffer B was used when only one slice was incubated, as described by Baerlocher et al. [6]. At the end of the uptake slices were removed, weighed, extracted and assayed for radioactivity as described previously [1,4]. The uptake was calculated by the technique of Rosenberg et al. [1,14] which has been employed in many reports from this laboratory [1—4]. The results are expressed as the distribution ratio, the cpm/ml intracel-

lular spaces used in the calculations were identical to those reported elsewhere [1,3,6,7]. Glycine uptake in isolated tubule fragments was studied by the method of Roth et al. [15].

In studies of dependence of velocity on substrate concentration, slices were incubated for 30 min and tubules for 10 min, as outlined above. Substrate concentrations were varied from 0.02 to 50 mM for slices and from 0.06 to 50 mM for tubules. Distribution ratios were calculated as described above. The data were initially plotted by the Lineweaver-Burk double-reciprocal method, and the lines fitted with the aid of a Monroe model 1775 calculator by the least-squares method. The data were then replotted by the Hofstee transformation which accentuates the multilimbed lines discerned by the double-reciprocal Lineweaver-Burk plots.

Unlabeled glycine was obtained from Mann Research Laboratories. Labeled compounds, [U-^{14}C]glycine (102 Ci/M) obtained from New England Nuclear Corp. and [*carboxy*-^{14}C]inulin (2.5 mCi/g) obtained from Amersham/Searle Corp. were used in both slice and tubule experiments. Collagenase purchased from Worthington Biochemical Corp. and fetal calf serum purchased from Flow Labs were used during tubule preparation and incubation.

Results

Relationship of rat strain to glycine uptake

The concentrative uptake of glycine by kidney cortex slices of both Sprague-Dawley and Long-Evans strains is shown in Fig. 1. This group of experiments with Sprague-Dawley tissue in bicarbonate buffer reproduces the previously published results from this laboratory [4] and shows again that there is no difference in the initial rate of glycine uptake by cortical slices between adult and newborn tissue. After 30 min of incubation the slices from the newborn accumulate the amino acid to a greater extent and a steady state is not reached by 90 min as it is in the adult. The results with Long-Evans rats under the same incubation conditions are identical to those of the Sprague-Dawley rat and differ from those of Baerlocher et al. [6,7] who reported slower initial uptake in cortex of immature Long-Evans rats.

The uptake of glycine over a wide range of concentration (0.02—50.00 mM) was examined in Buffer A using free-hand cortical slices from the newborn. Figs. 2A and 2B show Hofstee plots of the data for Sprague-Dawley and Long-Evans strains, respectively. The data for the Sprague-Dawley rat in these experiments (Fig. 2A) indicate the same two-limbed curve for both newborn and adult tissue uptake and are consistent with mediation by dual systems, a low K_m, low capacity and a high K_m, high capacity one. The apparent values for K_m and V in Table I are essentially the same as previously reported for Sprague-Dawley renal cortex [4]. The plots of the uptake data for Long-Evans (Fig. 2B) clearly show the same two-limbed curve and Table I reveals the apparent K_m and V for the two transport systems to be essentially the same for newborn and adult tissue. Thus, under identical conditions of incubation there is no difference in the glycine transport systems in the two rat strains. These data differ from those of Baerlocher et al. [6,7] who reported only a single system for uptake by Long-Evans cortical slices from immature animals.

