BBA 75722

TRANSPORT OF L-PROLINE AND α-AMINOISOBUTYRIC ACID IN THE ISOLATED RAT KIDNEY GLOMERULUS*

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(Received April 5th, 1971)

SUMMARY

Isolated rat kidney glomeruli were used to study amino acid transport *in vitro*. Uptake of L-proline and α-aminoisobutyric acid is slower in the glomerulus than in other parts of kidney. Amino acid uptake in glomeruli is dependent on tissue integrity, Na^+ and energy metabolism. The glomerulus can achieve concentrative uptake which can be supported by glycolysis. Uptake is saturable and two systems were identified for L-proline, the transport K_m values of which were 0.1 and 5.3 mM. These values were similar to those of systems serving L-proline transport in the rat kidney cortex slice. α-Aminoisobutyric acid uptake does not reach a steady state, behaving as if efflux were deficient. The K_m value for the α-aminoisobutyric acid mediation was 33 mM; a low-K_m component for α-aminoisobutyric acid transport identified in other tissues was apparently absent in the glomerulus. Glomeruli thus accumulate amino acids by mediations which resemble those of the tubule but which also have characteristics particular to the glomerulus.

INTRODUCTION

Investigation of amino acid transport in mammalian kidney has been characterized in recent years by many technical refinements. *In vitro* methods of investigation have been adapted to cortex slices[1], papilla[2], isolated tubules[3–5] and isolated tubule brush borders[6]. *In vivo* investigations have relied primarily on such measurements as renal clearance, net tubular absorption and arterio-venous extraction ratios[7–12]. However, correlation between *in vivo* and *in vitro* findings was limited[13,14] until the micropuncture technique[15] allowed more direct comparison of amino acid uptake kinetics by kidney fragments *in vitro* with events *in vivo* in the intact nephron. Taken together, these various approaches have yielded much information about amino acid uptake by kidney in general.

To the best of our knowledge other investigators have not yet published on amino acid transport in isolated glomeruli. Since the glomerulus is derived both from the nephron and vascular tissue, and since it serves a function quite different from

Abbreviation: AIB, α-aminoisobutyric acid.

* Publication No. 238, from the McGill University-Montreal Children's Hospital Research Institute.

that of the attached tubule, it seemed appropriate to investigate the characteristics of amino acid transport in this particular tissue. L-Proline and α-aminoisobutyric acid (AIB) were chosen as substrates since they have been used in our own recent work on transport in kidney[14,16], and because their transport has been well documented in various preparations of kidney. The present study is another facet of our examination into the expression of the genes which control proline and glycine transport in normal kidney[13,14,17–19], in mutant phenotypes[19–21] and during ontogeny[22]. A preliminary report on part of this work has appeared previously[23].

MATERIAL AND METHODS

Isolation of glomeruli

Female Long–Evans hooded rats weighing from 150 to 200 g were used in all experiments. After perfusion with buffer and removal of the kidneys, glomeruli were isolated according to the method described by FONG AND DRUMMOND[24], except that a Tris–Ringer–glucose buffer system (300 mosM, pH 7.4) was substituted for Krebs–Ringer–phosphate buffer. The glomerular tissue obtained from three rats was suspended in 16 ml of ice-cold buffer. Oxygen (100 %) was bubbled slowly through the suspension until aliquots of glomeruli were pipetted into prepared flasks.

Incubation and measurements of uptake of amino acids.

Covered siliconized erlenmeyer flasks (25 ml) were used for incubation of the tissue. When CO_2 collection was desired, parallel incubations were carried out in siliconized Warburg flasks, whose center wells contained fluted filter paper (2 cm^2), soaked with saturated KOH solution (50 μl) to absorb CO_2. Recovery of CO_2 from the medium was obtained by acidification with H_2SO_4 (9.25 M) and allowing 120 min equilibration. The efficiency of CO_2 collection by this method is 59 $\pm$ 4 %. Labelled and unlabelled amino acids were added to the flasks in amounts to provide the desired concentration of substrate in the total volume required by the experimental design (usually 4 or 16 ml). The final medium contained about 275000 disint./min per ml.

