CARNITINE BIOSYNTHESIS: THE FORMATION OF GLYCINE FROM CARBONS 1 AND 2 OF 6-N-TRIMETHYL-L-LYSINE

J. Bradley Hochalter and L. M. Henderson

Department of Biochemistry, University of Minnesota, St. Paul, MN 55108.

Received March 18,1976

Summary

The excreted hippuric acid from rats administered 6-N-trimethyl-L-[1-^{14}C]-lysine together with a dose of sodium benzoate was isolated and analyzed for radioactivity. From 21 to 30% of the isotope from metabolized substrate was incorporated into hippuric acid showing that there is a rather direct conversion of carbons 1 and 2 of 6-N-trimethyl-L-lysine to glycine.

Introduction

6-N-Trimethyl-L-lysine has recently been shown to be an intermediate in carnitine biosynthesis. In a lysine auxotroph of Neurospora crassa methyl labeled-trimethyllysine was incorporated in high yield (16 and 19%) into carnitine (1). Trimethyllysine has also been shown to be converted in high yield into carnitine in the rat (2,3). We have shown that when 6-N-[methyl-^{14}C]trimethyl-L-lysine is administered to a 12-day old chick embryo that the isotope is converted almost quantitatively into carnitine and γ-butyrobetaine by day 19 (unpublished data).

As a first step toward understanding more completely the mechanism of this conversion we have attempted to identify the 2-carbon fragment arising from the cleavage reaction. The high incorporation of label from trimethyllysine into hippuric acid shows that this 2-carbon fragment is glycine or a closely related compound.

Materials and Methods

Synthesis of 6-N-trimethyl-L-[1-^{14}C]lysine: 6-N-Benzoyl-DL-[1-^{14}C]lysine was prepared from the copper complex of DL-[1-^{14}C]lysine by the acid chloride method (4) and resolved with ε-lysine acylase isolated from Achromobacter pestifer EA (5). ε-N-Carbobenzoxy-L-[1-^{14}C]lysine was prepared through the copper complex, then acetylated at the α-position with acetic anhydride. After removal of the carbobenzoxy group with H_2/Pt, the α-N-Acetyl-L-[1-^{14}C]lysine was methylated with an excess of methyl iodide in aqueous barium hydroxide. This product was refluxed in 6 N HCl for 24 hr. to give, after ion exchange chromatography, pure 6-N-trimethyl-L-[1-^{14}C]lysine.

Copyright © 1976 by Academic Press, Inc.
All rights of reproduction in any form reserved.

Glycine trapping experiments: Male Weanling Sprague-Dawley rats were given 6-N-trimethyl-L-[1-^{14}C]lysine by intraperitoneal injection in a solution of 0.5 M or 1.0 M sodium benzoate (1 ml/100 g body wt.). Urine and CO_2 were collected for 24 hours. Hippuric acid was isolated from the 24-hour urine by continuous extraction with ether, diluted with 200 mg of unlabeled hippuric acid, and recrystallized to constant specific activity. Samples of the 24-hour urine were also used for ion exchange column chromatography. Urine samples were applied to a 1.2 x 50 cm column of Ag-50 (H^+), washed with 100 ml of water, and eluted with an increasing, linear HCl gradient (200 ml each of 1.5 N HCl and 5.0 N HCl). From the elution profile and subsequent paper electrophoresis at pH 3.7 it was shown that, in addition to hippuric acid, 6-N-trimethyl-L-[1-^{14}C]lysine and a ^{14}C-compound tentatively identified as the α-N-acetyl derivative of trimethyllysine were also excreted in the urine.