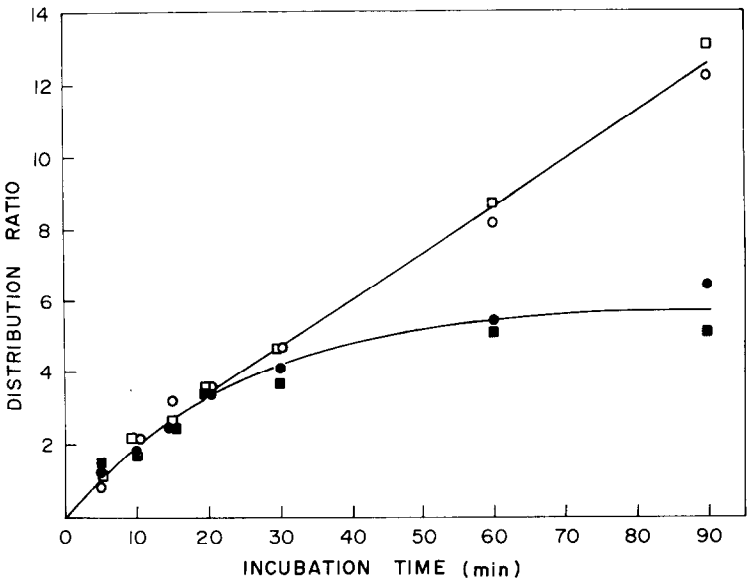

Fig. 1. Uptake of [^{14}C]glycine by rat kidney cortex slices. Sprague-Dawley, newborn (○) and adult (●), or Long-Evans newborn (□) and adult (■) slices were incubated in plastic flasks with 2 ml of Krebs-Ringer bicarbonate, pH 7.4, containing 0.1 μCi/ml labeled glycine and unlabeled glycine such that the final concentration was 0.065 mM at 37°C in a Dubnoff shaker. Free-hand slices of cortex from newborn kidneys were pooled and three slices weighing about 4 mg were added per flask. Three polar slices made with a Stadie-Riggs microtome weighing a total of about 45 mg were employed in adult incubations. The uptake is designated by the distribution ratio of cpm/ml intracellular fluid to cpm/ml medium using tissue water and inulin spaces appropriate for each age in making the calculation. Each point represents the average of triplicate determinations.

TABLE I

APPARENT K_m AND V FOR GLYCINE UPTAKE BY SPRAGUE-DAWLEY AND LONG-EVANS RAT KIDNEY CORTEX SLICES AND ISOLATED CORTICAL TUBULES

Values were calculated from the data shown in Figs. 2, 4 and 6 except for Sprague-Dawley isolated tubules which has previously been reported [15]. Radioactive glycine was diluted with unlabeled compound to give at least 12 concentrations over the range indicated. In each experiment the entire range was examined for both newborn and adult tissues at the same time. K_m is expressed in mmol/l and V as mmol/l per 30 min for slices and per 10 min for isolated tubules.

Strain (buffer)	Age	Concentration range (mM)	K_m	V
Slices				
Sprague-Dawley (Buffer A)	Newborn	0.03—0.50	0.65	3.36
		0.50—50.00	7.56	24.24
	Adult	0.02—0.50	0.94	4.36
		0.50—50.00	9.59	28.28
Long-Evans (Buffer A)	Newborn	0.03—0.50	1.14	5.63
		0.50—50.00	5.28	20.85
	Adult	0.02—0.20	0.79	3.22
		1.00—50.00	11.07	30.64
Long-Evans (Buffer B)	7-day	0.02—0.50	0.38	0.96
		1.00—50.00	9.79	9.47
Tubules				
Sprague-Dawley (Buffer C)	Newborn	0.04—5.00	7.70	30.48
		5.00—50.00	33.02	70.66
	Adult	0.04—0.29	0.74	2.68
		1.03—50.00	31.17	91.33
Long-Evans (Buffer C)	Newborn	0.05—5.00	3.47	16.00
		5.00—30.00	22.85	53.30
	Adult	0.04—0.50	2.10	10.00
		0.50—50.00	26.71	102.45