Incubations were carried out at 37° in a Dubnoff metabolic incubator, oscillating at 300 strokes/min to keep the glomeruli in suspension. At the end of incubation, an aliquot (about 1.3 ml) was pipetted from the flask onto a dry-weight tared, buffer-soaked Millipore filter (average pore size 0.45 μm) suspended over a suction flask. Approx. 6 ml of chilled buffer was added to the chamber and washed rapidly through the filter by vacuum suction. The tissue retained on the filter was washed again with two consecutive volumes (2 ml) of cold buffer. The tissue was then dried on the filters overnight at 80°. Triplicate aliquots were used throughout.

Constant humidity conditions are necessary for very accurate weight determinations of the dried filters and tissue. In the absence of such conditions, and as a control for the weight of buffer salts upon the filters, three control filters which contained no tissue but had been soaked with equivalent volumes of buffer were subjected to the entire experimental procedure. Gain in weight due to retention of buffer salts and variation in weight due to changes in ambient humidity, and the duration and temperature of the drying, were largely corrected for by this procedure.

Counting of ^{14}C radioactivity on the filters was performed with a Nuclear

Chicago gas flow counter operating at 30 % efficiency. Control Millipore filters were used to determine the amount of radioactive isotope adsorbed by the filters; these background counts were subtracted in calculating the radioactivity in tissue. Corrections were also made for the quenching effect of the filter on isotope counting. Measurement of isotope uptake by tissue was most reliable when at least 0.25 mg of dry tissue was present on the filters. When formation of $^{14}CO_2$ was studied, the filter papers from the well of the Warburg flasks were dried in scintillation vials under vacuum and then suspended in scintillation fluid comprising PPO (2,5-diphenyloxazole) 4 g, and POPOP (1,4-bis-[2-(5-phenyloxazolyl)]benzene), 0.1 g, per l of toluene. The vials were then counted in a Unilux II Nuclear Chicago liquid scintillation counter operating at 42 % efficiency.

Determination of tissue water

Glomeruli were prepared and incubated as described above, following which the tissue was scraped from the filters and placed in tared pans of aluminum foil which were immediately weighed to determine the wet tissue weight. After drying overnight at 80°, the pans were weighed to obtain constant dry tissue weight. Total tissue water was determined by subtracting the dry tissue weight from the wet tissue weight.

To determine the extracellular water space, the glomeruli were incubated in medium containing [^{14}C]sucrose 0.025 mM, and phlorizin 3 mM; the latter to inhibit sucrose uptake[25]. The volume of the extracellular space was calculated from the amount of [14C]sucrose retained by the tissue under steady-state conditions.

Sources of materials

Uniformly labelled sucrose (specific activity 5 mC/mmole), uniformly labelled L-proline (specific activity 210 mC/mmole) and α-amino[1-^{14}C] isobutyric acid (specific activity 8.59 mC/mmole) were obtained from New England Nuclear Corporation. Radiochemical purity was confirmed by chromatography in a butanol–acetic acid–water mixture (12:3:5, by vol.) followed by strip scanning. Phlorizin was obtained from J. T. Baker Company, and unlabelled amino acids were obtained from Mann Research Corporation (New York) and Nutritional Biochemical Corporation (Cleveland, Ohio).

Calculations

Distribution ratios, unless otherwise stated, indicate the isotope distribution ratio, that is (counts/min per ml intracellular fluid per unit time)/(counts/min per ml initial medium). The observed uptake velocity was corrected for uptake on the non-saturable component, by the methods of AKEDO AND CHRISTENSEN[26]. The Michaelis constant for the transport system was estimated from data obtained at or near steady-state conditions in experiments using L-proline. It has been shown[16,27] that a satisfactory estimate of the K_m value for binding of substrate with its reactive transport site can be obtained under these conditions. Two transformations of the Michaelis equation were used to plot concentration-dependent uptake data; we used the traditional Lineweaver–Burk method ($1/u$ *vs.* $1/[S]$) and the Eadie–Augustinsson method (u *vs.* $u/[S]$) which is said to be more reliable statistically[28] for evaluation of K_m constant. This was of importance as we had reason to believe that uptake of a single amino

acid in glomeruli might occur by more than one system, and clear evidence of this was desirable. When more than one mode of uptake was in evidence, the assumption was made that the observed uptake was the sum of transport on each component.

RESULTS

Preparation of glomeruli

Light microscopy revealed the preparation to be at least 90% glomerular in composition (Fig. 1). Occasional fragments of tubule were carried through in the preparation, and some glomeruli appeared to retain an intact Bowman's capsule; the proportion of glomeruli with and without capsules varied from one experiment to the next. An average of 8 mg of wet tissue was obtained from each rat.

