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EXCHANGE DIFFUSION OF DIBASIC AMINO ACIDS IN RAT-KIDNEY CORTEX SLICES

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SUMMARY

1. Lysine, arginine, ornithine, and diaminobutyric acid were found to participate in autoexchange and heteroexchange diffusion in rat-kidney cortex slices.

2. Neither cystine nor cysteine exchanged with the dibasic amino acids. However, at late incubation time points, tissues which had been preloaded with ornithine or lysine accumulated higher intracellular concentrations of cysteine than unloaded controls. This effect appeared to be due to a specific inhibition of cysteine efflux by ornithine and lysine.

INTRODUCTION

When Ehrlich ascites cells are allowed to accumulate a high concentration of non-radioactive glycine, the subsequent initial rate of uptake of a lower concentration of radioactive glycine is markedly stimulated[1]. This phenomenon, autoexchange diffusion, is strong evidence for the carrier-mediated transport concept[2,3]. A similar stimulation of glycine uptake occurs after preloading with a limited number of amino acids other than glycine[4]. The mutual participation of different amino acids in this latter event, heteroexchange diffusion, is an important method for the classification of amino acids into common membrane transport systems[5,6].

Previous studies *in vitro* have indicated the existence of a common transport mechanism for lysine, arginine, and ornithine in rat-kidney cortex slices[7]. This system has been examined with regard to competitive inhibition[7], saturation kinetics, pH sensitivity, Na^+ dependence, and response to metabolic inhibitors[8]. This paper presents the exchange diffusion characteristics of the dibasic amino acid transport system of rat-kidney slices. A formerly unrecognized relationship between lysine, ornitihne, arginine, cystine and cysteine transport is described.

EXPERIMENTAL PROCEDURE

The preparation of rat-kidney cortex slices, and the general incubation technique have been reported[9]. Each sample consisted of three slices, one from each of three different animals, with a total tissue weight of 60–100 mg. Total water and [^{14}C]inulin spaces were determined, and the uptake of radioactivity at various time intervals calculated as a distribution ratio of $\frac{\text{counts/min per ml intracellular fluid}}{\text{counts/min per ml extracellular fluid}}$, according to previously published methods[9,10].

In exchange diffusion experiments the tissues were preincubated for 60 min at pH 7.4 in 2 ml Krebs–Ringer bicarbonate buffer containing a 30 mM loading concentration of non-radioactive L-amino acid. Unloaded controls were preincubated without amino acid. After the preloading phase the tissues were blotted on filter paper and transferred to incubation flasks with 2 ml Krebs–Ringer bicarbonate buffer containing the radioactive L-amino acid (New England Nuclear Corp.) to be exchanged. High specific activity [^{14}C]amino acids were diluted with non-radioactive carrier to provide 13 mM stock solutions with 40 μC/ml. Incubation media contained 0.065 mM amino acid, 0.2 μC/ml. L-[^{35}S]Cystine (Schwarz BioResearch, Inc.) was prepared as a 2 mM stock solution with approx. 40 μC/ml. Incubation concentration of L-cystine was 0.07 mM, 0.2 μC/ml. All of the cysteine studies were performed using L-cystine with 2 mM dithiothreitol (Calif. Biochem. Corp.) in the incubation medium, according to the method of CRAWHALL AND SEGAL[11]. Cysteine concentration was 0.08 mM.

RESULTS

Exchange diffusion of dibasic amino acids

The time course of radioactive amino acid uptake into preloaded cells is compared with the simultaneously measured rate of uptake into control cells in Fig. 1. In each of these studies the initial rate of uptake was increased. With prolonged exchange time periods the equilibrium distribution ratios of preloaded and control cells were similar.

The percent increase of uptake due to preloading is shown in Table I. Preloading with lysine, arginine, or 2,4-diaminobutyric acid resulted in a marked increase of the 5-min distribution ratio of the ^{14}C isotope of the same amino acid (autoexchange).

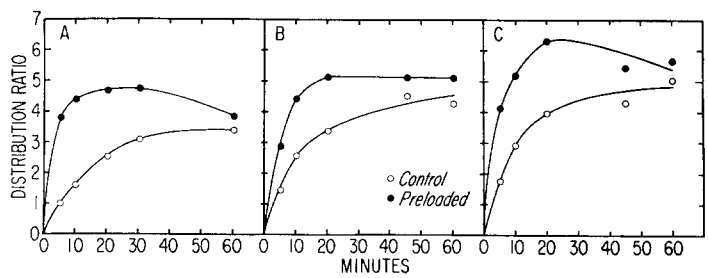


Fig. 1. Stimulation of uptake of 0.065 mM [^{14}C]amino acid into slices preloaded for 60 min at 37° in 30 mM non-radioactive amino acid. A, [^{14}C]lysine uptake into lysine-preloaded tissues; B, [^{14}C]arginine uptake into arginine-preloaded tissues; C, [^{14}C]arginine uptake into lysine-preloaded tissues. Curves are representative studies with duplicate paired samples for each point.