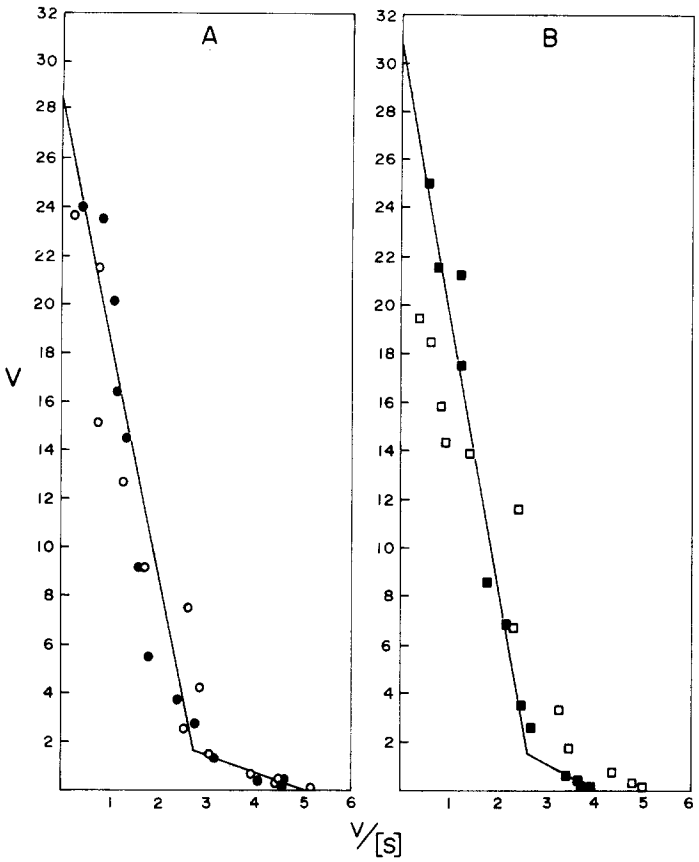

Fig. 2. Hofstee plots for the concentration dependence of glycine uptake. The velocity, V, is mmol/l per 30 min, and [S] is substrate concentration in mM. Slices from either Sprague-Dawley (A), newborn (○) or adult (●), or Long-Evans (B), Newborn (□) or adult (■) were prepared as described in Fig. 1. Slices were incubated at various concentrations of glycine (0.02—50.00mM) for 30 min at 37°C. The lines represent the best fit for the data of adult kidney. The data in B for the newborn tissue is best satisfied on statistical analysis by two lines similar to the adult but with slightly different kinetic parameters as noted in Table I, with lines for high and low K_m values statistically different ($P < 0.001$) [24].

TABLE II

EFFECT OF METHOD OF SLICE PREPARATION ON GLYCINE UPTAKE

Cortical tissue of 7-day-old animals were prepared either by using a razor blade to make free-hand slices or by using a Stadie-Riggs microtome to make slices as described under Materials and Methods. Three cortical tissue free-hand slices weighing a total of 4 mg or one slice weighing about 6 mg was incubated for 30 min in the presence of 0.065 mM glycine in Krebs-Ringer bicarbonate buffer, pH 7.4. The distribution ratio is the ratio of cpm/ml intracellular fluid to cpm/ml medium. Mean ± S.E.; shown in parentheses are the number of determinations.

Strain	Slice method	Distribution ratio
Sprague-Dawley	Freehand	6.20 ± 0.55 (3)
	Microtome	6.39 ± 0.33 (3)
Long-Evans	Freehand	5.82 ± 0.30 (6)
	Microtome	6.93 ± 0.60 (3)

TABLE III

EFFECT OF BUFFER ON GLYCINE UPTAKE BY KIDNEY CORTEX SLICES

Cortical slices of young and adult Sprague-Dawley and Long-Evans rats were incubated for 30 min in either Krebs-Ringer bicarbonate buffer (Buffer A) or Tris/glucose buffer (Buffer B) containing 0.065 mM glycine as described in Materials and Methods. Distribution ration is cpm/ml intracellular fluid to cpm/ml medium. Means ± S.E. of the number of determinations in parentheses.

Strain	Age	Distribution ratio	
		Buffer A	Buffer B
Sprague-Dawley	Newborn	5.77 ± 0.19 (9) *	4.48 ± 0.19 (7)
	7-day	6.33 ± 0.36 (8) *	5.06 ± 0.40 (3)
	Adult	4.59 ± 0.18 (16) *	3.66 ± 0.34 (6)
Long-Evans	Newborn	4.99 ± 0.40 (5)	4.18 ± 0.20 (10)
	7-day	5.68 ± 0.21 (17) ***	3.96 ± 0.32 (14)
	Adult	4.34 ± 0.36 (8) **	3.12 ± 0.20 (11)

Different from Buffer B-incubated slices: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$.