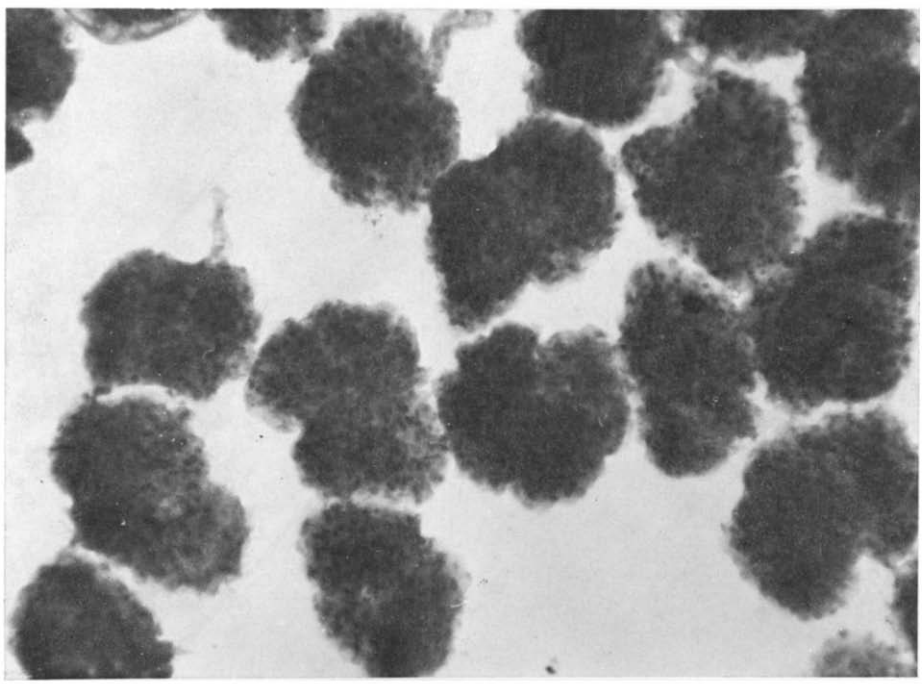

Fig. 1. Photomicrograph of rat glomeruli isolated by method described in text. Glomeruli stained with toluidine blue and photographed at × 156 magnification.

Tissue water

Total tissue water was 84.0 ± 4% of the wet tissue weight. When glomeruli were incubated in the presence of labelled sucrose and phlorizin, a constant sucrose distribution space was obtained within 5 min (Fig. 2); this space, which is equivalent to the extracellular fluid, was 17.6 ± 0.8% of the wet tissue weight. The intracellular fluid space was 66.4% of the wet tissue weight.

Time course of amino acid uptake

The uptake of L-proline and AIB during the course of incubation is shown in Fig. 3. Glomeruli require about 100 min to achieve a steady state for uptake of L-

proline at 0.5 mM in the medium; the isotope distribution ratio was about 4.2 under these conditions. Uptake of AIB at low initial concentrations did not reach a steady state even after incubation of glomeruli for 120 min at 37°. This non-metabolizable amino acid was accumulated against a chemical gradient in these experiments.

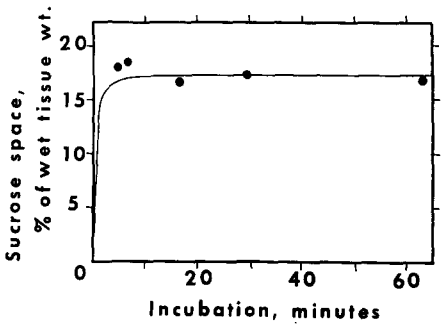

Fig. 2. Distribution of [^{14}C]sucrose (% of wet tissue weight) in the presence of 3 mM phlorizin. Incubations were carried out at 37° in Tris–electrolyte–glucose buffer at pH 7.4.