TABLE I

PERCENT INCREASE OF 5-min DISTRIBUTION RATIO DUE TO PRELOADING

After preloading for 60 min with 30 mM non-radioactive dibasic amino acid, the tissues were transferred to flasks containing 0.065 mM of the [^{14}C]amino acid to be exchanged, and incubated for 5 min.

Non-radioactive amino acid preloaded	*[^{14}C]Amino acid exchanged*		
	Lysine	*Arginine*	*Diamino-butyric acid*
Lysine	265	239	
Arginine	152	91	
Ornithine	246	222	
2,4-Diaminobutyric acid	210		96
2,3-Diaminopropionic acid	0		0

When the preloaded dibasic amino acid was other than the exchanged radioactive amino acid (heteroexchange), a similar phenomenon was seen. 2,3-Diaminopropionic acid did not participate in the heteroexchange of this system.

Several other amino acids were tested for their ability to exchange in the dibasic system. Preloading with lysine did not stimulate the 5-min uptake of histidine, phenylalanine, or 1-aminocyclopentane-1-carboxylic acid (cycloleucine). Preloading with glycine, leucine, or valine did not significantly enhance the subsequent 5-min uptake of lysine.

Autoexchange diffusion at 5 min was not demonstrated with any other amino acid tested by these methods. These include cycloleucine, valine, glycine, histidine, proline, phenylalanine, leucine, and methionine. In the methionine study the 5- and 10-min distribution ratios were decreased 32% and 40% by preloading.

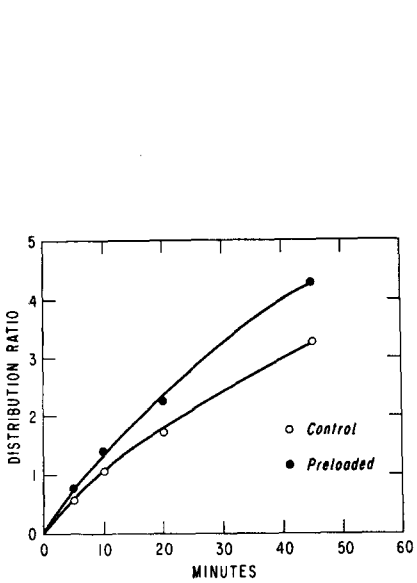
Conditions of exchange

Tissue water-space measurements were not significantly altered from previously published values[10] by the 60-min preincubation in the presence or absence of 30 mM lysine. The actual uptake of both 30 mM lysine and 30 mM arginine was measured using ^{14}C tracers, and the distribution ratios determined at timed intervals. Lysine distribution ratios at 60 min were consistently in the range of 1.7–2.0, indicating intracellular concentration of lysine even at this high incubation level. This finding is reported in greater detail elsewhere[8]. Arginine uptake at 60 min was 1.1–1.3.

The stimulation of uptake induced by preloading was directly related to the preloading concentration, a phenomenon previously described by HEINZ AND WALSH[4]. This was studied using lysine autoexchange with preloading concentrations of 2.5 mM to 30 mM. The percent stimulation of uptake at 5 min was: 2.5 mM, 75%; 5 mM, 100%; 10 mM, 175%; 15 mM, 200%; 30 mM, 260%.

Interrelationships between cystine, cysteine, and dibasic amino acid exchange diffusion

Neither cystine nor cysteine exchanged with the dibasic amino acids as defined by the 5-min distribution ratio in lysine, ornithine, or arginine preloaded cells. However, when [^{35}S]cystine or [^{35}S]cysteine exchange type experiments were carried out



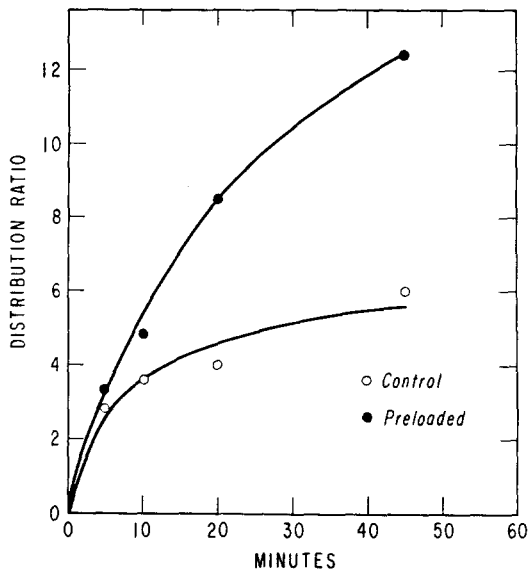


Fig. 2. Uptake of 0.07 mM [^{35}S]cystine into slices preloaded for 60 min in 30 mM lysine. Curves are a representative study with duplicate, paired samples for each point.

