PIPECOLIC ACID PATHWAY: THE MAJOR LYSINE METABOLIC ROUTE IN THE RAT BRAIN

Yung-Feng Chang

Department of Microbiology
University of Maryland Dental School
Baltimore, Maryland

Received January 15,1976

Summary: Lysine metabolism was studied in the rat brain by intraventricular injection of ^{14}C-labeled *L*- and *D*-lysine. The major metabolic intermediate in the brain labeled from either lysine isomer was found to be pipecolic acid which was characterized to be in the *L*-configuration. Small amounts of labeled α-aminoadipic acid could also be detected in the brain samples from rats 24 hours after injection with either lysine isomer. *L*- or *D*-lysine, therefore, appears to be metabolized in the rat brain via pipecolic acid to α-aminoadipic acid which differs from the major saccharopine pathway known to be operative in the liver or other mammalian tissues.

INTRODUCTION

Although the major pathway of *L*-lysine metabolism in mammals is in general believed to be via saccharopine (ε-N-[glutaryl-2]-*L*-lysine) (1-4), lysine utilization in animals and humans take many diversified routes (5,6). In spite of the fact that brain is known to be active in the metabolism of many amino acids, the metabolic fate of lysine in the brain has so far not yet been identified. As far as the major mammalian saccharopine pathway of lysine metabolism is concerned, the mammalian brain appears to be very inactive (3, 7). The difficulty to demonstrate *in vitro* lysine metabolism in the brain, as was experienced in this laboratory, may have contributed to the lack of knowledge in brain lysine metabolism. Through the use of the intraventricular injection technique, an active lysine metabolism was demonstrated in the rat brain in this report which indicates that *L*- and *D*-lysine are both metabolized to *L*-pipecolic acid as the major metabolic intermediate.

MATERIALS AND METHODS

Animal Procedures - Male Sprague-Dawley rats (ARS/Sprague-Dawley) weighing 150-180 g were fasted overnight before the experiments. Sodium pentobarbital (5 mg per 100 g body weight) was administered intraperitoneally to anesthesize the animal before placing it into a stereotaxic assembly for intraventricular injections of the brain. Ten µl portions of the labeled lysine at concentra-

tions indicated were injected into each lateral ventricle (i.e., two injections per animal). When urine samples were to be collected (i.e., animals kept for 24 hours after intraventricular injections) 1 mmole *L*-pipecolic acid (NaOH neutralized) was administered intraperitoneally immediately after brain injection for radioactive intermediate trapping. Animals were kept in metabolism cages provided with water only and their urine was collected in a petri dish in the presence of several drops of 0.5% pentachlorophenol in ethanol. At the termination of experiments, animals were sacrificed by decapitation. Brain and liver were homogenized in 5 volumes of 50% acetone with a Potter-Elvehjem glass homogenizer to extract soluble lysine metabolites. Urine samples were also deproteinized by acetone (50% final concentration). Precipitates in the above extracts were removed by centrifugation after overnight storage at 4°. The acetone extracts were concentrated under a stream of nitrogen gas at 50° and desalted through a Dowex 50 (H^+) column eluting with 1*N* NH_4OH prior to their application to the amino acid analyzer.

Analytical Procedures - Radioactive lysine metabolites in various samples were analyzed by a Technicon automatic amino acid analyzer equipped with a Durrum DC-1A column (0.63 x 57 cm). The column was eluted with the Pico-Buffer II system (Pierce) programmed as indicated in Fig. 1 at a flow rate of 35 ml per hour at 52°. Approximately one-half of the column effluent passed through the analyzer monitoring system, while the remainder passed into an automatic fraction collector. Each 1-minute fraction was mixed with 2.5 ml of Aquasol (New England Nuclear) for radioactivity counting in a Mark I liquid scintillation spectrometer. The counting efficiency of this instrument was about 90%.

The stereochemistry of the recovered ^{14}C-pipecolic acid was determined by incubation with *D*-amino acid oxidase in a reaction mixture (2 ml) containing the following ingredients: 0.3 µmole *DL*-pipecolate, 8000 units catalase, 0.85 unit *D*-amino acid oxidase (Sigma Chemical Co.), 0.02 µmole Na-pyrophosphate and approximately 20,000 cpm of ^{14}C-pipecolate obtained either from *L*- or *D*-^{14}C-lysine metabolism. The reaction was continued for 2 hours at 37° with aeration before termination by acidifying to pH 1 with 50% HCl. After removal of precipitated protein, the reaction mixtures were concentrated under reduced pressure to 1/10 of the original volume before analysis in the amino acid analyzer.

