CARNITINE SYNTHESIS IN RAT TISSUE SLICES

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Received December 3,1973

SUMMARY. The ability of rat liver, kidney, muscle, heart and testis tissue to carry out the in vitro synthesis of carnitine via ϵ-N-trimethyllysine and γ-butyrobetaine was studied. All tissues formed γ-butyrobetaine from trimethyllysine, but liver and testis also formed carnitine in about 7% and 1% yield respectively. Liver slices formed trimethyllysine from lysine in about 6% yield. These in vitro studies thus establish that liver has all the enzymes of the carnitine biosynthetic pathway. This tissue appears to be the primary site of carnitine synthesis in the rat as implied from whole animal studies in this and other laboratories.

INTRODUCTION. It has been established from appropriate isotopic labeling experiments that the essential amino acids methionine (1) and lysine (2, 3) contribute the nitrogen and carbon atoms of carnitine in a process in which ϵ-N-trimethyllysine is a key intermediate. As a first step in elucidating the individual steps of the carnitine biosynthetic pathway, we have sought to establish the primary site of carnitine biosynthesis in the rat. As briefly reported elsewhere (4) when ϵ-N-trimethyllysine was administered intraperitoneally to a series of lysine deficient rats, maximum incorporation of radioactivity into carnitine was found in the liver in only two hours, whereas at this time γ-butyrobetaine was maximal in the heart, skeletal muscle and the testes. The level of carnitine in the liver then rapidly decreased over the 24 hr test period with a concomitant rise in biosynthesized carnitine in the other tissues. These findings (4) were

interpreted to mean that carnitine is primarily biosynthesized in the liver and is then exported to the tissues. Direct evidence for this view is presented herein in which carnitine synthesis from ϵ-N-trimethyllysine is studied in vitro in several rat tissue slice systems.

EXPERIMENTAL PROCEDURE. Male weanling Sprague-Dawley rats (100-180 g) fed an ad libidum Purina Lab Chow diet were decapitated and the indicated tissue rapidly excised and rinsed in ice-cold Krebs-Ringer bicarbonate containing 0.1% glucose. A Stadie-Riggs tissue slicer was used to prepare 0.5mm thick slices of all tissues except testis. The slices were washed twice in the buffer, weighed, and then added to the incubation medium. The testes were prepared by removing the tunica albugenea, cutting into fourths, and then weighing. The incubation medium consisted of 29 μCi (1.27 moles) of ϵ-N-[methyl-^{3}H]-trimethyllysine in 10 ml of Krebs-Ringer bicarbonate containing 0.1% glucose. The tissues were incubated in a New Brunswick Water Bath Shaker equipped with a gassing hood at 37° C under a stream of 95% O_2 : 5% CO_2. The final pH was between 7.35 and 7.45.

At the indicated times buffer was filtered off and the slices washed with 5 ml of cold water, blotted dry and then frozen in liquid nitrogen. Carnitine and γ-butyrobetaine were extracted from these tissues and isolated by ion exchange chromatography by techniques described previously [cf. ref. in (3)]. Aliquots of each fraction were counted and the total amount of radioactivity eluted from the column was calculated. The amounts of carnitine and γ-butyrobetaine synthesized are expressed as percentages of this total. The amount of carnitine in each fraction was determined by the carnitine acetyltransferase assay (5).

ε-N-[methyl-^{3}H]-trimethyl-L-lysine was prepared from α-N-acetyl-L-lysine and [^{3}H] methyliodide according to the method of Mazzetti and Lemmon (6).

RESULTS AND DISCUSSION. Carnitine biosynthesis from ε-N-trimethyllysine was selected for study because of its efficient conversion to carnitine in the intact rat (3). As detailed above, slices of rat tissue were incubated under described conditions with trimethyllysine and tissue extracts subsequently applied to cation ion exchange columns such that trimethyllysine and its metabolites could be separated and quantitated. Typical experiments with kidney and liver slices are illustrated in Fig. 1, in which trimethyllysine had been incubated with these slices for 1 and 2 hrs respectively. Both systems formed γ-butyrobetaine from trimethyllysine, but carnitine was only synthesized in the liver slice system, lower frame Fig. 1, as shown by the coincidence of radioactivity derived from trimethyllysine with carnitine activity. These experiments are summarized in Table I together with related experiments with tissue slices obtained from heart and skeletal muscle and testis. Prolonged incubation of kidney slices (5 hours) with twice as much tissue did not result in carnitine synthesis, Table I. Muscle, heart, and testis also converted trimethyllysine to γ-butyrobetaine, and the testis also had some capacity to form carnitine, Table I. Testicular hydroxylase activity was confirmed by an experiment in which 1.03 g rat testes was incubated with 4.6 μCi (22.5 nmoles) [carboxy-^{14}C]-γ-butyrobetaine under the conditions described herein. Carnitine was formed in 3.04% yield. The experiments of Table I establish in in vitro tissue systems that the liver is the primary source of

