HYDROXYLATION OF γ-BUTYROBETAINE BY RAT AND OVINE TISSUES [1]

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SUMMARY: Ovine tissues were assayed for the capacity to synthesize carnitine from γ-butyrobetaine. Activity in liver, kidney and muscle was 0.25, 0.10 and 0.08 nmoles per mg protein per min, respectively. Heart was devoid of the enzyme. Of the rat tissues that were assayed only liver contained the hydroxylase (0.39 nmoles per mg per min). Although the specific activity of the enzyme was approximately three fold higher in sheep liver than in sheep skeletal muscle, on the basis of total activity, muscle would constitute the major portion of the total hydroxylase activity present in the body. The synthesis of carnitine in ovine skeletal muscle may in part explain the high level of carnitine found in that tissue and emphasizes the existence of species differences in the localization of carnitine synthesis.

The conversion of γ-butyrobetaine to carnitine was first demonstrated in rats *in vivo* by Lindstedt and Lindstedt (1). Later studies from the same laboratory (2) demonstrated that the *in vitro* hydroxylation of γ-butyrobetaine to carnitine was catalyzed by an enzyme which was localized in the cytosol of rat liver. A subsequent report (3) illustrated that rat liver but not muscle or kidney contained γ-butyrobetaine hydroxylase activity. The latter conclusion, that carnitine synthesis from γ-butyrobetaine takes place solely in the liver of the rat, was implied in *in vivo* isotope studies by Strength *et al.* (4) and in more recent studies (5,6) two laboratories have reiterated this speculation.

Studies from two laboratories have demonstrated that ε-N-trimethyllysine is the precursor of γ-butyrobetaine (7,8). The synthesis of γ-butyrobetaine from ε-N-trimethyllysine appears to occur in a number of rat tissues (9,10) however only liver and to a lesser extent testis can synthesize carnitine from

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ε-N-trimethyllysine by virtue of the fact that these tissues from the rat contain γ-butyrobetaine hydroxylase. The synthesis of carnitine from ε-N-trimethyllysine has been suggested to involve transport of intermediates to the liver for final conversion to carnitine. The present results of assays on the γ-butyrobetaine hydroxylase activity in ovine tissues negate the need for such a transport since kidney and in particular skeletal muscle contain the hydroxylase enzyme.

EXPERIMENTAL PROCEDURES

Animals: Sheep were 4 to 5 year old cull wethers. The animals were killed and tissue samples taken and placed on ice as soon as possible after death. Rats were 250 g males of the Sprague-Dawley strain.

Tissue extracts: Liver and kidney were homogenized in 2 vol of 0.25 mM sucrose with a Potter-Elvehjem homogenizer. Heart and skeletal muscle were homogenized in 2 and 5 vol respectively, of 0.25 M sucrose in a "Tissumizer" (O.H. Johns Co.). All extracts were centrifuged for 10 min at 12000 g to remove cell debris and mitochondria. The supernatant was recentrifuged for 60 min at 105,000 g. The cytosol from rat tissues was dialyzed overnight against 50 mM phosphate buffer (pH 7.0) prior to assay. Preparations from sheep tissues were treated with ammonium sulfate (75% saturation). The precipitate was dissolved in 50 mM phosphate buffer (pH 7.0) and assayed. Enzyme activity was also determined in dialyzed cytosol from sheep tissue.

Enzyme assay: The γ-butyrobetaine hydroxylase activity was assayed according to Lindstedt and Lindstedt (11). Incubations in 5 ml contained the following: potassium phosphate (pH 7.0), 20 mM; sodium 2-oxoglutarate, 3 mM; ferrous sulfate, 2 mM; sodium ascorbate, 10 mM; KCl, 20 mM and γ-butyrobetaine, 1 mM. Catalase was added at 0.8 mg per ml incubation. Protein added from tissue extracts ranged from 5 to 10 mg per ml. Aliquots (1 ml) taken at 0, 0.5, 1 and 2 hr from each incubation were boiled 5 min to denature protein. After centrifugation incubations were adjusted to pH 10 with KOH and heated to 90° for 5 min. Neutralized (with HCl) samples were passed through a milli-

pore filter. Carnitine in the filtrate was assayed according to Marquis and Fritz (12). Protein was determined by ultraviolet absorption (13).

