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Characteristics of lysine transport by isolated rat renal cortical tubule fragments

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The uptake of L-lysine was examined in isolated renal cortical tubules. Lysine was actively taken up by the renal tubule cells isolated from 7-week-old rats. No metabolism of the transported lysine was found. There was no evidence for sodium-dependence of lysine uptake. Concentration dependence studies revealed that the lysine was taken up by one saturable transport system with a K_m of 1.66 mmol/l and V_{max} of 7 mmol/l intracellular fluid per 10 min. Lysine also entered by a non-saturable pathway. Arginine and ornithine inhibited the initial uptake of lysine. Cystine increased the efflux of lysine from preloaded renal cells via hetero-exchange, indicating that a common system exists for these two amino acids.

Introduction

The human disorder cystinuria has focused attention on the nature of cystine and dibasic amino acid transport in the renal tubule. This disorder is characterized by excessive urinary excretion of cystine, lysine, arginine, and ornithine, but especially lysine [1]. Previous in vitro studies using renal cortical slices demonstrated a common system for dibasic amino acid transport, but failed to show any interaction between cystine and the dibasic amino acids [2,3].

However, the renal cortical slice is not the optimal system for studying amino acid transport because of problems related to slice thickness, substrate penetration, and tissue oxygenation. The isolated renal cortical tubule has been shown to be a better preparation for studies examining amino acid transport into intact cells. With this preparation, cystine has been shown to be taken up by renal tubule cells via two saturable systems [4,5]. The high-affinity system, which was not observed in studies employing slices, appears to be shared with lysine. Because the isolated tubule gave a more complete picture of cystine transport compared to the cortical slice, we have examined lysine transport using isolated renal cortical tubules from the rat. The results form the basis of this report.

Methods

The preparation of isolated renal cortical tubules was performed by a modification [6] of the procedure described by Burg and Orloff [7]. 7-week-old Sprague Dawley rats weighing 125–175 g were killed by decapitation. The kidneys were removed, decapsulated and placed in ice-cold Krebs-Ringer bicarbonate buffers (118.5 mmol/l NaCl, 25 mmol/l $NAHCO_3$, 4.7 mmol/l KCl, 2.56 mmol/l $CaCl_2$, 1.2 mmol/l potassium phos-

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phate), containing 10 mol/l sodium acetate at pH 7.4. Cortical slices were cut with a Stadie-Riggs microtome and the remainder of the cortex was dissected free by hand. The slices were homogenized with four strokes of a pestle in a 15 ml loose Dounce homogenizer and the suspension was centrifuged for 15 s at $800 \times g$ at room temperature. The pellet was resuspended in 10 ml of Krebs-Ringer buffer containing 18 mg of collagenase and 0.5 ml fetal calf serum. After a 45 min digestion at room temperature, the tubules were washed twice in iced Krebs-Ringer buffer. They were then resuspended in Krebs-Ringer buffer with 5% (v/v) fetal calf serum at a concentration of approx. 8–12 mg weight per ml for transport studies and filtered through three layers of surgical gauze.

Uptake studies of L-[^{14}C]lysine were performed in Krebs-Ringer buffer (pH 7.4), with 5% (v/v) fetal calf serum and 10 mmol/l sodium acetate (KRB-FCS) in Burg-Orloff flasks with continuous bubbling of a 95% O_2/5% CO_2 gas mixture at 37° C as previously described [6]. The tissue pool of radioactivity was extracted in 1 ml of water by boiling for 3 min, and 0.2 ml of media and the aqueous tissue extract were assayed for radioactivity by standard liquid scintillation techniques. All samples assayed for radioactivity had counts which were at least 20-fold above the background. The background value, which was determined for each experiment and was 20 cpm on average, was subtracted from the radioactivity of each sample prior to calculating the uptake value. Uptake was measured in terms of a distribution ratio of intracellular to extracellular radioactivity concentration as calculated by previous methods [4]. The intracellular fluid volume was determined as the difference between the total tissue fluid, the wet weight minus the weight after overnight dessication, and the fluid trapped between the tubules [6]. These volumes have been determined previously in isolated cortical tubules from adult male Sprague-Dawley rats and the total tissue fluid is 80% of the wet weight and the 'trapped fluid' is 26% of the wet weight [8].