Effect of tissue preparation on uptake

Table II shows a comparison of glycine uptake by cortex slices of 7-day-old rats prepared by free-hand razor blade or Stadie-Riggs microtome, the method employed, respectively, in this laboratory and Dr. C.R. Scriver's unit. There is no statistical difference in the distribution ratio after 30 min incubation between the two preparations.

Effects of buffer

Since Baerlocher et al. [6,7] employed Buffer B, uptake of glycine was compared in that buffer and in Buffer A routinely used in this laboratory. The results are seen in Table III which reveals that at low physiological glycine concentrations the uptake in Buffer B was significantly less than in Buffer A in most instances. Fig. 3 shows that the uptake of 5 mM glycine by adult cortical slices from Sprague-Dawley rats is considerably less in Buffer B. The early uptake values for Buffer B in Fig. 3 correspond to those of Baerlocher et al. [7]

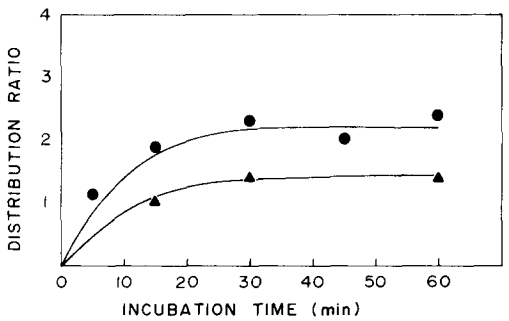

Fig. 3. Uptake of 5 mM glycine by adult Sprague-Dawley rat renal cortex slices. Adult cortical polar slices were prepared using a Stadie-Riggs microtome and were incubated either three slices per flask in Krebs-Ringer bicarbonate buffer (●) or a single slice per flask in Tris/glucose buffer (▲) for times indicated. Incubation conditions are described in Materials and Methods. The distribution ratio is the ratio of cpm/ml intracellular fluid to cpm/ml medium. Each point is the average of triplicate determination.

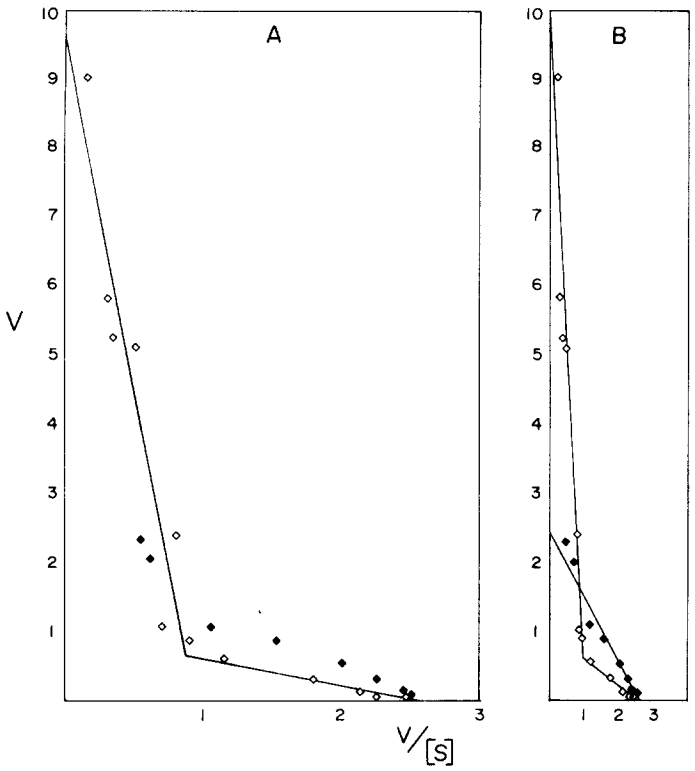

Fig. 4. Hofstee plots of concentration-dependent glycine uptake by kidney slices for 7-day-old Long-Evans rats (◇). Slices prepared via the Stadie-Riggs microtome method were incubated for 30 min in Tris/glucose, pH 7.4, with varying glycine concentrations (0.03—50.00 mM). The points are the average of at least three determinations. The previous glycine uptake data for 7-day-old Long-Evans rat slices reported by Baerlocher et al. [6,7] over glycine concentration (0.01—6.0 mM) is inserted (◆) for comparison. B is a replot of the data shown in A using the ratio of coordinate employed by Baerlocher et al. [6,7] with the line which those investigators published to fit their data. *V* is velocity, nmol/l per 30 min. [*S*] is the substrate concentration in mM.

but the steady-state distribution ratio is lower.