Ascending paper chromatography was performed on 3 MM Whatman filter paper in the solvent systems specified. Paper electrophoresis was conducted on a Savant flat plate high voltage electrophoresis system using the buffer indicated.

Chemicals Prepared - *D*-[2-^{14}C]lysine was prepared from *DL*-[2-^{14}C]lysine (New England Nuclear) by the procedures detailed earlier (8). Both the prepared *D*-[2-^{14}C]lysine and the commercially obtained *L*-[U-^{14}C]lysine (New England Nuclear) were purified through the amino acid analyzer and desalted by a Dowex 50 (H^+) column. Saccharopine was prepared according to the procedure of Larson (9).

RESULTS

Labeling of Pipecolate from ^{14}C-Lysine in the Brain - The formation of labeled pipecolic acid in the rat brain as a result of both *L*- and *D*-^{14}C-lysine metabolism is demonstrated in the experimental results shown in Fig. 1. It can be seen in Fig. 1B that pipecolic acid was the only radioactive *L*-lysine metab-

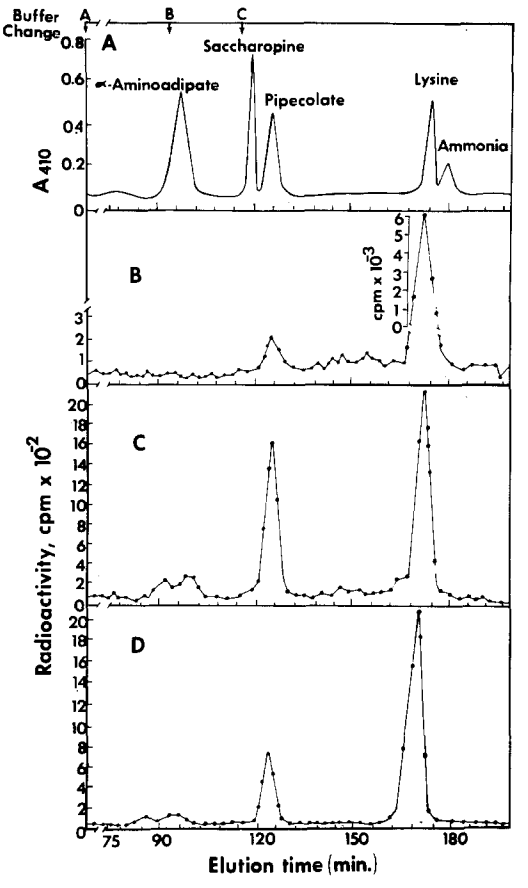

Fig. 1. Elution positions of ^{14}C-lysine metabolites from brain samples analyzed by a Technicon automatic amino acid analyzer. Fifty microliters (1/10 of total) of brain sample were applied to the analyzer column for analysis. A. Standard amino acids: α-aminoadipate, 50 nmoles; saccharopine, 250 nmoles; pipecolate, 12.5 μmoles; and lysine, 100 nmoles. B. 4 hours after injection of 4 x 10^6 cpm *L*-[U-^{14}C]lysine (300 Ci/mole). C. 24 hours after injection of 96.8 x 10^6 cpm *L*-[U-^{14}C]lysine (300 Ci/mole) and 1 mmole *L*-pipecolate. D. 24 hours after injection of 19.6 x 10^6 cpm *D*-[2-^{14}C]lysine (1.1 Ci/mole) and 1 mmole *L*-pipecolate. Radioactive lysine was injected intraventricularly and the overloading *L*-pipecolate intraperitoneally.

olite identifiable in the brain extracts 4 hours after intraventricular injection. In fact, labeled pipecolic acid could be detected in the brain extracts as early as 1 hour after injection. The 24-hour brain samples from animals injected with *L*- or *D*-^{14}C-lysine also showed pipecolic acid as the only labeled

Table 1. Quantitation of Labeled Metabolites Recovered from Brain, Urine and Liver after Intraventricular Injection with ^{14}C-lysine

Compound Injected	Sample	Labeled Metabolites Recovered		
		α-Aminoadipate	Pipecolate	Lysine
			cpm	
L-[U-^{14}C]lysine	Brain	28,750(0.03)	170,780(0.18)	281,014(0.29)
	Urine	trace	1,715,386(1.78)	1,164,145(1.21)
	Liver	trace	4,232(0.004)	4,542(0.01)
D-[2-^{14}C]lysine	Brain	17,204(0.02)	71,622(0.07)	208,288(0.22)
	Urine	trace	907,994(0.94)	199,709(0.21)
	Liver	trace	5,405(0.01)	5,542(0.01)