Analysis of the intracellular pool of radioactivity was performed by incubating isolated tubules in KRB-FCS at 37° for 30 min with 0.1 μCi/ml L-[^{14}C]lysine followed by centrifugation at 4° C and $33000 \times g$ for 10 min. The pellet was resuspended in 10% (w/v) trichloroacetic acid. After sitting in an ice water bath for 10 min the denatured tubule suspension was centrifuged for 10 min at 4° C and $33000 \times g$ and the supernatant was removed for chromatography. The supernatant was extracted with ether three times to remove any trichloroacetic acid and 10 μl were chromatographed on 3 mm Watman chromatography paper with a solvent system of butanol/acetic acid/water (12 : 3 : 5, v/v). After developing and cutting the chromatogram into 1 cm segments, each segment was assayed for radioactivity. The segments were identified by location of a simultaneously chromatographed lysine standard stained with ninhydrin.

In concentration dependence studies, tubules were incubated for 10 min with 0.1 μCi/ml L-[^{14}C]lysine plus unlabelled lysine to give the desired final concentration over the concentration range of from 0.01 to 50 mmol/l. The observed transport kinetic parameters were determined from a Hofstee plot of the transport data using the least squares method to obtain the best fit. To determine the effect of other amino acids on lysine uptake, tubules were incubated with 0.025 mmol/l, 0.1 μCi/ml L-[^{14}C]lysine in the presence and absence of various amino acids. The means of the distribution ratios of ^{14}C were calculated and compared using Student's *t*-test for unpaired data [9].

The effect of sodium on lysine transport was determined by modifying the Krebs-Ringer buffer by replacing the NaCl and $NaHCO_3$ with choline chloride and choline bicarbonate (mmole for mmole). Sodium acetate was omitted from both the control and sodium free buffers for this study. The uptake of 0.025 mmol/l L-[^{14}C]lysine in these two buffers was then compared.

To study efflux in the presence and absence of cystine, the tubules were incubated for 15 min with 0.025 mmol/l, 0.1 μCi/ml L-[^{14}C]lysine. The tubule suspension was removed and spun at $800 \times g$ for 15 s. The supernatant was discarded and the tubules resuspended in Krebs-Ringer buffer with and without 0.5 mmol/l cystine. Samples were taken at 0, 5 and 15 min and the residual tissue radioactivity was determined. From these data the intracellular concentration of radioactive amino

acid at time zero and the percent loss of radioactivity with time were calculated.

All chemicals were of the highest purity available. L-[^{14}C]Lysine (300 mCi/mol), chromatographically pure, was obtained from ICN Pharmaceuticals, Irvine, CA. Unlabelled lysine, cystine, arginine and ornithine were obtained from Sigma Chemical Co., St. Louis, MO. The phosphor used in liquid scintillation counting was Biofluor from New England Nuclear, Boston, MA.

Results

Lysine uptake with time

The renal tubule fragments actively took up 0.025 mmol/l L-lysine as shown in Fig. 1. There was a rapid initial uptake such that the distribution ratio of intracellular to extracellular radioactivity was 4.66 (S.E. 0.39, $n = 8$) by 5 min of incubation. By 60 min a distribution ratio of 6.94 (S.E. 0.21, $n = 8$) was reached. The distribution ratios represent chemical gradients of lysine since no metabolism of the transported label was demonstrated with chromatography of the intracellular contents after 30 min of incubation.