A concentration dependence experiment was performed just as described by Baerlocher et al. [6,7], using kidney cortex slices from 7-day-old Long-Evans rats made with a Stadie-Riggs microtome and Buffer B. A Hofstee plot of the data is shown in Fig. 4A. The curve is shifted to the left and the height is compressed when compared to the curves of uptake in Buffer A (Fig. 2). This is due to the much slower velocity of uptake in Buffer B at all concentrations and not to any difference due to age since the uptake by the 7-day-old tissue is not less than that of the newborn [4]. The most important aspect is that a two-limbed curve is present with apparent K_m and V parameters shown in Table I. In this laboratory, the developmental pattern described by Baerlocher et al. [6,7] has not been obtained even when their conditions were closely reporduced.

Glycine uptake by isolated tubules

In order to further examine the question of developmental changes in

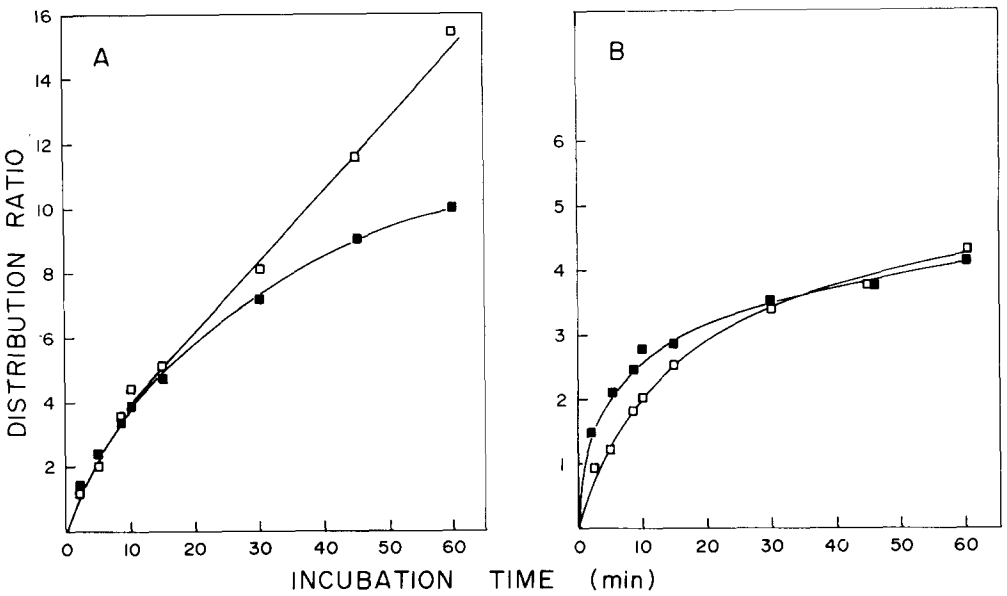

Fig. 5. Uptake of glycine by isolated renal cortical tubules of newborn (□) or adult (■) Long-Evans rats. Tubules were incubated in Krebs-Ringer bicarbonate buffer plus acetate in the presence of either (A) 0.133 mM or (B) 5.0 mM glycine as described in Materials and Methods. Each point is the average of at least six determinations.

glycine uptake, another kidney cortex preparation, the isolated tubule fragment, has been employed. Published data, using Sprague-Dawley tissue [10,15] indicate no initial rate differences in glycine uptake due to age and the presence of two transport systems [15]. Such studies have now been performed with Long-Evans rat kidney tubules and the results shown in Figs. 5 and 6. While the uptake of glycine at a low physiologic concentration shows the newborn tissue to have the same rate as the adult (Fig. 5A), the uptake of 5 mM glycine has a slower rate in the newborn than the adult (Fig. 5B). The concentration dependence of uptake (Fig. 6) reveals two systems for glycine transport in the newborn. In this kidney preparation, the maximum velocity of the newborn high K_m system is less than that of the adult (Table I).

