Carnitine and Trimethylaminobutyrate Synthesis in Rat Tissues

By RICHARD A. COX and CHARLES L. HOPPEL

Departments of Pharmacology and Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106, U.S.A.

(Received 3 June 1974)

Liver and testis slices convert 6-*N*-trimethyl-lysine into 4-*N*-trimethylaminobutyrate and carnitine. Adipose, skeletal muscle, heart, or kidney tissues metabolize trimethyl-lysine into trimethylaminobutyrate but not into carnitine. Trimethylaminobutyrate hydroxylation, forming carnitine, occurs in liver and to a minor degree in testis. Liver is the primary site of carnitine biosynthesis in the rat.

Carnitine(3-hydroxy-4-*N*-trimethylaminobutyrate) is present in a wide variety of animals, plants and micro-organisms (Fraenkel & Friedman, 1957); it is found in virtually all organs (Pearson & Tubbs, 1967). The major function of carnitine is concerned with the transport of long-chain fatty acids, as acylcarnitines, into mitochondria.

In the rat carnitine and trimethylaminobutyrate are synthesized from lysine and methionine, with lysine contributing the main butyrate carbon chain and methionine the 4-*N*-methyl groups (Cox & Hoppel, 1973*a*; Horne & Broquist, 1973). Similarly, 6-*N*-trimethyl-lysine is synthesized via the methylation of protein-bound lysine by *S*-adenosylmethionine. This reaction is catalysed by protein (lysine) methyltransferase(s) (EC 2.1.1.-) (Paik & Kim, 1971). Trimethyl-lysine is thought to be an intermediate in the synthesis of carnitine, since it is converted into carnitine and 4-*N*-trimethylaminobutyrate in the rat (Cox & Hoppel, 1973*b*; Horne & Broquist, 1973). Trimethylaminobutyrate is rapidly hydroxylated to carnitine in the rat by the hepatic enzyme trimethylaminobutyrate (butyrobetaine) hydroxylase (γ -butyrobetaine,2-oxoglutarate dioxygenase, EC 1.14.11.1) (Lindstedt, 1967). Therefore carnitine appears to be synthesized through the following transformations: lysine+methionine $\rightarrow$ trimethyl-lysine $\rightarrow$ trimethylaminobutyrate $\rightarrow$ carnitine.

Indirect evidence from studies in rats has suggested that liver is the site of carnitine biosynthesis. After a single injection of radioactive methionine (Strength *et al.*, 1965) or trimethyl-lysine (Broquist & Tanphaichitr, 1973), radioactive carnitine first appeared in the liver and later accumulated in extrahepatic tissues. Further, the conversion of trimethyl-lysine into carnitine has been shown in liver slices, and trimethylaminobutyrate was an intermediate in this conversion (Cox & Hoppel, 1974). By using slices from several tissues of the rat, the present study demonstrates that the liver is the primary site of carnitine biosynthesis from trimethyl-lysine; however, extrahepatic tissues are capable of synthesizing trimethylaminobutyrate.

Material and methods

Male Wistar rats (Carworth Farms, New City, N.Y., U.S.A.) weighing 125–200g were killed by decapitation, and the desired organs removed and rinsed in ice-cold 0.9% NaCl. Liver was sliced to 0.5mm thickness by using a McIlwain tissue chopper (Brinkman Instrument Co., Cleveland, Ohio, U.S.A.). Kidney and heart slices were cut with a Stadie-Riggs tissue slicer. Pieces of epididymal fat-pad and testis were cut with a razor such that 8 to 12 pieces weighed 0.5g. Rat hemidiaphragms were used as the source of skeletal muscle. For each experiment organ slices were taken from two rats in approximately equal proportions to make a total of 0.5g (wet weight). The tissue slices were incubated, homogenized and extracted as described in Cox & Hoppel (1974). All organs were handled in the same manner except fat-pads. Instead of HClO₄, 0.5ml of 10M-KOH was added to the homogenized adipose tissue. This solution was heated at 60°C for 1 h to hydrolyse both long-chain and short-chain acylcarnitines. The hydrolysate was then adjusted to pH 2–3 with 60% (w/v) HClO₄ and centrifuged. After a second extraction of the insoluble material with 2ml of 1% (w/v) HClO₄, the combined supernatants were neutralized with 10M-KOH, cooled in ice and centrifuged to remove the KClO₄.

At this point 10mg each of carrier carnitine and trimethylaminobutyrate was added to the various organ extracts. In experiments using radioactive trimethyl-lysine the solutions were chromatographed on columns of AG-50 resin (H⁺ form; Bio-Rad Laboratories, Richmond, Calif., U.S.A.), eluted with a gradient of 1.5–4.0M-HCl as described by Cox & Hoppel (1974). For experiments using radioactive trimethylaminobutyrate the columns were eluted with 250ml of 2M-HCl. Elution was at room temperature and 5ml fractions were collected. Carnitine was assayed enzymically by the method of Marquis & Fritz (1964), and trimethylaminobutyrate was measured chemically by using the periodide assay (Wall *et al.*, 1960).

Radioactive carnitine and trimethylaminobutyrate were further identified by chromatography on

cellulose thin-layer plates as described previously (Cox & Hoppel, 1974). 6-*N*-[Me - ^{14}C]Trimethyl-lysine (25 $\mu Ci/\mu mol$) and 4-*N*-[Me - 3H]trimethylaminobutyrate (86.8 $\mu Ci/\mu mol$) were synthesized from the parent primary amines by using radioactive methyl iodide (Cox & Hoppel, 1973b).