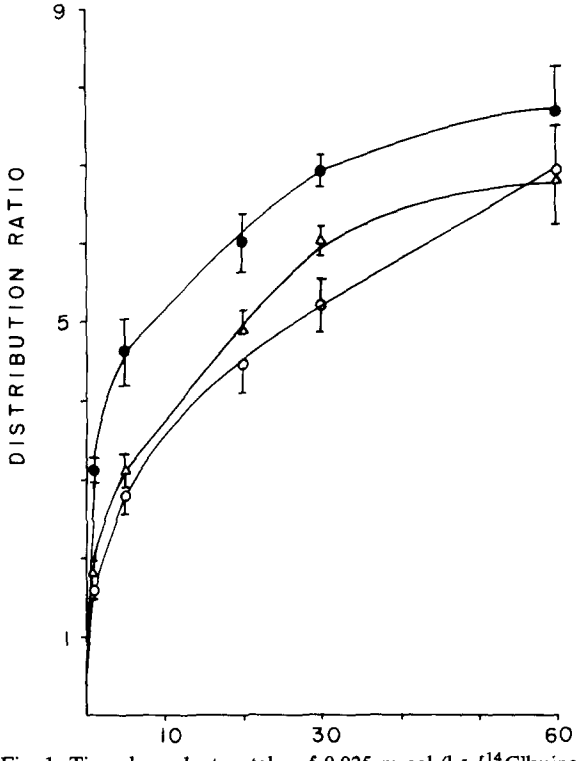

Fig. 1. Time-dependent uptake of 0.025 mmol/l L-[^{14}C]lysine by isolated rat renal cortical tubules. Uptake of L-[^{14}C]lysine alone is represented by closed circles (●), in the presence of 1.0 mmol/l arginine, by open circles (○) and in the presence of 1.0 mmol/l ornithine, by triangles (△). Each point represents the mean ± S.E. of at least eight determinations done in duplicate. Standard errors not shown are within the size of the point.

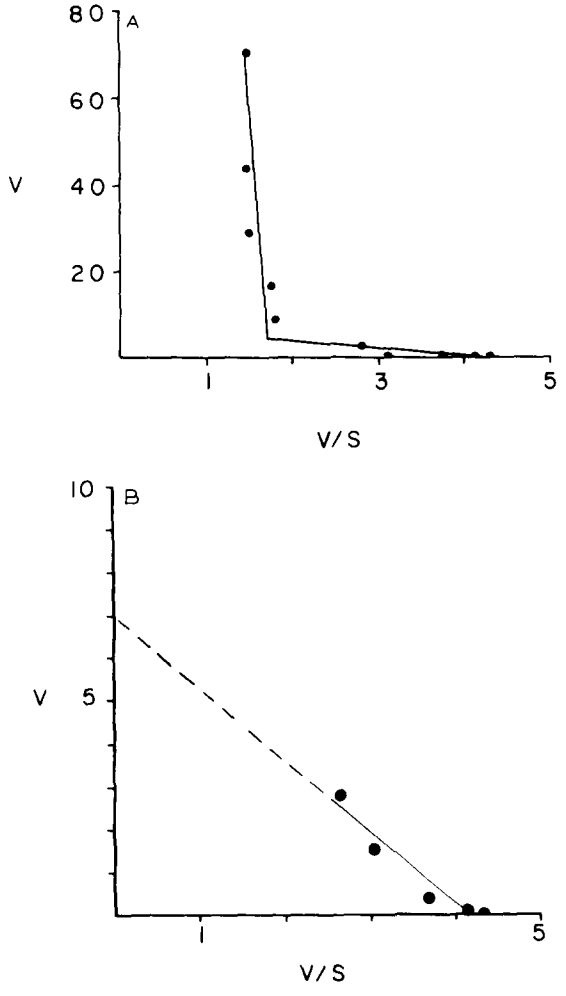

Fig. 2. A Hofstee plot of the concentration dependence of lysine uptake by isolated rat renal cortical tubules. Tubules were incubated for 10 min with 0.1 μCi/ml L-[^{14}C]lysine and sufficient unlabelled lysine to give the desired concentrations. (A) represents the entire concentration range from 0.1 to 50 mmol/l. (B) represents the concentration range from 0.1 to 1.0 mmol/l. *V* represents the velocity of uptake in mmol/l intracellular fluid per 10 min. *S* represents the mean ± S.E. of at least four determinations done in duplicate.

Concentration dependence of uptake

The concentration dependence of lysine uptake was studied over the concentration range of from 0.01 mmol/l to 50 mmol/l (Fig. 2A). As can be seen in Fig. 2B an expanded graph of the transport data in the 0.01–1.0 mmol/l range gave a linear plot indicating a single saturable pathway for lysine entry into the renal tubule cell. The observed transport parameters of this system were a K_m of 1.66 mmol/l and V_{max} of 7.0 mmol/l intracellular fluid per 10 min. There was little change in the ratio of velocity over substrate concentration (V/S) with increasing concentrations of lysine over 1.0 mmol/l suggesting a diffusional pathway or a saturable system with a very high K_m. Correction for the diffusion component in the concentration range 0.01–1.0 mM gave a K_m of 0.7 mmol/l and V_{max} of 2.2 mmol/l intracellular fluid per 10 min.

