A COMMON RENAL TRANSPORT SYSTEM FOR LYSINE, ORNITHINE, ARGININE AND CYSTEINE

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This communication presents in vitro evidence of a specific interaction between the transport of lysine, ornithine, arginine, and cysteine in rat kidney slices. An association between these amino acids has not previously been found in isolated renal tissue.

Much of the interest in this area relates to human cystinuria, an inherited disease characterized by the excessive urinary excretion of cystine, lysine, ornithine, and arginine. In 1951, Dent and Rose postulated a single transport mechanism mediating the renal tubular reabsorption of lysine, arginine, and cystine, and that the disease is a consequence of an inborn error in the normal transport pathway. In vitro studies in kidney cortex slices obtained from cystinuric patients undergoing surgery have not supported this hypothesis (Fox, et al., 1964). Cystine uptake was found to be normal in the presence of a defect in lysine and arginine accumulation. Furthermore, there has been no evidence of mutual competition between cystine and dibasic amino acid transport in normal or cystinuric human kidney slices.

In rat kidney cortex slices a common transport pathway for lysine, ornithine, and arginine has been demonstrated (Rosenberg, Downing, and Segal, 1962). As in the human, all previous work in rat kidney has shown no relationship between cystine influx, and that of the dibasic amino acids.

Separate systems have been distinguished by competitive inhibitor studies, sodium dependence, pH sensitivity, and response to metabolic inhibitors. The experiments reported below arose from observations made during an evaluation of the exchange diffusion of dibasic amino acids in this tissue (Schwartzman, Blair, and Segal, 1966, in preparation). There was no flow-counterflow relationship between cystine or cysteine and the actively exchanging system for dibasic amino acids. Although an increase in intracellular cysteine was found in lysine or ornithine preloaded cells, the increase was kinetically dissimilar from that of exchange diffusion. This phenomenon, due to an inhibition of cysteine efflux by the dibasic amino acids, is reported in this paper.

METHODS

Efflux studies were performed by a modification of the technique previously described (Rosenberg, Blair, and Segal, 1961). Rat kidney slices were preloaded for 30 minutes with the radioactive amino acid to be studied for efflux kinetics, and simultaneously with a nonradioactive amino acid to be tested for inhibitory action. The tissues were then transferred to flasks containing only buffer, and at four minute intervals the medium was sampled for radioactivity. After 20 minutes (efflux period) the residual tissue radioactivity was determined. From these data the intracellular concentration of radioactive amino acid at efflux time zero, and the percent loss of radioactivity with time, were calculated. Efflux time zero concentrations were confirmed by independent uptake studies. The efflux time zero concentrations of test inhibitors were determined in separate uptake experiments using radioactive tracers under identical conditions to the preloading phase of the efflux experiments.

RESULTS

In all of the cysteine experiments, commercial cystine was reduced and maintained as cysteine by the addition of 2mM dithiothreitol (DTT)

to the preloading medium, as well as to the efflux buffer (Crawhall and Segal, 1966). The efflux of S^{35}-cysteine following cysteine preloading, and the effect of ornithine, lysine, and arginine on the efflux are shown in Figure 1. In Table I, the data are expressed as fractional rate