Fig. 3. Uptake of 0.08 mM [^{35}S]cysteine into slices preloaded for 60 min in 30 mM lysine. Curves are a representative study with duplicate, paired samples for each point.

for longer time periods a new phenomenon was observed. Figs. 2 and 3 demonstrate the effect of preloading with 30 mM lysine on the time course of cystine and cysteine uptake. For both amino acids, but particularly cysteine, there was a markedly increased accumulation of radioactivity at later time points. To investigate the specificity of this phenomenon several other amino acids were preloaded, and the subsequent 45-min uptake of cystine and cysteine determined (Table II). Ornithine preloading had an even more pronounced effect than lysine. Neither arginine, glycine, nor valine increased the 45-min distribution ratios.

The increased accumulation due to preloading in these studies differs from the exchange diffusion experiments shown in Fig. 1, in that, (1) the difference between

TABLE II

PERCENT CHANGE OF 45-min DISTRIBUTION RATIO DUE TO PRELOADING

After preloading for 60 min with 30 mM non-radioactive amino acid, the tissues were transferred to flasks containing 0.07 mM [^{35}S]cystine, or 0.08 mM [^{35}S]cysteine, and incubated for 45 min.

Non-radioactive amino acid preloaded	*[^{35}S]Amino acid exchanged*	
	Cystine	*Cysteine*
Lysine	+33	+119
Arginine	−16	0
Ornithine	+19	+190
Glycine	−31	0
Valine	−23	0

preloaded and control tissues increases with time, and (2) the preloaded tissues do not reach equilibrium during the 45-min study.

Using 5- and 10-min time points the uptake of radioactive cystine was determined after preloading with various other amino acids. Valine, glycine, leucine, S-methylcysteine, and S-ethylcysteine did not increase the subsequent cystine uptake. Preloading with 0.6 mM cystine did not induce autoexchange diffusion of cystine. Higher preloading concentrations of cystine were not attempted because of solubility limitations.

Cysteine efflux from tissues preloaded with dibasic amino acids

We have previously reported a specific inhibition of cysteine efflux from kidney slices containing high intracellular concentrations of lysine, ornithine, or arginine[12]. The kinetics of the studies shown in Figs. 2 and 3 are consistent with an efflux inhibition, rather than an influx stimulation as seen in the exchange diffusion experiments for lysine and arginine (Fig. 1). Therefore, efflux studies similar to those previously reported[12] were carried out under the conditions of the present experiments (Fig.4). From these data the fractional rate constants of the efflux, and standard deviations, were computed by least squares fit of the 8- to 20-min values to a linear semilog plot. Two of the control studies were set as dependent parameters for normalization of the paired experiments; therefore, the calculated rate constants were not directly obtained from the average points as presented in Fig. 4. These values are: control, 0.0222 ± 0.0017; ornithine, 0.0109 ± 0.0029; lysine, 0.0133 ± 0.0029. In the presence of arginine, the cysteine efflux constant was 0.0169 ± 0.0029. There was no effect of lysine on valine efflux in the presence or absence of dithiothreitol by this method, and no effect of valine on cysteine efflux. These results indicate that dibasic amino acid inhibition of cysteine efflux probably accounts for the enhanced accumulation of cysteine in lysine and ornithine preloaded tissues.