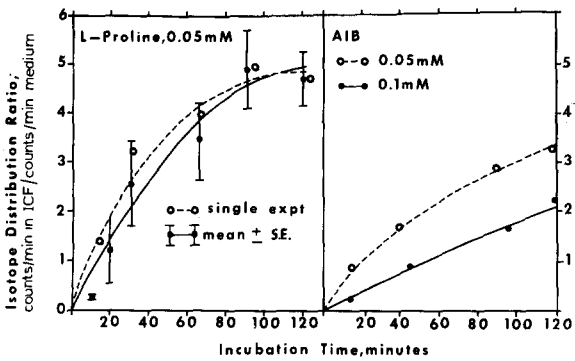

Fig. 3. Accumulation of labelled L-proline and AIB from the medium by isolated rat glomeruli. Incubations were carried out as described in the text. The isotope distribution ratio is (counts/min per ml intracellular fluid)/(counts/min per ml extracellular fluid). The mean ± S.E. for distribution ratio of L-proline uptake at 0.05 mM is shown (●). An average of 5 determinations (range 3–7) were obtained for each point. A single representative experiment is indicated by O. Uptake of AIB is shown at 2 initial concentrations in the medium.

Concentration-dependent uptake

L-Proline. Uptake into glomeruli was examined at 120 min at concentrations of the substrate in the initial medium between 0.04 and 16 mM. Saturable and non-saturable systems are used for L-proline uptake under steady-state conditions as revealed by the Akedo–Christensen plot (Fig. 4). The ordinate intercept for L-proline in Fig. 4 is 1.0 after 120 min incubation; when correction was made for the contribution to total uptake made by the non-saturable component, analysis of the uptake kinetics revealed that glomeruli accumulate L-proline by more than one mediation (Fig. 5). At physiological concentrations (below 0.4 mM in the extracellular fluid) the apparent transport K_m for glomerular uptake of L-proline is 0.17 mM; above this concentration range the Michaelis constant is 4.8 mM.

The theoretical rates of uptake calculated for the two apparent L-proline trans-

port systems were compared with the observed rate of uptake on the combined system (Table I). Twelve different initial concentrations of substrate were used to obtain these measurements. When the calculated uptakes were plotted in relation to

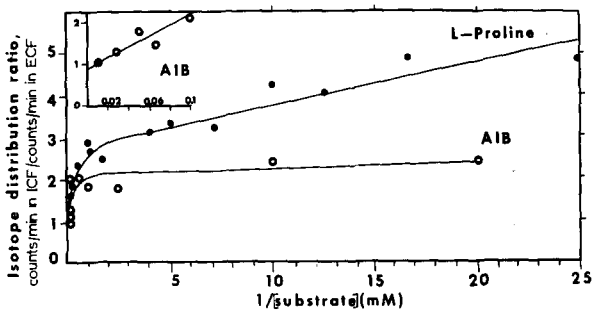

Fig. 4. Relation of the isotope distribution ratio for L-proline and AIB to the reciprocal of the substrate concentration as proposed by AKEDO AND CHRISTENSEN[26] to indicate saturable transport. Incubations were carried out for 120 min in pH 7.4 Tris–electrolyte–glucose buffer at 37°. The isotope distribution ratio is (counts/min per ml intracellular fluid)/(counts/min per ml extracellular fluid). Inset indicates ordinate intercept for AIB under conditions where a steady state had not been achieved. The average numbers of determinations for each point were 16 for the L-proline data and 10 for the AIB data.

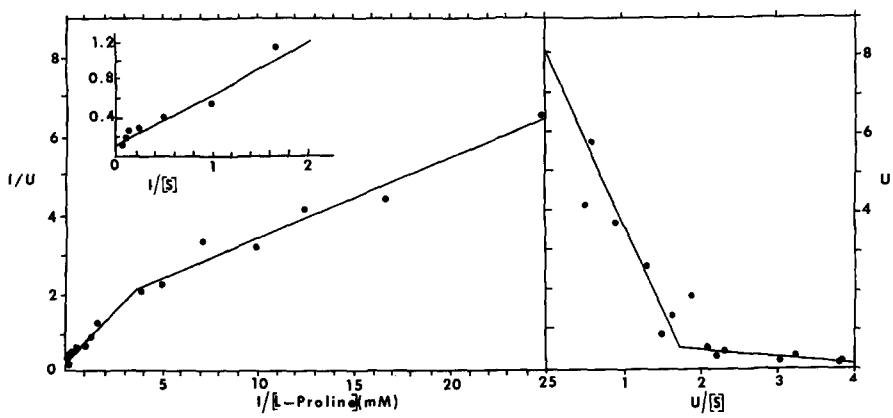

Fig. 5. Concentration-dependent uptake of L-proline by isolated rat glomeruli under steady-state conditions. Incubations were carried out for 120 min at 37° in pH 7.4 Tris–electrolyte–glucose buffer The observed rate of uptake was corrected for uptake on the non-saturable component to obtain the accumulation on the saturable component. L-proline concentration in the medium was varied between 0.04 and 16 mM. Left: Lineweaver–Burk plot ($1/u$ *vs.* $1/[S]$); Right: Eadie–Augustinsson transformation (u *vs.* $u/[S]$). The two sets of data are taken from the same experiments. Uptake on more than one mediation is indicated.