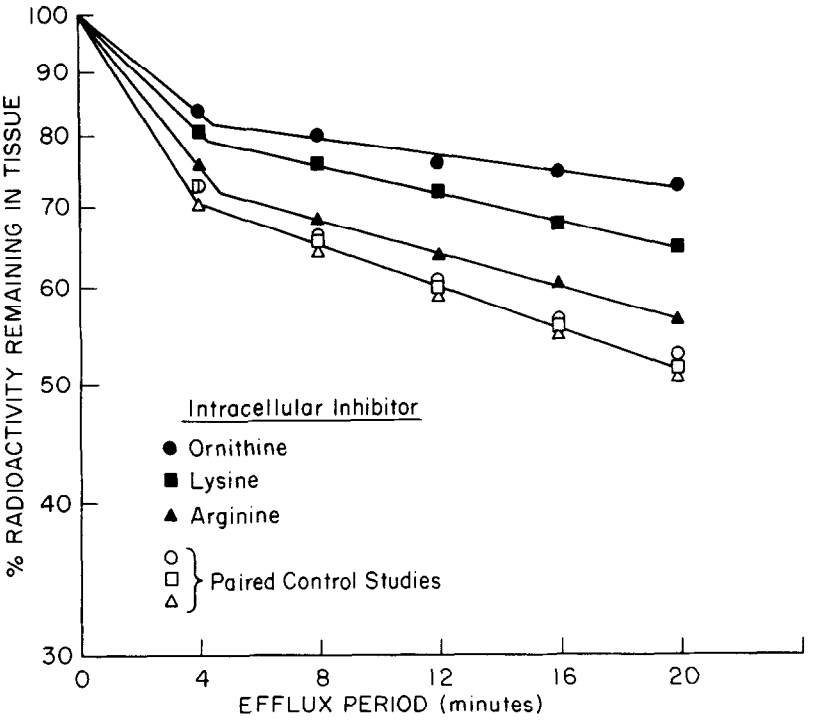

Fig. 1. Effect of dibasic amino acids on S^{35}-cysteine efflux. Each point represents an average two separate determinations. Control and inhibitor studies were paired for each experiment. Curves shown were drawn by eye.

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constants of the efflux, computed by least squares fit of the 8 to 20 minute points to a linear semilog plot. The results indicate marked dibasic amino acid inhibition of efflux of radioactivity from tissues preloaded with S^{35}-cysteine. Glycine and valine do not have thie effect. Ornithine does not alter the efflux of glycine or valine.

The S^{35}-cystine experiments were carried out as for cysteine,

except DTT was not added to the preincubation or efflux media. The relative effect of each test inhibitor on S^{35} efflux kinetics is similar for cysteine and cystine studies, although statistical significance is not present for the lysine and arginine inhibitions of the latter. Crawhall

Table I. Amino Acid Efflux

PRELOADED FOR INHIBITION		PRELOADED FOR EFFLUX						
		S^{35}-CYSTEINE		S^{35}-CYSTINE			C^{14}-VAL	C^{14}-GLY
Amino Acid	Conc. ICF	F.R.C. ± S.D.	Conc* ICF	F.R.C. ± S.D.	Conc* ICF		F.R.C.	F.R.C.
None	-	.0190 ± .0006	.47	.0134 ± .0011	.30		.0302	.0204
Orn A	-	.0079 ± .0011	.75	.0083 ± .0019	.26		.0302	.0192
Orn B	-	.0100 ± .0011	.33	-	-			
Lys	20	.0137 ± .0011	.66	.0107 ± .0019	.26			
Arg	15	.0155 ± .0011	.50	.0112 ± .0015	.18			
Val	15	.0182	.32	.0124	.22			
Gly	28	.0192	.45	.0127	.18			

Efflux rates are expressed as fractional rate constants (F.R.C.). Preloading concentrations were: inhibitors, 15mM; cysteine, .08mM; cysteine in Orn B study, .04mM; cystine, .07mM. *Intracellular fluid (ICF) concentrations for both the cysteine and cystine studies are expressed in mM cysteine, calculated from total intracellular counts recovered, and corrected for 90% cysteine after cysteine load, and 80% cysteine after cystine load.

and Segal (1966) have shown that following either cystine or cysteine (cystine + DTT) loading of kidney slices, the intracellular form of the amino acid is 80-90% cysteine. Therefore, in both the cysteine and cystine efflux experiments reported in Table I, the tissues contain largely intracellular cysteine at the start of the efflux period. The difference between the efflux rate constants for cysteine and cystine appears to be a function of DTT in efflux medium of the cysteine experiments. This

was confirmed by a study in which S^{35}-cystine was preloaded, and the efflux compared into buffer alone, and buffer with DTT. The rate constants were .0150 and .0210, respectively, values similar to the control efflux constants for cystine and cysteine shown in Table I. The DTT effect is specific to the efflux of cysteine. DTT has no effect on the efflux of valine or lysine.

