Biochemical Pharmacology, Vol. 28, pp. 2691-2692.
Pergamon Press Ltd. Printed in Great Britain.

METABOLISM OF L-HOMOCITRULLINE BY A MOUSE LIVER ENZYME

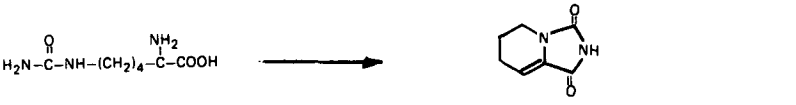
Ronald W. Wilson and Arthur F. Kohrman

Department of Human Development, Michigan State University

East Lansing, MI 48824, U.S.A.

(Received 27 April 1979; accepted 14 June 1979)

We recently reported that L-homocitrulline can be converted to a previously unknown metabolite by living rats and mice and in vitro by snake venom L-aminoacid oxidase (1). Mass spectrometric and nuclear magnetic resonance studies revealed that the compound (see below) was cyclic (2). We postulated by analogy from previous studies (3,4) that the initial intermediate molecular species formed was probably an alpha-ketoacid. The purpose of the present investigation was to explore the nature of the enzymatic reaction responsible for this conversion.



L-HOMOCITRULLINE

6,7-DIHYDROIMIDAZO (1,5-α) PYRIDINE-1,3 (2H,5H) -DIONE

The enzyme reaction was assessed by following the conversion of radioactive L-homocitrulline to the newly described derivative. The amount of product formed was determined by measuring the radioactivity present in the 1st fraction eluted from the cation-exchange column (1). The identity of the product was confirmed by chromatography of the 1st fraction in 3 different solvent systems (1). Tissue homogenates were prepared by grinding 1 g of mouse liver in 5 ml of water in a Potter homogenizer. The homogenate was used without further treatment. The reaction mixture, based on previous studies of glutamine transaminase (5), consisted of: L-homocitrulline, 20 μmoles, plus 0.1 μCi of L- [ureido-^{14}C] homocitrulline (sp. act., 5.0 mCi/mmole); sodium glyoxylate, 20 μmoles; Tris-HCl (pH 9.0), 100 μmoles; pyridoxal phosphate, 1.0 μmole; and homogenate to a final volume of 1.0 ml. Incubation was generally for 90 min at 35°. Controls without homogenate were treated in an identical fashion.

Since the newly described metabolite can be produced by the action of L-aminoacid oxidase, we deduced that the initial product was an alpha-ketoacid. This suggested that the compound was produced in vivo by either a deamination or transamination.

With this in mind, a variety of mouse tissue homogenates were tested under conditions known to be optimal for deamination reactions. In no case was homocitrulline converted to any identifiable product.

Next it was decided to test for a transamination reaction by the addition of an amino-group acceptor to the reaction mixture. Therefore, glyoxylate was added to a liver preparation which contained labeled homocitrulline. The results showed that a significant amount (5%) of the substrate was converted to the newly described metabolite. Product formation was proportionate to protein concentration and was linear for nearly 2 hr of incubation. The pH maximum for the glyoxylate-stimulated reaction was approximately 9.0. Several other ketoacids were tested as amino-group acceptors, and their relative effectiveness was compared to glyoxylate (100%): pyruvate (100%), α-ketobutyrate (55%), α-ketoisocaproate (54%), oxalacetate (51%), α-ketomalonate (33%), and α-ketoglutarate (42%). There was no conversion of homocitrulline in controls which lacked an α-ketoacid. The omission of pyridoxal phosphate led to a reduction of product formation. The lack of a total requirement for this co-factor is probably due to the endogenous levels present in the homogenate. Under optimal conditions of the assay, a small amount (1%) of homocitrulline was converted by mouse kidney homogenate, but no activity could be found in homogenates of brain, spleen, lung, muscle, intestine or intestinal contents.

While the cyclic derivative of the homocitrulline can be produced in vitro by L-aminoacid oxidase (1,2), the results presented here suggest that a transamination reaction may be operative in mouse liver homogenates.

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ACKNOWLEDGEMENTS

This research was supported by NIH Grant No. HD-08456 to A.F. Kohrman.