the appropriate substrate concentration (Fig. 6), it was evident that L-proline transport into glomeruli is divided equally between high- and low-K_m systems when its external concentration is about 0.1 mM. Above this concentration the low-K_m system is virtually saturated and uptake occurs almost exclusively on the second high-K_m system. The corrected K_m values for the two transport systems are 0.1 and 5.3 mM, respectively.

TABLE I

OBSERVED AND THEORETICAL UPTAKES OF L-PROLINE (μmoles/ml INTRACELLULAR FLUID PER 120 min) BY TWO SYSTEMS IDENTIFIED IN THE ISOLATED RAT GLOMERULUS

Observed and calculated uptake rates for L-proline by component and total systems in isolated rat glomeruli incubated for 120 min in oxygenated Tris–Ringer–phosphate buffer at 37°, pH 7.4. The individual contributions of the two components to the total uptake were calculated by methods described earlier[17]. u_1, represents uptake on a system whose corrected K_m is 0.1 mM; u_2, defines a system for which the corrected K_m is 5.3 mM.

Initial concn. of L-proline in medium (mM)	*Calculated uptake by each system*		*Combined uptake* $u_1 + u_2$	*Observed uptake*
	u_1	u_2		
0.04	0.09	0.07	0.16	0.15
0.06	0.12	0.10	0.21	0.23
0.08	0.14	0.13	0.27	0.24
0 10	0.16	0.17	0.33	0.32
0.14	0.19	0.23	0.42	0.31
0.20	0.22	0.33	0.55	0.46
0.25	0.24	0.41	0.65	0.53
0.60	0.29	0.94	1.23	0.90
0.80	0.30	1.22	1.52	1.34
1.00	0.30	1.43	1.73	1.89
2.00	0.32	2.50	2.82	2.60
10.00	0.34	5.88	6.21	5.80

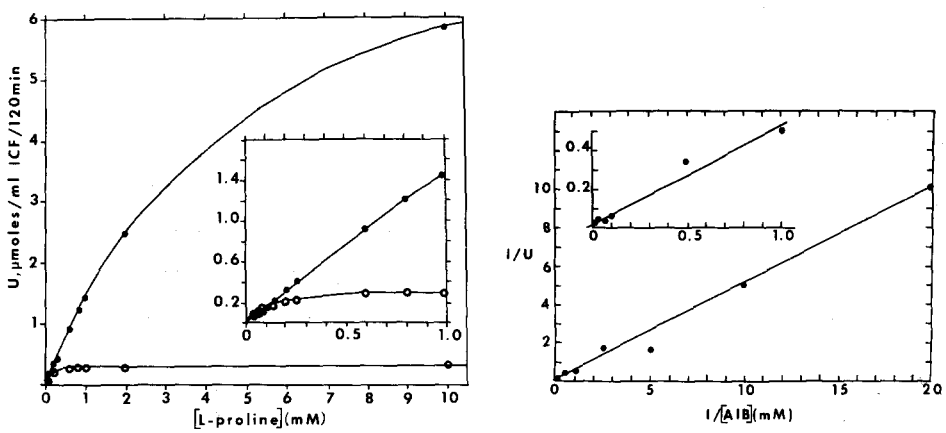

Fig. 6. Michaelis plots of the relationship between substrate concentration ([S]) and velocity of uptake (u = μmoles/ml intracellular fluid per 120 min) for each of two systems mediating the uptake of L-proline by isolated rat glomeruli. Data are taken from Table I after resolving the components of uptake revealed in Fig. 5 by a computer method. Two systems are revealed; one with low capacity, another with high capacity.