Discussion

We have examined in a systematic way numerous experimental conditions that might explain the reported discrepant observations regarding glycine uptake by renal cortical slices of young rats so that the nature of developmental transport changes in the kidney may be clarified. The question of rat strain influence was approached by reproducing the previously published work with kidney slices from newborn Sprague-Dawley rats [4] which shows dual transport systems identical to that of adult tissue. Concomitant parallel studies with tissue of Long-Evans rat, indeed, showed a similar finding suggesting that investigation of other aspects of the experimental design would have to provide an explanation why Baerlocher et al. [6,7] failed to observe two K_m components

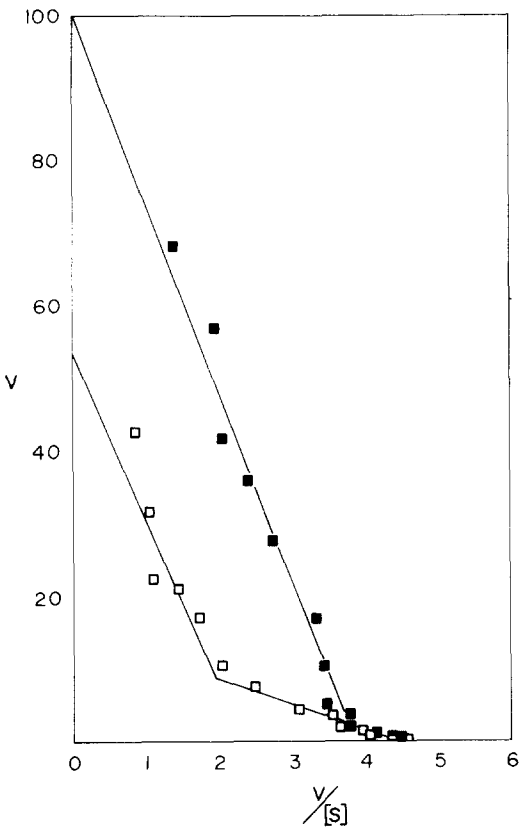

Fig. 6. Hofstee plot of the concentration dependence of uptake of glycine by newborn and adult Long-Evans isolated kidney tubules. Newborn (□) and adult (■) tubules incubated in glycine concentration from 0.06 to 50.00 mM glycine incubated for 10 min in Krebs-Ringer bicarbonate buffer plus acetate as described in Materials and Methods. *V* is velocity in mM/l per 10 min. [*S*] is in mM. Each point is the average of at least four determinations. The data is best satisfied on statistical analysis by two lines. Kinetic parameters are noted in Table I, with lines for high and low K_m values statistically different ($P < 0.001$) [24].

to the glycine uptake process. Assessment of the slicing techniques revealed no difference in uptake whether small free-hand razor slices weighing 1 mg or Stadie-Riggs microtome slices weighing several milligrams were used.

The comparison of buffers, Krebs-Ringer bicarbonate employed in this laboratory and Tris/glucose-containing buffer used in Scriver's laboratory, showed a difference; the velocity of uptake in Buffer B was significantly slower. We have routinely used Krebs-Ringer bicarbonate buffer at pH 7.4 for the following reasons: (1) it closely resembles the plasma buffer system, (2) the regulation of acid-base by the kidney is related to HCO_3^- diffusion, and (3) it is stable and easily prepared. On the other hand, Buffer B with a pK_a of 8.4 is a relatively poor buffer at pH 7.4 with marked temperature and concentration dependence. (Increase in temperature between 22 and 37°C is associated with a drop in pH of 0.025 units per degree.) in the present studies Buffer B was meticulously prepared to account for these factors but still was associated with a

lower glycine uptake. The explanation for this has not been explored but we feel this may be due to the fact that Baerlocher, whose directions were followed, used 95% O_2 : 5% CO_2 to gas the buffer. When Buffer B has been used in this laboratory at higher pH [13,16] 100% O_2 was employed for gassing and oxygenation since there is no need for CO_2 in the system. We found many years ago that the use of 95% O_2 : 5% CO_2 with Buffer B caused low values of amino acid uptake by kidney slices.

