Derivation of Pipecolic Acid from L-Lysine by Familial Hyperlysinemics

N. C. WOODY^[13] AND M. B. PUPENE

Department of Pediatrics, Tulane University School of Medicine, New Orleans, Louisiana, USA

Extract

Normal children and children with familial hyperlysinemia were given L-(U-¹⁴C)-lysine by intramuscular injection. Labeled pipecolic acid and *N*- α -acetyllysine were found in the urines, indicating derivation of these substances from lysine. The percentage of administered radioactivity excreted as ¹⁴CO₂ and as urinary lysine varied markedly between controls and those with hyperlysinemia, but the percentage excreted in the urine in the form of various metabolites of lysine was about the same for controls (1.7-2.4) and those with hyperlysinemia (1.9-2.2). The proportion of these urinary lysine metabolites comprising pipecolic acid, homocitrulline, homoarginine, *N*- ϵ -acetyllysine and *N*- α -acetyllysine was two to seven times greater for children with hyperlysinemia than for controls. These findings suggest that these pathways for lysine degradation are being maximally utilized by patients with familial hyperlysinemia who cannot degrade lysine by way of the saccharopine pathway.

Speculation

L-Pipecolic acid is derived in humans, at least in part, from L-lysine. The availability of a pathway for lysine degradation by way of pipecolic acid may play some part in the fact that three of four children with hyperlysinemia in this family had no clear somatic expression of biochemical disorder.

Introduction

When pipecolic acid was found in the urine of young infants and of patients with familial hyperlysinemia, it was postulated that it derived from metabolism of L-lysine [8]. However, Grove and Henderson [3], in reviewing the earlier studies of Rothstein and Miller [4], demonstrated that rats derived more L-pipecolic acid from D-lysine than from L-lysine (31.1% of D-lysine being so converted as compared with 1.1% of L-lysine). This finding suggested that pipecolic acid excreted by normal humans [2, 8] might be derived from dietary D-lysine. In fact, the observation [2] that oral administration of L-lysine to normal subjects did not alter plasma pipecolate levels would support this idea.

To determine whether normal children and children with familial hyperlysinemia could convert L-lysine to pipecolate, the excretion of labeled pipecolic acid and other lysine metabolites was measured following administration of L-(U-¹⁴C)-lysine.

Materials and Methods

Two siblings with familial hyperlysinemia and two appropriate control children were given 25 μ Ci of L-(U-¹⁴C)-lysine [10] by intramuscular injection [11]. This was prepared, sterilized, and tested for purity as previously described [7]. Urines were collected every 1.5 hr for 12 hr; 10% thymol in isopropanol was added, and specimens were stored at -50° until ana-

Table I. Percentage of administered radioactivity excreted during first 6 hr

	Control subjects		Patients with hyperlysinemia	
	A	B	I	II
$^{14}\text{CO}_2$	7.4	9.1	0.6	0.4
Urine				
Lysine	1.1	1.5	14.4	1.8
Lysine metabolites	1.7	2.0	1.6	1.5

lyzed. Urine concentrates were separated by the technique of Spackman, Stein, and Moore [5, 8] into identifiable fractions by stream splitting. Four-fifths of the sample was diverted to a fraction collector and gathered in 2-ml portions. Each fraction was placed in a scintillation vial and dried on a 60° hot plate in the hood. The dried residue was dissolved in 0.5 ml of water. Twenty milliliters of scintillation fluid (a mixture of 67% toluene and 33% methylcellulose with PPO 0.4% and POPOP 0.01%) were added to each vial. Vials were counted in a scintillation counter [9] with a 50% efficiency for ^{14}C . When ^{14}C -glycine was serially diluted in water, processed as above, and counted, recoveries averaged 90% and were linear; when dissolved in the 0.2 N Na citrate buffers, pH 3.25 and pH 4.25, used in the amino acid analyzer, recoveries were, respectively, 86 and 64% and were also linear. No correction was made for this quenching effect. Methods for counting $^{14}\text{CO}_2$ and total lysine and lysine metabolites in urine have been previously described [7].

Results

Percentage of radioactivity excreted during the 6 hr following administration of L-(U- ^{14}C)-lysine is shown in Table I. This table restates, in somewhat more de-

tail, data previously gathered in collaboration with others [7]. Of interest here is the fact that the percentage excreted in the urine as lysine metabolites is about the same for all the subjects although excretion of $^{14}\text{CO}_2$ and urinary lysine varied markedly between the controls and patients with hyperlysinemia.

Table II compares radioactivity measured in lysine and in the lysine metabolites in the urine. Values for homocitrulline, homoarginine, and N- ϵ -acetyllysine have been reported [8] and are included here for comparison with those found for pipecolic acid and N- α -acetyllysine. The percentage of lysine metabolites excreted in the form of pipecolic acid, homocitrulline, homoarginine, N- ϵ -acetyllysine, and N- α -acetyllysine were two to seven times greater for patients with hyperlysinemia than for controls.

Discussion and Summary

Although control subjects and patients with hyperlysinemia both excreted about the same amount of the administered radioactivity in the form of lysine metabolites in the urine, the children with hyperlysinemia excreted a larger proportion of the label in the specific metabolites listed in Table II. This difference supports

Fig. 1. Major and minor degradation pathways for lysine in man. Site of the block in familial hyperlysinemia is shown [1].

Table II. Lysine and lysine metabolites in urine¹

Compound	Control subjects				Patients with hyperlysinemia			
	Patient A		Patient B		I		II	
	Preload ²	Postload	Preload	Postload				
Lysine	3,588	4,635	5,106	4,458	28,883		10,117	
Pipecolic acid	(2.3) ³ 92	(6.1) 221	(4.7) 207		(21.8) 540		(10.6) 392	
Homocitrulline	0	(2.7) 100	0	(2.4) 86	(15.5) 381		(16.2) 600	
Homoarginine	0	(0.8) 28	0	(1.1) 40	(4.5) 112		(3.0) 110	
N- ϵ -acetyllysine	0	(2.7) 97	(3.4) 149	(6.8) 246	(13.2) 324		(9.0) 334	
N- α -acetyllysine	(5.8) 233	(4.9) 180	(3.8) 167	(6.5) 238	(15.5) 381		(19.3) 714	
Total lysine metabolites	4,002	3,640	4,375	3,640	2,463		3,698	

¹ Values are cpm/mg of urinary creatinine.

² With 150 mg/kg of L-lysine orally.

³ Figures in parentheses are percentage of total lysine metabolites.

the observation of Dancis *et al.* [1] that normal children have a metabolic pathway, through the formation of saccharopine, which is open to them for the metabolism of lysine and which is not available to children presenting with familial hyperlysinemia. The latter are utilizing alternate pathways via the substances shown in Figure 1. The availability of these alternate paths, especially that by way of pipecolic acid, may explain the fact that three of the four children with hyperlysinemia in this family had normal growth and were not mentally affected by their metabolic disease [6].

Demonstration of radioactivity in urinary pipecolate and *N*- α -acetyllysine of children and patients with hyperlysinemia following parenteral administration of L-(U-¹⁴C)-lysine indicates that these substances are derived from L-lysine in the human.

References and Notes

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9. Nuclear Chicago, model 720, Des Plaines, Ill.
10. Chemtrac, Cambridge, Mass.
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13. Requests for reprints should be addressed to: N. C. Woody, M.D., Department of Pediatrics, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, La. 70112 (USA).
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