Biochem. J. (1970) **118**, 895–897
Printed in Great Britain

Short Communications

Metabolism of Pyrrolidonecarboxylic Acid in the Rat

By M. RAMAKRISHNA, P. R. KRISHNASWAMY* and D. RAJAGOPAL RAO
Discipline of Biochemistry, Central Food Technological Research Institute, Mysore-2A, India

(*Received* 12 *May* 1970)

L-Pyrrolidonecarboxylic acid occurs as the *N*-terminal residue of a number of biologically important protein molecules (Blombäck & Doolittle, 1963; Ikenaka & Schmid, 1965; Hood, Gray & Dreyer, 1966; Blombäck, 1967) and is also present in the free form in human skin (Laden & Spitzer, 1967). Formation of L- and D-pyrrolidonecarboxylic acid in several enzymic reactions involving glutamic acid, glutamine and glutaminyl-peptides has been reported in the literature (Wilson & Koeppe, 1961; Krishnaswamy, Pamiljans & Meister, 1962; Meister, Bukenberger & Strassburger, 1963; Orlowski, Richman & Meister, 1969; Messer & Ottesen, 1965; Niwaguchi, Motohashi & Strecker, 1965; Bernfield & Nestor, 1968). A pyrrolidonecarboxylic acid peptidase has been reported from rat liver and bacterial sources (Armentrout & Doolittle, 1969; Doolittle & Armentrout, 1969; Armentrout, 1969). Ratner (1944) reported that the rat excreted D-pyrrolidonecarboxylic acid in urine after an oral dose of D-glutamic acid.

In view of these findings it was decided to determine whether D- and L-pyrrolidonecarboxylic acid undergo metabolic degradation in mammalian systems. Our present experiments demonstrate that the L-isomer is rapidly metabolized in the rat and also in kidney and liver slices.

Materials and methods. L-[U-^{14}C]Glutamic acid (sp. radioactivity 55mCi/mmol) was obtained from Bhaba Atomic Research Centre, Trombay, India. D-[5-^{14}C]Glutamic acid was prepared from DL-[5-^{14}C]glutamic acid (sp. radioactivity 0.78mCi/mmol; product of Bhaba Atomic Research Centre) by the action of *Escherichia coli* glutamate decarboxylase and purification on a column (10cm × 1.9cm) of Dowex-50 (X4; H^+ form; 100–200 mesh). L-[U-^{14}C]- and D-[5-^{14}C]Pyrrolidonecarboxylic acid were prepared from labelled L- or D-glutamic acid by modification of the procedure of Uliana & Doolittle (1969). DL-[U-^{14}C]Pyrrolidonecarboxylic acid was prepared by heating L-[U-^{14}C]glutamic acid at 200°C in an oil bath for 4h and purifying the product on a column (10cm × 1.9cm) of Dowex-50 (H^+ form). The radiochemical purity of labelled pyrrolidonecarboxylic acid was established by paper chromatography in several solvents (Ramakrishna & Krishnaswamy, 1967) and radioautography. The stereochemical purity was established by acid hydrolysis of L- or D-pyrrolidonecarboxylic acid and susceptibility of the product to *E. coli* glutamate decarboxylase. All radioactivity determinations were made in a Tracerlab windowless gas-flow counter (model SC-16). The efficiency of the counter was about 40%.

Male albino rats (weight range 100–200g) from the stock colony of the institute were used in all experiments. Respiratory CO_2 was collected in an apparatus as described by Weinhouse & Friedmann (1951) and various tissues were processed as described by Gholson, Rao, Henderson, Hill & Koeppe (1958). Glutamic acid was isolated from protein hydrolysates by ion-exchange chromatography on a column (25cm × 2.5cm) of Dowex-1 (X4; acetate form; 100–200 mesh). Brain, liver and kidney slices (0.5mm thick) were cut freehand in a Stadie–Riggs tissue slicer. Liver cells were isolated by the technique described by Jacob & Bhargava (1962).