Interaction with amino acids

Since previous studies have suggested a common transport system for cystine and dibasic amino acids [4,10,11], studies were performed to examine the interactions of these amino acids. In Fig. 1 the effect of 1.0 mmol/l arginine and ornithine on the uptake of 0.025 mmol/l lysine is shown. At 1 min arginine inhibited lysine uptake by 50% and ornithine inhibited lysine uptake by 40%. This inhibition was statistically significant at $P < 0.01$ ($n = 7$). These two amino acids also significantly inhibited lysine uptake after 5, 15 and 30 min of incubation. After 60 min of incubation, there was no statistical difference between the distribution ratio for lysine alone and in the presence of either arginine or ornithine. In contrast, 1.0 mmol/l proline or 1.0 mmol/l aminoisobutyric acid had no effect on the uptake of 0.025 mmol/l lysine.

In Fig. 3 no effect of 0.5 mmol/l cystine on 0.025 mmol/l lysine is seen at the early time points but by 15 min there was a 20% reduction in the distribution ratio, and at 30 min, a 37% reduction compared to the distribution ratio in absence of cystine. This was statistically significant at $P < 0.05$ ($n = 11$). This suggested that the cystine con-

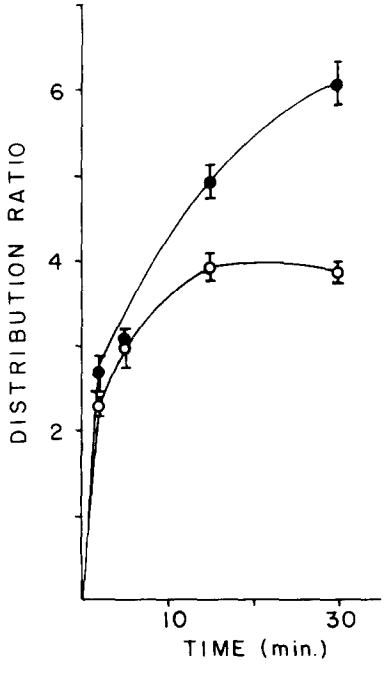

Fig. 3. Time-dependent uptake of 0.025 mmol/l L-[^{14}C]lysine by isolated rate renal cortical tubules. Open circles (○) represent uptake in the presence of 0.5 mmol/l cystine and closed circles (●) represent uptake in the absence of cystine. Each point represents the mean ± S.E. of at least six determinations done in duplicate.

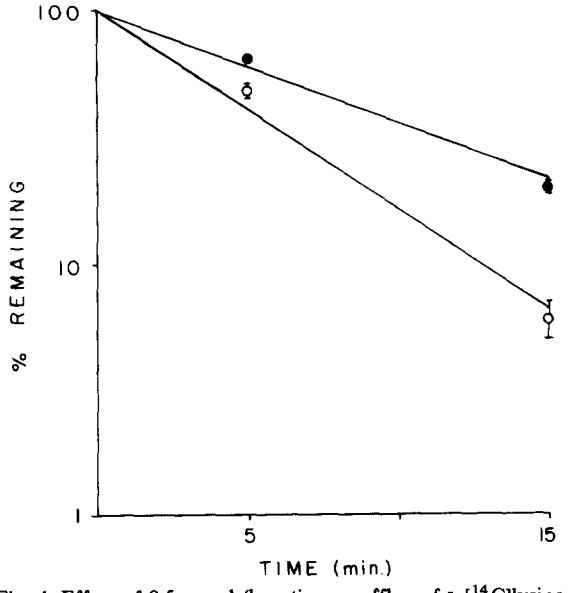

Fig. 4. Effect of 0.5 mmol/l cystine on efflux of L-[^{14}C]lysine. Isolated renal tubules were preloaded by incubating for 15 min with 0.025 mmol/l lysine. Open circles (○) represent efflux in the presence of cystine and closed circles (●) represent efflux in the absence of cystine. Each point represents three determinations.

centration may not be high enough to inhibit lysine influx but was at a level sufficient to cause accelerated lysine efflux. In Fig. 4 the effect of the medium cystine on the efflux of lysine from tubules pre-loaded with lysine is seen. There was a significant increase in efflux at 5 and 15 min. ($P < 0.01$, $n = 6$) when cystine was present in the incubation media. This can be explained by hetero-exchange diffusion and supports the idea of a shared transport system, although cystine did not inhibit the entry of lysine in these experiments.