Results and Discussion

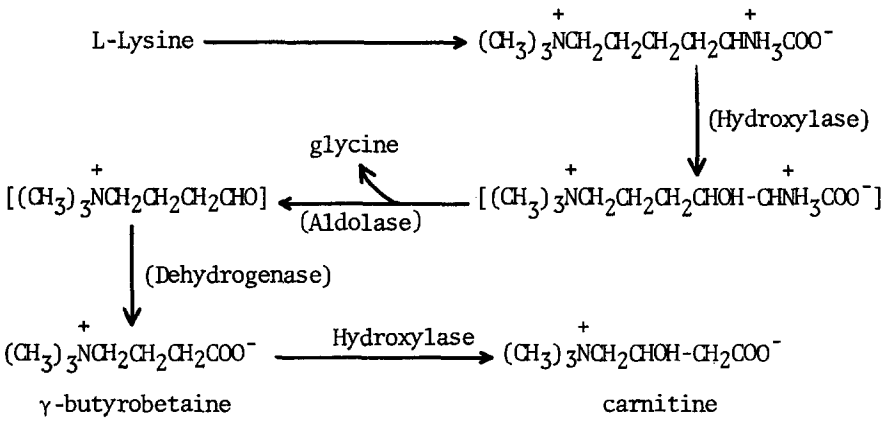
Table I summarizes the results of three experiments in which rats were given 6-N-trimethyl-L-[1-^{14}C]lysine together with a dose of sodium benzoate. From 6.7 to 14.9% of the ^{14}C was incorporated into hippuric acid. Since a large fraction of the isotope recovered in the urine was present in unmetabolized substrate (either trimethyllysine or its derivative) the value for the per cent incorporation was recalculated based only on metabolized trimethyllysine. From 21.6 to 30.3% of the ^{14}C from the metabolized substrate was incorporated into hippuric acid.

TABLE I

Degree of Labeling of Urinary Hippurate in the Rat from 6-N-Trimethyl-L-lysine-[1-^{14}C]

Rat No.	1	2	3
Weight gm.	120	113	116
Na Benzoate, mmoles	0.60	0.57	1.16
TML-[1-^{14}C] μmoles	7.6*	1.7**	1.7**
24-hour $^{14}CO_2$ % of dose	6.5	10.1	11.6
24-hour urine % of dose	84.6	65.4	53.8
^{14}C recovered % of dose	91.1	75.5	65.4
^{14}C in hippurate % of dose	6.7	14.9	12.7
μmoles uncleaved ^{14}C-TML	5.9	0.86	0.70
μmoles ^{14}C-TML cleaved	1.7	0.84	1.0
μmoles ^{14}C Hippurate	0.51	0.25	0.22
% of cleaved TML ^{14}C in Hippurate	30.3	30.1	21.6

* SA = 0.68 μCi per μmole
** SA = 0.74 μCi per μmole



Scheme 1. Postulated pathway of carnitine biosynthesis.

This high incorporation of isotope from trimethyllysine into hippuric acid shows that there is rather direct conversion of carbons 1 and 2 to glycine. From what is known about metabolic pathways leading to urinary hippurate (6) it appears that either glyoxylate or glycine is the 2-carbon fragment arising from C_1 and C_2 of trimethyllysine. It seems more likely that glycine is involved, since a corresponding reaction catalyzed by threonine aldolase has been described (7) for the fission of L-threonine to give acetaldehyde and glycine. The reaction sequence postulated for the conversion of trimethyllysine to γ-butyrobetaine is presented in Scheme 1. It involves the hydroxylation of trimethyllysine in the 3-position, aldol cleavage, and dehydrogenation of 4-trimethylaminobutyraldehyde, to give the well recognized intermediate γ-butyrobetaine whose hydroxylation to carnitine has been carefully documented (8).

References

1. Horne, D. W. and Broquist, H. P. (1973) J. Biol. Chem. 248, 2170-2175.
2. Cox, R. A. and Hoppel, C. L. (1973) Biochem. J. 136, 1083-1090.
3. Tanphaichitr, V. and Broquist, H. P. (1973) J. Biol. Chem. 248, 2176-2181.
4. Neuberger, A. and Sanger, F. (1943) Biochem. J. 37, 515-518.
5. Chibata, I., Ishikawa, T. and Tose, T. Methods in Enzymology, Vol XIX pp. 756-762.
6. Weinhouse, S. and Friedman, B. (1951) J. Biol. Chem. 191, 707-717.
7. Braunshtein, A. E. and Vilenkina, G. Y. (1949) Doklady Akad. Nauk. S.S.S.R. 66, 1243.
8. Lindstedt, G. (1967) Biochemistry 6, 1271-1282.