Fig. 7. Lineweaver–Burk plot ($1/u$ *vs.* $1/[S]$) describing concentration-dependent uptake of AIB by isolated rat glomeruli. Incubations were carried out for 120 min at 37° in Tris–electrolyte–glucose buffer. AIB concentration in the medium was varied from 0.05 to 80 mM. The contribution to uptake by the non-saturable component under these conditions was considered (*viz.* Fig. 4) to determine the uptake of AIB on the saturable component.

AIB. Glomerular uptake of this amino acid after 120 min incubation was studied at concentrations from 0.05 to 80 mM. Although a steady-state was not achieved within this period of incubation, the diffusion constant was calculated by the method of AKEDO AND CHRISTENSEN[26] from the ordinate intercept (inset Fig. 4) and uptake by the saturable component was then calculated. The Lineweaver–Burk transformation revealed that AIB uptake in the glomerulus occurred on a single system only (Fig. 7). The K_m value for this system was about 33 mM.

Effect of metabolism on transport into glomeruli

Metabolic conversion of substrate. There is active metabolism of L-proline in mammalian kidney cortex[14]. Consequently its chemical distribution ratio during transport studies is significantly different from its isotope distribution ratio in this tissue. When the effect of proline conversion upon its own transport into isolated glomeruli was examined, we observed the following: L-Proline was vigorously oxidized to CO_2 over a wide range of substrate concentration (0.01–10 mM). The respective fraction of the labelled substrate which was lost by intracellular oxidation, varied between 73 and 30 % per 120 min incubation. About 5 % of the intracellular counts derived from L-proline were found in a water-insoluble residue of boiled glomeruli.

Only 29 to 67 % of the soluble counts in the boiled supernatant were proline after 120 min of incubation when the initial medium concentration was varied between 1.3 and 10 mM, respectively. The non-proline counts were predominantly glutamic acid and ornithine.

TABLE II

INHIBITION OF STEADY-STATE ACCUMULATION OF AMINO ACIDS IN ISOLATED GLOMERULI FROM RAT KIDNEY

Values are the mean of at least triplicate observations. Glomeruli were pooled from 2 animals and divided into control and test aliquots. Controls were incubated without inhibitor; experimental samples were exposed to inhibitor in parallel incubations. Net uptake was measured after 120 minutes at 37°. The statistical significance of the observations was determined by analysis of variance.

Treatment	*Isotope distribution ratio*			
	L-Proline (0.1 mM)		*AIB (0.1 mM)*	
	Control	*Test*	*Control*	*Test*
Boiling (10 min)	5.38	0.09 *		
Freeze and thaw (3 times)	6.04	0.95 *		
NaCN (10^{-4} M)	3.76	1.47 **	1.76	0.85 *
NaCN (10^{-2} M)	5.38	0.63 *	2.33	0.03 *
2, 4-dinitrophenol (10^{-4} M)	5.92	2.70 *		
KF (10^{-2} M)			1.76	0.60 *
N_2 replacing O_2 in gaseous phase	4.70	4.61 ***		
Iodoacetamide (10^{-2} M)	5.92	0.26 *		
Ouabain ($5 \cdot 10^{-4}$ M)	5.38	2.87 *		
Choline chloride (144 mequiv/l), replacing NaCl	4.70	0.90 *	4.09	0.07 *

* $P < 0.01$.
** $P < 0.02$.
*** No significant difference.

When the soluble isotope distribution ratio in the glomerulus was corrected for metabolism, the subsequent "chemical" distribution ratio at 120 min averaged about 1.0, but occasionally it was as low as 0.8. Thus, L-proline did not always enter glomerular cells against a chemical gradient under the conditions of our experiments.

Effect of metabolic inhibitors. Boiling for 10 min and repeated freezing and thawing impaired the ability of glomeruli to take up substrate (Table II). This indicates that non-specific binding does not account for the uptake of L-proline by intact glomeruli. Cyanide and 2,4-dinitrophenol inhibited the uptake of L-proline and AIB, indicating that a source of energy is coupled to the transport of these substances into glomeruli. However, when oxygen in the buffer and atmosphere was replaced by nitrogen during incubation, there was no inhibition of substrate accumulation. This suggests that glycolysis can provide some of the energy which is used for transport in glomeruli, an interpretation supported by the inhibitory effect of fluoride on transport into glomeruli. L-Proline uptake was inhibited by iodoacetamide, indicating that sulphydryl-dependent reactions participate in transport. Uptake was inhibited by ouabain and by complete removal of Na^+ and replacement by choline in the medium, indicating that amino acid transport is Na^+-dependent in glomeruli.