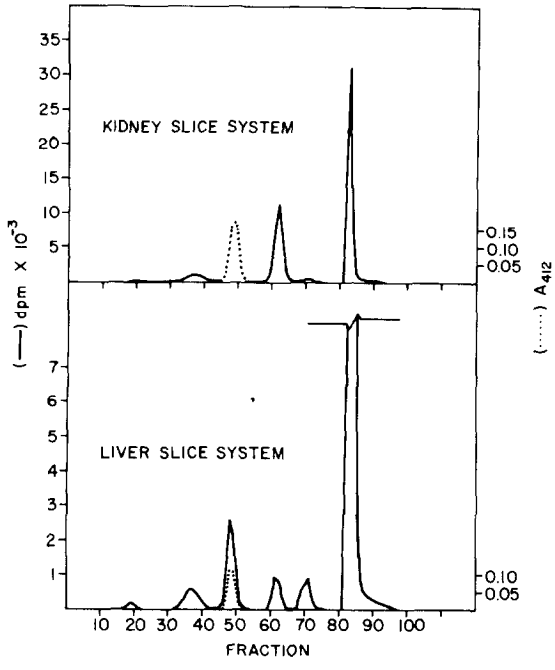

Fig. 1. ε-N-Trimethyllysine metabolism in rat kidney and liver slice systems. Full experimental details are given in the text. Slices of rat kidney and liver were incubated with trimethyllysine and extracts subsequently prepared and chromatographed on Dowex-50 under conditions appropriate for the separation of trimethyllysine (fractions 80-88), γ-butyrobetaine (fractions 59-64), and carnitine (fractions 46-52). In the kidney slice experiment, to gain additional confirmation of the nature of the material in tubes 59-64, these fractions were pooled, desalted, and whosn to be identical with authentic γ-butyrobetaine by co-chromatography in two thin layer chromatography systems by methods previously described (3).

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carnitine in the rat in agreement with the interpretation of earlier in vivo studies (4, 7, 8), and are consistent with the observation of Lindstedt and Lindstedt (9) of the inability of rat muscle and kidney homogenates to hydroxylate γ-butyrobetaine forming carnitine. The physiological significance of the limited ability of the testis to synthesize carnitine is not known.

Repeated attempts to synthesize carnitine in the liver slice system from lysine have failed to date, although trimethyllysine was found in

TABLE I

γ-Butyrobetaine and Carnitine Synthesis from ∈-N-Trimethyllysine In Rat Tissue Slices

Experimental details are described in the Experimental Procedure

Tissue and wet weight of slice		Incubation Time	% Conversion trimethyllysine in γ-butyrobetaine	carnitine
Liver	1.48 g	1 Hr.	2.11	6.42
	1.49	2	2.20	7.06
Kidney	0.84	1	33.59	0.00
	1.55	5	30.89	0.00
Muscle	1.51	2	0.21	0.00
	1.52	5	0.75	0.00
Heart	0.48	5	9.92	0.00
Testis	1.02	2	10.48	0.98
	1.03	2	12.58	1.01

significant yield (6.48% at 5 hours). Thus it is clear that liver has all the enzymes concerned in the lysine ——→ carnitine transformations. It was estimated (3) that about 0.1% dietary lysine, but 22% trimethyllysine, is utilized for the synthesis of the carnitine of 100 g of rat muscle. Hence it is clear that lysine, in contrast to trimethyllysine, has so many priorities in the animal that technical and physiological considerations may preclude a demonstration of carnitine synthesis directly from lysine in liver slices. Other radioactive compounds accumulating from trimethyllysine in the liver slice system (i.e. fractions 32 – 42 and 68 – 72, lower frame, Fig. 1) are under investigation, as they may possibly provide details of the trimethyllysine ——→ γ-butyrobetaine transformations.

ACKNOWLEDGEMENTS. This work was supported by National Institutes of Health Grants 5 T01 AM05441 and 5 R01 AM 16019.

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