Rats were injected with either *L*-[U-^{14}C]lysine or *D*-[2-^{14}C]lysine and neutralized *L*-pipecolate as indicated in Fig. 1C and D. The animals were kept for 24 hours before sacrifice. Radioactivity of each metabolite was computed by integration of the corresponding peak area minus the background from the amino acid analysis data shown in Fig. 1. Figures in parenthesis indicate % of radioactivity in each recovered metabolite out of the total injected; "trace" indicates radioactivity less than 1,000 cpm. Other details are described in the text.

major metabolic intermediate (Fig. 1C and 1D). Besides pipecolic acid, the 24-hour brain samples also displayed a trace of radioactivity eluting under the standard *L*-α-aminoadipic acid peak.

Quantitation of Labeled Metabolites Recovered from Brain, Urine and Liver

After 24 hours of brain injection with either *L*- or *D*-^{14}C-lysine, pipecolic acid appeared to be the highest ^{14}C-labeled metabolite in the brain, urine or liver samples analyzed (Table 1). In fact, in the urine samples the radioactivity recovered in pipecolic acid was higher than that in lysine (pipecolate to lysine ratio 1.75 and 5.15, respectively for *L*- and *D*-^{14}C-lysine injected). Labeled α-aminoadipate could only be detected in the brain samples obtained from either *L*- or *D*-^{14}C-lysine injection. The liver samples had very low levels of radioactive pipecolate and also retained very low levels of radioactive lysine in both experiments.

Characterization of Lysine Metabolic Intermediates - The radioactive com-

pound recovered under the pipecolic acid peak either obtained from *L*- or *D*-^{14}C-lysine injection was identified as pipecolic acid by autoradiography of paper chromatograms developed in the following solvent systems: methanol-n-butanol-benzene-water (2:1:1:1 with 1% acetic acid) (R_f 0.65), n-butanol-pyridine-water (1:1:1) (R_f 0.91) and phenol-water-acetic acid (5:1:6) (R_f 0.49); it also coelectrophoresed with authentic *L*-pipecolic acid in 0.02 *M* sodium barbital buffer, pH 8.6. When ^{14}C-pipecolic acid, either recovered from *L*- or *D*-^{14}C-lysine metabolism, was treated with commercial *D*-amino acid oxidase the radioactivity remained in pipecolic acid, whereas externally added *D*-pipecolic acid was readily converted to Δ^1-piperideine-2-carboxylic acid (α-keto-ε-amino-caproic acid) as was detected by the amino acid analyzer. These results indicate that both *L*- and *D*-lysine gave rise to *L*-pipecolic acid in the rat brain.

Due to the low radioactivity in the compound recovered under the α-amino-adipic acid peak further characterization employing other methods was not successful. However, the radioactive compound eluting under α-aminoadipic acid in the amino acid analysis shown in Fig. 1 also could be detected eluting under the same compound when Pico-Buffer C alone was used as running buffer.

DISCUSSION

The experimental results presented above indicate that *L*- and *D*-lysine are both metabolized in the rat brain to *L*-pipecolic acid as a major intermediate. Since no labeled saccharopine was detected in the brain samples after various times of brain injection (Fig. 1), the possibility of either lysine isomer being metabolized through saccharopine, as was established by earlier reports in other tissues (1-4), appears to be precluded. This observation is consistent with the reported evidence that mammalian brain contains very low level of lysine-α-ketoglutarate reductase, the enzyme responsible for the conversion of lysine to saccharopine, as compared to the liver, heart or kidney (3, 7). In view of the early appearance (within 1 hour of injection) of labeled pipecolic acid in the brain and the lack of other detectable lysine

metabolites, it is quite unlikely that *L*-pipecolic acid would have arisen from *L*-lysine via saccharopine and Δ^1-piperideine-6-carboxylate (α-aminoadipic semialdehyde) through the reduction of the aldimine.

The formation of pipecolic acid from *L*-lysine appears to be as active as from *D*-lysine (Table 1). This is in contrast to the results obtained from intraperitoneal injection of ^{14}C-lysine in which high levels of labeled pipecolic acid was only recovered from urine of animals receiving *D*-^{14}C-lysine with an *L*-pipecolate overload (10, 11; Y. F. Chang unpublished results). The presence of labeled α-aminoadipate in the brain samples but not other samples from rats receiving ^{14}C-lysine intraventricularly may indicate that either this compound was less actively metabolized in the brain than other body tissues or it was metabolized further before it was transferred out of the brain.