DISCUSSION

Reasonable amounts of glomerular tissue can be isolated from the kidneys of small laboratory animals by the method used in this investigation and by previous workers[24]. However, we have learned (W. A. WEBBER, personal communication) that electron microscopic examination of isolated glomeruli reveals tissue damage not visible to light microscopy. Some inconstancy in the integrity of our tissue preparation was evident in the variable proportion of glomeruli still having an attached Bowman's capsule. Cellular damage may explain the greater intracellular and total tissue water in the glomerulus, when compared with the cortex slice obtained from the same rat species[13]. Some of the variability which was encountered in our studies of glomerular transport (*viz.* Fig. 3) may be the result of this tissue damage. FONG AND DRUMMOND[24,30], in their studies of glomerular metabolism observed variability in their data, and W. A. WEBBER (personal communication) has encountered the same finding in his studies of amino acid uptake by isolated glomeruli. We attempted to control any additional variability arising from the technical artifacts of Millipore methods. Such difficulties limited some aspects of our investigation.

The principal characteristics of glomerular transport are compared with the corresponding features which have been identified in the cortex slice, papilla, isolated tubule and tubule brush border (Table III). The comparisons reveal that glomerular transport of L-proline and AIB has many similarities with the rest of kidney, and yet some significant differences. Physical and chemical agents affect the glomerular uptake of amino acids in a manner similar to that observed in other parts of the kidney. Glomerular transport of L-proline and AIB is mediated by saturable sites which are coupled to aerobic energy metabolism and are sodium-dependent. However, the glomerulus, like the papilla[2] can also accumulate these amino acids under anaerobic conditions. This characteristic is not shared by the cortex slice[1,2] or by the isolated tubule[3]. This provides indirect evidence that glomeruli probably contribute little to the observed total uptake in cortex slices under the usual aerobic conditions.

TABLE III

TRANSPORT OF L-PROLINE AND AIB IN DIFFERENT REGIONS OF MAMMALIAN KIDNEY

Condition	*Cortex slice (rat)*[14,16]	*Papilla slice (rat)*[2]	*Glomerulus (rat)*	*Tubule (rabbit)*[3,5,35]	*Brush border of tubule (rabbit)*[6]
Time (min) to achieve steady-state					
L-Proline (0.05 mM)	40		100	20	5
AIB (0.1 mM)	60	90[a])	>120	20[b])	
Distribution ratio					
(1) 2-min value					
L-proline (0.05 mM)	0.4		0.32	2.8	
AIB (0.1 mM)	0.5	0.5	0.04	1.0[b])	
(2) Steady-state value					
L-proline (0.05 mM)	2.8		4.75	4.7	
AIB (0.1 mM)	4.25	5.0	2.10[c])	2.0[b])	
K_m values for saturable uptake					
L-Proline	0.1; 5		0.1; 5.3	0.3; 0.8; 30	30
AIB	4; 25	~1	33		
Saturable uptake					
(1) Occurs under anaerobic conditions	No	Yes	Yes	No	
(2) Inhibited by 2,4-dinitrophenol	Yes		Yes	Yes	No
(3) Is Na^+ dependent	Yes	Yes	Yes	Yes	Yes

[a]) AIB concentration was 0.065 mM.
[b]) AIB concentration was 0.02 mM.
[c]) Steady state not achieved; value shown is for 120 min incubation.

Glomeruli accumulate AIB against a concentration gradient and inhibitors of energy metabolism suppress the active transport of this amino acid. L-Proline is not consistently taken up against a chemical gradient because its rate of uptake is apparently not sufficient to offset the removal of L-proline by intracellular metabolism.

Uptake ratios of AIB and L-proline after short incubations, are lower in the glomerulus than in the rest of the kidney (Table III). This feature is not an artifact of solute permeation into the complex interstices of the glomerulus, since sucrose can permeate this tissue rapidly (*viz.* Fig. 2). Thus the low early uptake rates in the glomerulus probably reflect a reduced number of transport sites per cell mass or a slower rate of transfer on the sites which are present. This is supported also by the low uptake ratio of AIB after prolonged incubation.