Results and discussion. L-[U-^{14}C]Pyrrolidonecarboxylic acid is rapidly metabolized by the intact rat (Table 1). In this experiment 64% of the administered radioactivity was recovered in the various fractions. Nearly 87% of the recovered radioactivity was found in respiratory CO_2, of which 80% was exhaled in 1h. In a short-term experiment (1h) it was found that the patterns of distribution of radioactivity in various tissues were similar. In both of the experiments glutamic acid was the only amino acid that was found predominantly labelled in the liver proteins. Experiments with D-[5-^{14}C]pyrrolidonecarboxylic acid revealed that most of the compound was excreted unchanged in the urine and there was little radioactivity in the respiratory CO_2. It was therefore apparent that D-pyrrolidonecarboxylic acid does not undergo metabolic degradation in the rat, confirming the earlier findings (Ratner, 1944; Wilson & Koeppe, 1961).

In order to study whether different tissues of the

* Present address: Protein Foods Association of India, Mahalaxmi Chambers, 22 Bhulabhai Desai Road, Bombay-26, India.

Table 1. *Distribution of radioactivity in various fractions of rat tissues and excretory products after administration of* L-[U-^{14}C]*pyrrolidonecarboxylic acid*

A male adult albino rat weighing 106g was injected intraperitoneally with 134μCi of L-[U-^{14}C]pyrrolidonecarboxylic acid (sp. radioactivity 55mCi/mmol) and kept in a glass metabolism unit (Weinhouse & Friedmann, 1951) for 8h. At the end of the period, the rat was decapitated, tissues were quickly dissected and processed as described under 'Materials and methods'.

Fraction	$10^{-5}\times$Total radioactivity (c.p.m.)
CO_2	710
Urine	60
Liver proteins	20
Liver trichloroacetic acid supernatant	14
Carcass lipids	8.6
Carcass proteins	5
Kidney proteins	2.4
Faeces	0.44
Brain trichloroacetic acid supernatant	0.36
Kidney trichloroacetic acid supernatant	0.17
Brain proteins	0.10

rat can metabolize pyrrolidonecarboxylic acid, experiments were carried out with slices of liver, kidney and brain. In a typical experiment kidney slices (360mg) or liver slices (780mg) were incubated with DL-[U-^{14}C]pyrrolidonecarboxylic acid (10μmol, 1.75×10^6 c.p.m.) in the presence of Ca^{2+}-free Krebs–Ringer phosphate buffer, pH 7 (3ml) (Dawson, Elliott, Elliott & Jones, 1959), for 4h at 37°C in Warburg vessels provided with a potassium hydroxide trap. Of the radioactivity, 16% and 3% were found in metabolic CO_2 in kidney and liver slices respectively. After deproteinization, the reaction mixture was passed through a column of Dowex-50 (H^+ form). In the free amino acid pool of the media 4% of the radioactivity with liver, and 5% with kidney, were found. A considerable amount of the radioactivity (50%) was found in glutamic acid and in addition about 1% of the radioactivity was found in an unidentified acidic compound. Rat brain slices did not oxidize D- or L-pyrrolidonecarboxylic acid. Kidney slices from sheep, rabbit, guinea pig and chicken were also able to oxidize DL-pyrrolidonecarboxylic acid. The rates of liberation of radioactive CO_2 were 5%, 10%, 3% and 10% respectively. Oxidation of DL-pyrrolidonecarboxylic acid could also be demonstrated with liver cells under the above experimental conditions (4% of the radioactivity in metabolic CO_2 in 4h). Metabolism of L-pyrrolidonecarboxylic acid by rat liver or kidney slices was inhibited by electron-transport inhibitors such as antimycin (20μg/ml), sodium azide (1mM) and 2,4-dinitrophenol (0.33mM). D-[5-^{14}C]Pyrrolidonecarboxylic acid was not metabolized by rat liver or kidney slices. L-[U-^{14}C]-Pyrrolidonecarboxylic acid is metabolized in the same way as DL-[U-^{14}C]pyrrolidonecarboxylic acid by liver and kidney slices.