RESULTS AND DISCUSSION

The synthesis of carnitine from γ-butyrobetaine was determined in several ovine tissues and compared to rat liver (Fig. 1). The linearity of carnitine synthesis with time is illustrated. The activity in rat liver was determined on dialyzed supernatant while the activity in ovine tissues was assayed on a 0 to 75% saturated ammonium sulfate fraction. In all cases the activity was calculated back to the original wet weight of tissue, however no correction was made for any loss in activity of ovine tissues as a result of fractiona-

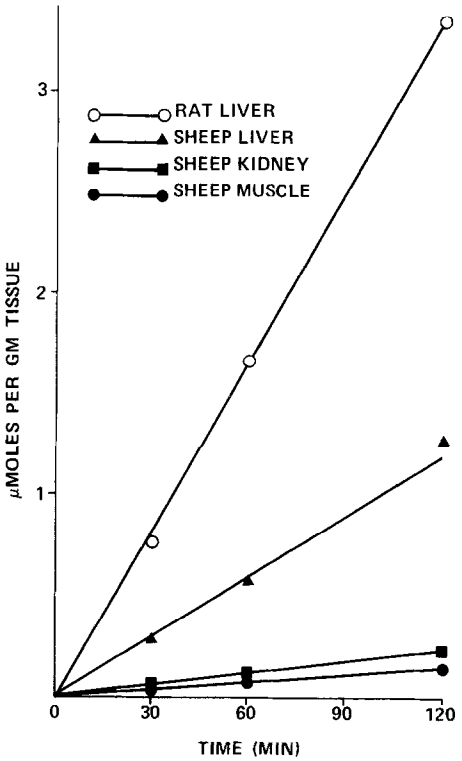

Fig. 1. The time dependence of carnitine synthesis from γ-butyrobetaine by tissue extracts of rat and sheep. Rat liver fraction assayed was a dialyzed, high speed supernatant. Sheep tissue extracts were ammonium sulfate fractions (0 to 75% saturation). The details of the incubation and the method of carnitine assay are described in Experimental Procedures.

tion. Thus Fig. 1 serves mainly to illustrate the efficacy of the assay and to compare activities between the sheep tissues.

The results in Table 1 illustrate the specific activity of γ-butyrobetaine hydroxylase found in rat and sheep tissues. Whether assayed on the dialyzed supernatants or the ammonium sulfate fractions, kidney and skeletal muscle from the sheep contained almost a third of the activity found in liver of that species. The activity of liver of rat and sheep was not greatly different. The hydroxylase activity found in kidney and particularly skeletal muscle of sheep represents the major proportion of that enzyme in the body.

The distribution of the hydroxylase enzyme in rat tissue is in agreement with the proposed site of carnitine synthesis in that species (5,6). Although some hydroxylase activity has been reported in rat testis (6,9) it is considerably less than that found in liver. In the rat it has been postulated that

TABLE 1

Specific Activity of γ-Butyrobetaine Hydroxylase from Rat and Sheep Tissue Extracts.

Details of the assay method and preparations of tissue extracts are described in the Experimental Procedures.

Species	Tissue	Preparation	Specific Activity (nmoles/min/mg)
Rat	Liver	Supernatant*	0.39
Sheep	Liver	Supernatant	0.25
Sheep	Kidney	Supernatant	0.10
Sheep	Muscle	Supernatant	0.08
Sheep	Liver	A.S. Fraction**	0.18
Sheep	Kidney	A.S. Fraction	0.08
Sheep	Muscle	A.S. Fraction	0.07

* Supernatant was dialyzed against 50 mM phosphate (pH 7.0) for 16 hrs. prior to assay.

** 0 to 75% saturated ammonium sulfate fraction.

conversion of ε-N-trimethyllysine to γ-butyrobetaine takes place in a number of tissues (9) but that the final step of carnitine synthesis is relegated to the liver (5,6,9). This mechanism for synthesis implies transport of intermediates from extrahepatic tissue to liver and transport of carnitine back. Such a mechanism need not be postulated for the ovine species since presumably γ-butyrobetaine synthesis from ε-N-trimethyllysine can occur in muscle or kidney. The considerably higher concentrations of carnitine found in ovine (14,15) and perhaps also those in bovine (15) tissues could in part result from the difference in the localization of the carnitine hydroxylase between ovine and rat tissues.

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