The dibasic amino acid inhibition of cysteine transport appears to be limited to the efflux step. In Table 2, cysteine uptakes are

Table II. Amino Acid Uptake

INHIBITOR	S^{35}-CYSTEINE 10 min	30 min	S^{35}-CYSTINE 30 min
NONE	2.44	6.44	2.65
ORNITHINE	3.18**	10.33*	2.23*
LYSINE	3.27**	9.15*	2.23*
ARGININE	2.82	6.98*	1.68*
VALINE	1.97	4.54*	1.88*
GLYCINE	2.44	6.20	1.65*

$\frac{\text{intracellular}}{\text{extracellular}}$

Table II. Distribution ratios of radioactivity after cysteine and cystine incubations. The "cystine" ratios are expressed as radioactivity ratios for comparison with previously published studies. These are not true concentration ratios since the intracellular form after cystine incubation is at least 80% cysteine. Incubation concentrations used for these studies were: inhibitors, 15mM; cysteine, .08mM; cystine, .07mM. *$p<.01$, **$p<.05$ by t test for paired data.

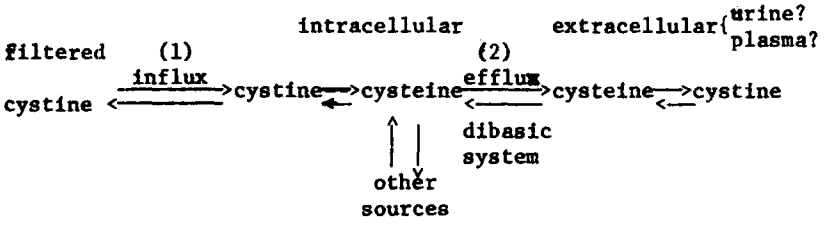
expressed as radioactivity distribution ratios at 10 and 30 minutes. The inhibition of efflux, shown in these studies as an increase in distribution ratio, is seen as early as 10 minutes. In contrast, the uptake of cystine is significantly decreased by each of the amino acids studied at this concentration. The difference between cysteine and cystine

sensitivity to inhibitors suggests separate influx pathways for these two. The sensitivity of cystine influx to all of these amino acids is not explained, but it does not indicate a specific cystine and dibasic amino acid transport system.

DISCUSSION

In addition to the *in vitro* work in rat, human, and human cystinuric kidney, certain *in vivo* findings do not support the common transport hypothesis of Dent and Rose. (i) Lysine infusion into dogs produces cystinuria, but with a renal cystine clearance in excess of the glomerular filtration rate (Webber, Brown, and Pitts, 1961). That is, the effect of lysine involves, at least in part, what may be the tubular secretion of cystine. (ii) Several cystinuric patients are reported in whom renal cystine clearance exceeds GFR, suggesting the natural occurrence of tubular cystine secretion in this disorder (Arrow and Westall, 1958; Frimpter, et.al., 1962; Crawhall and Thompson, 1965).

The common efflux pathway described in this paper may explain these findings. It is tempting to speculate the following scheme for the renal reabsorption of cystine.



If the efflux of cysteine, Step (2), were retarded by a competitive concentration of a dibasic amino acid, one would anticipate (i) *in vitro*, an increase of intracellular cysteine, (ii) *in vivo*, a shift of flow to the left. If Step (2) were defective in cystinuria, a similar sequence of events should ensue. This hypothesis supports the concept of cystinuria as a single genetic error of dibasic amino acid renal

transport. However, the mechanism of excessive urinary cystine excretion is considered to be impaired cysteine efflux, rather than impaired cystine influx.

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