In spite of the high activity of the pipecolic acid pathway in the intact rat brain, this pathway could not be demonstrated in the *in vitro* systems tested in this laboratory which employed various buffers, cofactors and coenzymes under different reaction conditions. In the absence of a working *in vitro* system, the actual mechanism of pipecolic acid formation from lysine in the brain, therefore, cannot yet be elucidated. However, the most likely mechanism by which *L*-lysine or *D*-lysine can be converted to *L*-pipecolic acid is by loss of the α-amino group (through transamination or deamination) and cyclization to Δ^1-piperideine-2-carboxylate followed by reduction of this unsaturated ketimine. Although the postulated intermediate Δ^1-piperideine-2-carboxylate has not yet been demonstrated in this system, the enzyme, Δ^1-piperideine-2-carboxylate reductase, responsible for its conversion to *L*-pipecolic acid has been reported in the rat brain (12) and was also observed in this laboratory.

Pipecolic acid was found not only in the urine of young infants (13) and children with hyperlysinemia (13, 14) but also in the brain of the dog (15) and an infant with hyperpipecolatemia (16, 17). Pipecolic acid has also been shown to be a precurser of piperidine (18, 19), an alicyclic amine which is

known to have nicotine-like action, and to be involved in the sleep-awake effect of animals (20, 21) and in the hibernation of mollusks (22). In view of these facts, the pipecolic acid pathway in the mammalian brain may, therefore, play a far more active and significant role in the nervous system than was previously realized.

Acknowledgement: I thank Dr. P. D. Thut for help in the intraventricular injections and the use of the stereotaxic instrument. This work was supported by a U.S. Public Health Service Grant NS 11822 from the National Institute of Neurological Disease and Stroke.

REFERENCES

1. Higashino, K., Tsukada, K. and Lieberman, J., Biochem. Biophys. Res. Commun., 20, 285 (1965).
2. Higashino, K., Fujioka, M., Aoki, T. and Yamamura, Y., Biochem. Biophys. Res. Commun., 29, 95 (1967).
3. Hutzler, J. and Dancis, J., Biochim. Biophys. Acta, 158, 62 (1968).
4. Higashino, K., Fujioka, M. and Yamamura, Y., Arch. Biochem. Biophys., 142, 606 (1971).
5. Meister, A., in Biochemistry of the Amino Acids 2nd ed., Vol. II, p. 941-951, Academic Press, New York (1965).
6. Scriver, C. R. and Rosenberg, L. E., in Amino Acid Metabolism and its Disorders p. 250-255, W. B. Saunders, Philadelphia (1973).
7. Hutzler, J. and Dancis, J., Biochim. Biophys. Acta, 377, 42 (1975).
8. Chang, Y. F. and Adams, E., J. Bacteriol., 117, 753 (1974).
9. Larson, P. O., Acta Chem. Scand., 26, 2562 (1972).
10. Grove, J. and Henderson, L. M., Biochim. Biophys. Acta, 165, 113 (1968).
11. Grove, J., Gilbertson, T. J., Hammerstedt, R. H. and Henderson, L. M., Biochim. Biophys. Acta 184, 329 (1969).
12. Meister, A., Radhakrishnan, A. N. and Buckley, S. O., J. Biol. Chem. 229, 789 (1957).
13. Woody, N. C. and Pupene, M. B., Pediat. Res., 4, 89 (1970).
14. Woody, N. C., Amer. J. Dis. Child. 108, 542 (1964).
15. Kase, Y., Kataoka, M., Miyata, T. and Okano, Y., Life Sci., 13, 867 (1973).
16. Gatfield, P. D. and Taller, E., Brain Res., 29, 170 (1971).
17. Gatfield, P. D., Taller, E., Hinton, G. G., Wallace, A. C., Abdelnour, G. M. and Haust, M. D., Canad. Med. Ass. J., 99, 1215 (1968).
18. Kase, Y., Kataoka, M. and Miyata, T., Life Sci. 6, 2427 (1967).
19. Kase, Y., Okano, Y., Yamanishi, Y., Kataoka, M., Kitahara, K. and Miyata, T., Life Sci., 9, 1381 (1970).
20. Stepita-Klauco, M., Dolezalova, H. and Fairweather, R., Science, 183, 536 (1974).
21. Drucker-Colín, R. and Giacobini, E., Brain Res., 88, 186 (1975).
22. Dolezalova, H., Stepita-Klauco, M. and Fairweather, R., Brain Res., 72, 115 (1974).