Results and discussion

Slices from extrahepatic tissues metabolize 6-*N*-[Me - ^{14}C]trimethyl-lysine, but only testis and liver slices produced carnitine. As shown in Table 1, testis slices produced only 6% as much carnitine as did liver slices incubated under the same conditions. There was no evidence of carnitine production from trimethyl-lysine in incubations of adipose tissue, skeletal muscle, heart or kidney slices (Table 1). In these tissues, a very small amount of radioactivity was present in the fractions containing carnitine, but this radioactivity did not co-chromatograph with carrier carnitine on t.l.c. In liver and testes slice experiments the radioactivity in the carnitine fractions chromatographed in the above t.l.c. system precisely with carrier carnitine (R_f 0.50).

Slices from all tissues tested converted trimethyl-lysine into trimethylaminobutyrate (Table 1). The trimethylaminobutyrate isolated in these experiments was identified by chromatography on the AG-50 resin columns and cellulose thin-layer plates and by reaction with trimethylaminobutyrate hydroxylase. As previously described (Cox & Hoppel, 1973b), synthetic [Me - 3H]trimethylaminobutyrate and [^{14}C]trimethylaminobutyrate biosynthesized in the testis, heart, and kidney slice experi-

ments were incubated with calf liver hydroxylase. After incubation, trimethylaminobutyrate and carnitine were separated on a column of AG-50 resin eluted with 2M-HCl. The same proportion of 3H - and ^{14}C -labelled material was hydroxylated to carnitine, further establishing the identity of the ^{14}C -labelled metabolite as trimethylaminobutyrate. After 2h of incubation, the amount of radioactive trimethylaminobutyrate produced by kidney slices was more than 10 times the sum of carnitine and trimethylaminobutyrate radioactivity produced by liver slices under the same conditions (Table 1); the other tissues produced less than 5% of the trimethylaminobutyrate found in the kidney slice experiments (Table 1). However, optimal conditions for the conversion of trimethyl-lysine into trimethylaminobutyrate or carnitine were not determined for any of the tissues.

Testes, fat-pad, skeletal muscle, heart, and kidney slices metabolize 6-[Me - ^{14}C]trimethyl-lysine into unidentified metabolites, as previously reported for liver slices (Cox & Hoppel, 1974) and as found in the urine of lysine-deficient rats injected with radioactive trimethyl-lysine (Cox & Hoppel, 1973b). When 5mg of trimethylaminobutyrate was added to heart or kidney slices incubated with 6-[Me - ^{14}C]trimethyl-lysine, there was no major change in the amount of radioactivity in these metabolites or in trimethylaminobutyrate. Therefore the unidentified peaks do not appear to be metabolites of the biosynthesized trimethylaminobutyrate.

When slices from extrahepatic tissues were incubated with [Me - ^{14}C]trimethylaminobutyrate, carnitine was produced only by testis slices (Table 1). After 2h of incubation, testis slices made about

Table 1. Formation of 4-*N*-trimethylaminobutyrate and carnitine by rat tissue slices

Expt. A: for each experiment tissue slices (0.5g) were taken from two rats in approximately equal proportions. Duplicate experiments were done with different pairs of rats. The tissue slices were incubated for 2h with 12.1 μCi (0.5 μmol) of 6-[Me - ^{14}C]trimethyl-lysine, and the radioactive metabolites separated as described in the text. Expt. B: in each experiment tissue slices (0.5g) were incubated with 11.4 μCi (0.13 μmol) of 4-[Me - ^{14}C]trimethylaminobutyrate. Carnitine was isolated as described in the text. The values given represent the average of two determinations given in parentheses.

Tissue	A % of radioactive trimethyl-lysine incorporated into:		B % of radioactive trimethylaminobutyrate incorporated into:
	Carnitine	Trimethylaminobutyrate	Carnitine
Liver	1.35 (1.32, 1.37)	0.36 (0.33, 0.38)	29.5 (32.1, 27.0)
Testes	0.075 (0.073, 0.076)	0.70 (0.68, 0.72)	0.49 (0.51, 0.46)
Adipose tissue	0 (0, 0)	0.36 (0.42, 0.30)	0.04 (0, 0.08)
Skeletal muscle	0 (0, 0)	0.46 (0.45, 0.46)	0.03 (0, 0.06)
Heart	0 (0, 0)	0.39 (0.29, 0.48)	0 (0, 0)
Kidney	0 (0, 0)	17.9 (16.2, 19.5)	0 (0, 0)

1.5% as much carnitine as did liver slices incubated with radioactive trimethylaminobutyrate under the same conditions. These data agree with the observed production of carnitine from trimethyl-lysine by slices of only liver and testis.

Kidney slices effected the largest conversion of trimethyl-lysine into trimethylaminobutyrate; however, the large bulk of skeletal muscle in mammals may enable this tissue to produce significant total amounts of trimethylaminobutyrate *in vivo*. These studies, however, did not measure the capacity of the liver or extrahepatic tissues to produce trimethylaminobutyrate from trimethyl-lysine. All of the above tissues contain the enzyme protein (lysine) methyltransferase (Paik *et al.*, 1971) and are capable of forming trimethyl-lysine. Therefore it would appear that both liver and extrahepatic tissues can independently form trimethylaminobutyrate from lysine and methionine. A physiological function of trimethylaminobutyrate other than as a precursor of carnitine has not been determined.

We thank Julie S. Turkaly and Geri Bedford for their technical assistance. This work was supported by grants from the National Institutes of Health (5 RO1 AM 15804 and 5 TO1-GM 00661) and the Diabetes Association of Greater Cleveland and was taken from the thesis submitted by R. A. C. to the Graduate School of Case

Western Reserve University in partial fulfilment of requirements for the degree of Ph.D. C. L. H. is a recipient of a Research Career Development Award (5 KO4 GM 35759) from the National Institutes of Health.

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