The possibility that one of the transport systems observed with young tissue in bicarbonate buffer might not function in Buffer B prompted us to repeat as closely as possible the experiment of Baerlocher et al. of concentration vs. velocity relationship with tissue from the kindeys of 7-day-old Long-Evans rats, microtome slices and Buffer B gassed with 95% O_2 : 5% CO_2 [6,7]. Despite the low velocities a two-limbed Hofstee plot was obtained. There was, however, one design difference between this experiment and that of Baerlocher et al.; that is, we used a wider range of substrate concentration up to 50 mM compared to their maximum of 6 mM. Fig. 4A shows a plot of the data of Baerlocher et al. in relation to our own. A great similarity of velocity values is observed up to 6 mM substrate, the highest concentration used in their experiments. The second high K_m system, however, is more clearly delineated by the use of substrate concentrations above 6 mM. That Baerlocher et al. [6,7] would interpret the presence of only a single system with one K_m value stems also from the nature of their plot. In Fig. 4B is seen a plot of the data with the ratio of ordinate to abscissa used by Baerlocher et al, [6,7] which compresses the abscissa and obscures the fact that the last two points with a V/S value below one might be part of another line. Under these circumstances it appeared appropriate to draw a single line for their data as we did in Fig. 4B. We feel justified in concluding that there are, indeed, two functioning systems for glycine uptake by the immature renal cortical cells of Long-Evans rats.

The main difference in glycine uptake between immature and adult renal cortical slices is the much greater concentration gradients formed by young tissues. The consensus is that this is due to diminished efflux from the cells [4,6, 7]. When, however, we examine glycine uptake by isolated renal tubule fragments some differences other than the higher concentration gradients achieved in the young are observed. In tubules of both Sprague-Dawley and Long-Evans rats there appears to be a decreased velocity (V) associated with the high K_m system in the young. This might be interpreted as being due to a diminished number of binding sites or lesser efficiency of the same number as in the adult. In both strains the velocity of initial uptake at lower physiologic glycine levels is the same as the adult although in Sprague-Dawley newborn tubules both the K_m and the V of the higher affinity system is higher than that of the adult.

The correlation of these findings with the physiological aminoaciduria of the newborn rat [17] and man [18] is uncertain. Since at physiological levels of glycine a significant uptake occurs via the high K_m system, a decreased efficiency of this system seen in newborn isolated tubules could reflect decreased reabsorption in vivo of glycine from plasma filtrate at the liminal membrane. On the other hand, the decreased efflux might reflect diminished exit at the basal-lateral membrane with the concomitant increase in intracellular glycine effecting in a secondary fashion fluxes across the luminal bursbborder as has

been suggested for proline by Scriver et al. [19] in the PRO/Re mouse kidney. The increase in intracellular glycine in renal tubule cells of the immature animal has been suggested [20] and documented in our own laboratory for tubules [15] and fresh slices where the newborn cortex contained 3.41 ± 0.10 and the adult 2.94 ± 0.12 μmol/g wet weight ($P < 0.01$ for 6 and 9 determinations, respectively). These speculations have been made with the realization that the known polarity of cells of the proximal convoluted tubule may be disrupted in vitro in preparations such as the cortex slice or isolated tubule. Recent data would support the inference that uptake does occur at the brushborder membrane. McNamara et al. [21] have demonstrated that the uptake of glycine by isolated rat brushborder membrane vesicles occurs by two systems with K_m values similar to those reported here for slices and tubules. In addition, these processes in brushborder vesicles [21] and slices [13] are sodium dependent whereas uptake of amino acids by vesicles of isolated basal-lateral membranes are not [22,23].

Acknowledgements

This work was supported by N.I.H. Grants AM10894 and HD08536. The authors wish to thank Dr. Charles R. Scriver of Montreal for reading the manuscript and making helpful comments.

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