Transport in Na^+-free medium

The medium sodium concentration has been shown to be important for initial influx of cystine with renal cortical slices [12] and in isolated tubules [4]. In contrast, no inhibition of early influx of lysine was found in rat renal cortical slices although the steady state values for lysine were lower in the sodium free buffer [13]. Incubation of isolated tubules in sodium free buffer resulted in an accelerated initial influx of 0.025 mmol/l lysine (Fig. 5). After 2 min of incubation the distribution ratio was 4.38 (S.E. 0.23; $n = 3$) in the sodium free buffer vs. 2.64 (S.E. 0.03, $n = 3$) in the control ($P < 0.02$). There was no statistically significant difference between control and experimental values after 5 min of incubation.

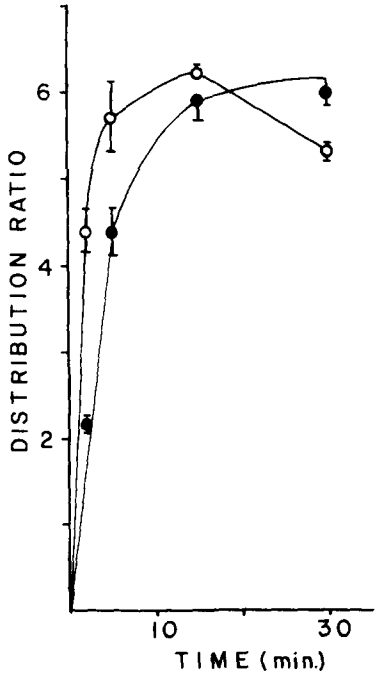

Fig. 5. Time-dependent uptake of 0.025 mmol/l L-[^{14}C]lysine by isolated rat renal cortical tubules in the presence (●) and absence (○) of sodium. The Na^+-free Krebs-Ringer buffer was made by replacing the NaCl and $NaHCO_3$ with choline chloride and choline bicarbonate. Sodium acetate was omitted from both the control and sodium free buffers. Each point represents the mean ± S.E. at least six determinations.

Discussion

Lysine, ornithine, arginine and cystine are thought to share a common reabsorptive pathway which is defective in cystinuria. Support for this shared transport system initially came from studies by Robson and Rose [14] showing increased excretion of ornithine, arginine, lysine and cystine when lysine was infused into normal subjects. In vitro data using the renal cortical slice did show a common system for the dibasic amino acids but no interaction between cystine and the dibasic amino acids could be demonstrated [2,3]. The slice has been shown not to be an optimal system for exploring these transport interactions. More recent studies using isolated renal cortical tubules, which have better substrate penetration and tissue oxygenation, have shown that dibasic amino acids inhibit cystine transport [4,5].

The present studies examined the nature of lysine uptake in the renal tubule. Lysine was actively transported into the renal cell via at least one saturable system. With concentrations above 1 mmol/l a significant portion of the lysine taken up by the cell appeared to enter by diffusion or via a system with a very high K_m. This is similar to data found in our laboratory using isolated renal brushborder vesicles, suggesting that transport into the renal tubule fragments is via the luminal membrane [10]. Earlier studies with human and rat renal cortical slices indicated that there were two saturable transport systems for lysine uptake [13,15]. The low K_m for lysine was 2.4 mmol/l. However, the K_m for the second system for lysine was 50 mmol/l which is markedly higher than normal physiologic concentrations in the plasma and luminal fluid and corresponds to the second system observed in the present studies. The findings presented here resemble data published previously on lysine uptake in tubules isolated from the dog where transport was via a

single mediated system with a K_m of 0.56 mmol/l and a V_{max} of 6.1 mmol/l per 5 min [15].