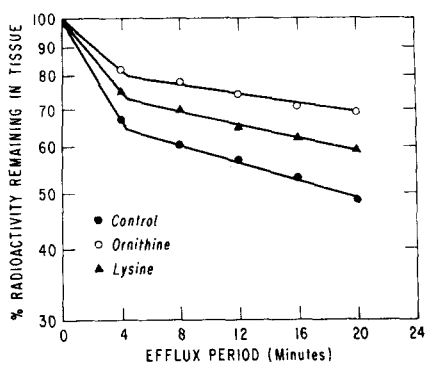


Fig. 4. Inhibition of [^{35}S]cysteine efflux from tissues preloaded with lysine or ornithine. Tissues were preloaded with 30 mM lysine or ornithine as before, transferred to flasks containing 0.08 mM [^{35}S]cysteine, and allowed to accumulate radioactivity for 45 min. At the end of the radioactivity uptake period the tissues were dipped twice in saline, and transferred to flasks containing 3 ml Krebs–Ringer bicarbonate with 2 mM dithiothreitol at 37° in a metabolic shaker. The medium was sampled in 0.2-ml aliquots, every 4 min for 20 min, for the appearance of radioactivity. At the end of the efflux period residual tissue radioactivity was determined, corrected for extracellular space radioactivity, and efflux expressed as percent of calculated initial radioactivity remaining in the intracellular space with time. Each point on the lysine and ornithine curves represents the mean of 4 samples; each control point is the mean of the 8 paired samples.

DISCUSSION

The dibasic amino acids have been shown to share a common transport pathway, *in vitro*, in Ehrlich ascites tumor cells[13], *Escherichia coli*[14], in mammalian intestine[15–21] and kidney[7,22]. The pathway appears to be distinct from the mechanisms mediating neutral amino acid permeation in each of these cell types. However, these dibasic systems are not absolutely specific for the diamino acids. In Ehrlich ascites cells CHRISTENSEN[13] has found inhibition of lysine accumulation by many amino acids, particularly leucine, histidine, methionine, phenylalanine, and valine. Using the everted small intestinal sac method, HAGIHARA *et al.*[15] in hamsters, and LARSEN, ROSS AND TAPLEY[16] in rats, have demonstrated inhibition of transcellular lysine migration by leucine, methionine, and phenylalanine. The overlapping affinities of these dibasic systems have also been shown by heteroexchange diffusion experiments. MUNCK[23] has reported a leucine–lysine heteroexchange in rat gut; CHRISTENSEN[13] has shown leucine–lysine, and phenylalanine–2,4-diaminobutyric acid heteroexchange in ascites cells.

The lysine, arginine, ornithine, 2,4-diaminobutyric acid-mediating system of rat renal cortex is more specific for the diamino acids. Previously published work of ROSENBERG, DOWNING AND SEGAL[7] has shown some leucine inhibition of lysine uptake in the kidney slice. However, phenylalanine and histidine, both of which are potent inhibitors in other systems, do not decrease lysine accumulation in this preparation. The exchange diffusion data presented in this communication are further evidence for specificity. Heteroexchange was limited to the dibasic amino acids with at least a C_3 chain separating the amino groups.

Autoexchange was not demonstrated for any of the other amino acids studied. These included most of the amino acids which have been reported to exhibit exchange in other experimental systems.

The role of the dibasic amino acid system in renal cystine transport is of particular current interest. In mammalian intestine, a common cystine and dibasic amino acid influx pathway has been defined by the feeding experiments *in vivo* of MILNE *et al.*[24], LONDON AND FOLEY[25], and ROSENBERG, DURANT AND HOLLAND[26], and by the studies *in vitro* of HAGIHARA *et al.*[15], THIER *et al.*[19,21], and MCCARTHY *et al.*[20]. A similar cystine–diamino acid relationship in kidney was originally suggested by studies of renal tubular reabsorption *in vivo*; this has been reviewed by KNOX[27]. However, studies *in vitro* in rat- and human-kidney cortex slices have consistently indicated separate transport influx pathways for the dibasic amino acids, cystine, and more recently cysteine[11]. These findings have led to a speculation that cystinuria, an inherited disease manifested by excessive urinary loss of cystine, lysine, arginine, and ornithine, is not caused by a single genetic error of a membrane transport process[22]. Related evidence for the existence of distinct transport mechanisms for cystine and the dibasic amino acids has also been reported by MAAS in *E. coli*[14]. He has described a permease mutant with defective accumulation of lysine, arginine, and ornithine, but with a normal uptake of cystine.

The failure of both cystine and cysteine to exchange with the dibasic amino acids is further support for the lack of a common influx pathway in kidney tissue. However, the marked inhibition of cysteine efflux by the dibasic amino acids suggests a common efflux pathway. CRAWHALL AND SEGAL[28] have recently shown that follow-

ing either cystine or cysteine incubations in renal cortex slices, the intracellular form of the amino acid is virtually all cysteine. That is, at some phase of cystine influx, a reduction to intracellular cysteine occurs. Therefore, a cysteine–dibasic amino acid interaction at the efflux site could influence the net transport of both cystine and cysteine. On the basis of this phenomenon we have proposed that the abnormality in genetic and experimental cystinuria may be the result of the impaired cysteine efflux which accompanies a single genetic or induced defect in the dibasic amino acid transport system[12].

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