The accumulation of amino acids by the glomerulus after prolonged incubation is also different when compared with other areas in the kidney. The isotope distribution ratio for proline is higher in the glomerulus than in the slice and AIB never achieved a steady-state in glomerulus. This suggests that efflux of labelled material is reduced in the glomerulus. Impaired efflux of AIB occurs in other tissues[31] and L-proline efflux

is reduced in newborn kidney[22], where the mass of glomeruli relative to tubules is increased compared to mature kidney.

L-Proline transport in kidney is achieved by more than one system *in vitro*[5,6,14,17,22] and *in vivo* [18,20,32]. The same is also true of AIB transport *in vitro*[16]. In general it may be said that transport at low substrate concentration take place on low-K_m systems with low capacity and high specificity, while at high concentrations high-K_m systems with high capacity and broader specificities serve uptake of these two amino acids. The two types of transport appear to be under independent genetic control[20,32]. Glomerular transport of L-proline is also heterogeneous. We found non-linear kinetics for its uptake over a wide concentration range. The K_m values for the L-proline transport in the glomerulus, expressed either as first approximations, or corrected for simultaneous uptake on multiple systems, were very similar to those measured in the rat cortex slice[14]. There is some evidence for a system with a very high K_m for L-proline uptake in the rat cortex slice (F. MOHYUDDIN AND C. R. SCRIVER, unpublished data), which resembles that identified in rabbit tubule[5]. Further resolution of this system was not attempted in glomerulus. Intracellular metabolism does not influence estimation of the transport K_m[14]. Thus relative differences in metabolism of L-proline in glomerulus, slice and tubule do not influence the estimates of the relevant transport kinetics for L-proline.

Concentration-dependent uptake of AIB into glomerulus occurs on a single mode, in contrast to the two systems identified in the cortex slice[16]. The K_m of the glomerular system is similar to that of the high-K_m system in the cortex slice. Absence of the low-K_m AIB system in glomerulus may account for its low rate of uptake at low external concentration (*viz.* Fig. 3). If only the high-K_m AIB system is present in the glomerulus, and efflux of AIB is depressed in this tissue, it is possible that the low-K_m system is responsible for the majority of efflux at low concentrations. However, the K_m for AIB uptake in muscle[31,33,34] is equivalent to that of the low-K_m AIB system in kidney[16]. Yet this system in muscle does not readily accomodate efflux in that tissue; thus its absence is unlikely to account for the low efflux rate in glomerulus. One must assume that AIB transport in glomerulus is fundamentally different from its transport in other cells.

Attempts were made to study interactions between amino acids presumed to use the AIB and proline transport systems in glomeruli. Substrate preferences of the relevant transport sites have been defined for the kidney cortex slice *in vitro*[14,16], for whole kidney *in vivo*[13,18-20] and for the isolated tubule preparation[5,6]. As reported previously[23] uptake of 0.1 mM L-proline, by glomeruli is inhibited by 10-fold concentrations of AIB and hydroxy-L-proline, but not by L-leucine. Exclusion of L-leucine from interaction with the proline sites reveals that the specificity of L-proline uptake in glomerulus is in accord with that of the cortex slice[14]. AIB is normally excluded from the low-K_m system for L-proline in kidney[14]. However, uptake of L-proline at 0.1 mM in glomerulus and slice is equally divided between the high-K_m and low-K_m systems (Table I). Thus about half of the 0.1 mM L-proline uptake should be inhibited by AIB; this was observed[23].

The glomerulus is an organ of filtration through which solutes and water of plasma pass to be reabsorbed in large part by the luminal brush border of tubular epithelial cells. Some of these solutes will be taken up in transit, by glomerular cells, presumably into the epithelial cell in particular; this cell must expend energy to

synthesize and maintain the glomerular basement membrane. Glomerular cells can take up two representative amino acids by energy and Na^+-coupled membrane sites. The systems serving L-proline transport are not as active as in the tubule but they are evidently as elaborately differentiated as they are elsewhere in kidney. This is in keeping with the common embryologic origin of the epithelial glomerular components and the tubules. On the other hand, glomerulus retains a transport identity of its own, as revealed by the characteristics of AIB transport which are not mere copies of those identified elsewhere in kidney.

ACKNOWLEDGEMENTS

We thank Dr. K. N. Drummond and Dr. J. S. C. Fong for helpful discussion, Dr. J. L. Neal for help with computer programs and Mrs. A. Shannon for providing the animals.

This work was supported in part by Grant MT-1085 from the Medical Research Council of Canada and a grant from the National Genetics Foundation (New York N. Y.)

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