The present investigations strongly indicate that pyrrolidonecarboxylic acid is rapidly metabolized in mammalian systems. The rate at which radioactive CO_2 is exhaled by the rat after administration of L-pyrrolidonecarboxylic acid is comparable with the rate of oxidation of L-[2-^{14}C]-glutamic acid (Wilson & Koeppe, 1961). Our attempts to obtain cell-free systems from mammalian tissues (liver and kidney) to form glutamate or other metabolites from pyrrolidonecarboxylic acid under a variety of conditions have been unsuccessful so far. Although different pathways (Moruyama & Nomura, 1956; Kawai, Aida & Uemura, 1969) have been postulated for the bacterial metabolism of pyrrolidonecarboxylic acid, the nature of the initial enzymic reactions involved has remained obscure. Further information is needed to identify the enzymic reactions concerned in the mammalian metabolism of L-pyrrolidonecarboxylic acid.

The investigations were partly supported by the Public Law 480 Funds of the United States of America.

Armentrout, R. W. (1969). *Biochim. biophys. Acta*, **191**, 756.
Armentrout, R. W. & Doolittle, R. F. (1969). *Archs Biochem. Biophys.* **132**, 80.
Bernfield, M. R. & Nestor, L. (1968). *Biochem. biophys. Res. Commun.* **33**, 843.
Blombäck, B. (1967). In *Methods in Enzymology*, vol. 11, p. 398. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Blombäck, B. & Doolittle, R. F. (1963). *Acta chem. scand.* **17**, 1816.
Dawson, R. M. C., Elliott, D. C., Elliott, W. H. & Jones, K. M. (1959). *Data for Biochemical Research*, p. 208. London: Oxford University Press.
Doolittle, R. F. & Armentrout, R. W. (1968). *Biochemistry, Easton*, **7**, 516.
Gholson, R. K., Rao, D. R., Henderson, L. M., Hill, R. J. & Koeppe, R. E. (1958). *J. biol. Chem.* **230**, 179.
Hood, L., Gray, W. R. & Dreyer, W. J. (1966). *J. molec. Biol.* **22**, 179.
Ikenaka, T. & Schmid, K. (1965). *Proc. Soc. exp. Biol. Med.* **120**, 749.
Jacob, S. T. & Bhargava, P. M. (1962). *Expl Cell Res.* **27**, 453.
Kawai, Y., Aida, K. & Uemura, T. (1969). *Agric. biol. Chem., Tokyo*, **33**, 212.
Krishnaswamy, P. R., Pamiljans, V. & Meister, A. (1962). *J. biol. Chem.* **237**, 2932.
Laden, K. & Spitzer, R. (1967). *J. Soc. cosmet. Chem.* **18**, 351.

Meister, A., Bukenberger, M. W. & Strassburger, M. (1963). *Biochem. Z.* **338**, 217.

Messer, M. & Ottesen, M. (1965). *C. r. Trav. Lab. Carlsberg, Ser. Chim.* **35**, 1.

Moruyama, Y. & Nomura, M. (1956). *J. Biochem., Tokyo*, **43**, 327.

Niwaguchi, T., Motohashi, N. & Strecker, H. J. (1965). *Biochem. Z.* **342**, 469.

Orlowski, M., Richman, P. G. & Meister, A. (1969). *Biochemistry, Easton*, **8**, 1048.

Ramakrishna, M. & Krishnaswamy, P. R. (1967). *Analyt. Biochem.* **19**, 338.

Ratner, S. (1944). *J. biol. Chem.* **152**, 559.

Uliana, J. A. & Doolittle, R. F. (1969). *Archs Biochem. Biophys.* **131**, 561.

Weinhouse, S. & Friedmann, B. (1951). *J. biol. Chem.* **191**, 707.

Wilson, W. E. & Koeppe, R. E. (1961). *J. biol. Chem.* **236**, 365.