Most investigators have found only a single mediated transport system for dibasic amino acid uptake across the brushborder membrane. McNamara et al. [10] found a single system for lysine into the renal brush-border vesicle with a K_m of 0.04 mmol/l. Stieger et al. [11] also noted a single system with a K_m of 0.3–0.7 mmol/l in rat brush-border vesicles. Silbernagl and Deetjen [17] found only a single mediated system for arginine with a K_m of 1.2 mmol/l in microperfused rat proximal tubules.

Similarly, Hammerman [18] using rabbit brush-border vesicles and Jean et al. [19] using rat brush-border vesicles, identified only a single mediated system for arginine uptake with a K_m of 0.16 mmol/l and 0.03–0.06 mmol/l, respectively. In contrast, Busse [20], using rabbit brush-border vesicles, noted two mediated systems for arginine transport with a K_m of 0.07–0.1 for the high-affinity system and 3.5–4.0 mmol/l for the low-affinity system. Ullrich et al. noted only a single mediated system for ornithine with a K_m of 1.8 mmol/l in the microperfused rat proximal tubule. It appears that the lysine K_m of isolated tubule fragments and microperfused tubules are similar but differ from the much lower K_m values observed with brush-border vesicles.

The carrier-mediated portion of lysine uptake was inhibited by arginine and ornithine as has been shown in other studies [4,10,11,16]. The inhibition occurred at early time points suggesting that it was the influx of lysine which was affected. In contrast, cystine inhibition of lysine transport did not occur until later time points, which suggests that the effect of cystine was primarily on efflux events. Direct examination of the effect of cystine on lysine efflux confirmed this interpretation. This acceleration of lysine efflux by cystine supports the concept of a shared system for cystine and the diabasic amino acids although a direct effect of cystine on lysine influx could not be established. In previously reported studies with dog tubules cystine did inhibit initial lysine uptake [16].

Why an effect of cystine on lysine influx could not be demonstrated when an effect on efflux was observed is not clear. It may be that a medium cystine concentration greater than 0.5 mmol/l would significantly inhibit lysine influx. However, this is not possible at pH 7.4 because 0.5 mmol/l is the limit of cystine solubility. Previous studies have indicated that the exchange diffusion of lysine can be more easily affected than influx [22]. Indeed, removal of sodium from the media had no effect on lysine influx, but did lead to a marked inhibition of the exchange diffusion of lysine [21].

Sodium appears not to be necessary for the uptake of lysine in isolated rat tubules, a finding consistent with data found in the rat renal cortical slice [13]. Indeed, replacement of sodium with choline in the incubation buffer led to a more rapid initial rate of uptake. There has been evidence both for [23–25] and against co-transport of sodium with dibasic amino acids in brush-border membrane vesicles. McNamara et al. [10] found that the initial uptake of lysine was not dependent on the presence of sodium nor was the affinity of the carrier in rat renal brushborder vesicles. Most of the evidence now indicates that it is the membrane potential, and not sodium, which has the greatest effect on the uptake of dibasic amino acids [10,18–20]. Dibasic amino acids carry a net positive charge at physiologic pH which may serve to substitute for sodium in the binding requirements of the amino acid carrier. The negative charge of the interior of the renal tubule cell would also favor the uptake of dibasic amino acids. Podevin et al. [26] have shown that substituting choline for sodium in the buffer made the intracellular potential of the renal tubule cell more negative. This may explain the more rapid initial uptake of lysine in the sodium-free buffer.

There are a number of studies which provide evidence that the uptake of lysine by tubule fragments mirrors transport at the brush-border membrane although, in this preparation, as in other intact renal cell systems, a contribution at the antiluminal membrane cannot be excluded. The characteristics of uptake of cystine [4], proline [27], glycine [28] and taurine [29] by renal cortical tubule cells are similar to those observed in isolated brush-border vesicles for these same amino acids [21,30,31]. Accelerated exchange between cystine and lysine has been demonstrated in brush-border preparations and these are akin to the accelerated efflux demonstrated in the present

paper with intact cells [10]. There have been as yet no reports of lysine entry into renal basolateral membrane vesicles.

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