γ-BUTYROBETAINE HYDROXYLASE ACTIVITY IN HUMAN AND OVINE LIVER AND SKELETAL MUSCLE TISSUE

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1. Introduction

It has been shown [1–3] that labelled γ-butyrobetaine administered to animals was rapidly transformed to carnitine. The reaction was catalyzed by an enzyme (γ-butyrobetaine hydroxylase, EC 1.14.11.1) in the soluble fraction of rat liver homogenates [4–6]. In recent work with normal and diabetic human liver (unpublished) we have also recovered this enzymic activity in the soluble fraction of cell homogenates. It was shown [5] that homogenates from rat liver but not from muscle or kidney hydroxylated γ-butyrobetaine to carnitine, and no conversion of γ-butyrobetaine to carnitine was found [7], when the liver was excluded from the circulation. The ability of rat liver, kidney, muscle, heart and testis to form carnitine in vitro was tested [8]. All tissues carried out the synthesis of γ-butyrobetaine from trimethyllysine, but only liver tissue, and to a limited extent also testis tissue, formed carnitine. These studies suggested that, in the rat, the liver is the main site of the conversion of γ-butyrobetaine to carnitine.

It was reported [9] that ovine kidney and skeletal muscle but not heart muscle had the ability to form carnitine from γ-butyrobetaine. Although the activity of γ-butyrobetaine hydroxylase in skeletal muscle was only 1/3rd of that in the liver calculated per g soluble protein, the major portion of the total hydroxylase activity of the body would be present in the skeletal muscle. This finding implied that the present concept that carnitine is transported to muscle rather than synthesized within this tissue is incorrect. Ovine [10] and human skeletal muscle [11] have a very high carnitine concentration compared to rat [11,12] and mouse muscle [13]. In man and sheep, the carnitine concentration in heart muscle is lower than in the skeletal muscle [10,13] in contrast to what is found in the rat [12]. In view of these species differences, it was of interest to examine human skeletal muscle for γ-butyrobetaine hydroxylase activity; we also attempted to demonstrate such activity in ovine skeletal muscle.

2. Experimental procedures

2.1. *Materials*

γ-Butyrobetaine chloride was obtained from E. Merck AG, Darmstadt. γ-[*methyl*-$^{14}C_3$]Butyrobetaine was synthesized as in [5].

2.2. *Sources of tissues*

Four female patients were studied, age 18, 35, 48 and 75 years. They were admitted to the hospital for operation of inguinal hernia or uncomplicated gallstone disease. Three liver biopsies [14] and four muscle biopsies from rectus abdominis and gastrocnemius muscles were taken [15]. The patients had no evidence of liver damage as judged from the determination of serum concentration of bilirubin, aspartate and alanine aminotransferases, and alkaline

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phosphatase. Informed consent was obtained from all patients. The sheep were 1.5–3 years old. Tissue samples were taken from liver and the tibialis anterior muscle as soon as possible after death. Visible fat and fascia was rapidly removed from the specimens, which were placed immediately in ice-cold 0.25 mol/l sucrose. The procedure lasted 15–30 min.

2.3. *Preparation of tissue extracts*

Homogenates were prepared in cold 0.25 mol/l sucrose in a Potter-Elvehjem homogenizer with a glass or a Teflon pestle under cooling. The concentration (wet wt/vol.) was: 33% for liver tissue, 25% for human muscle, 17% and 33% for sheep muscle tissue. The homogenates from human muscle were first centrifuged at 800 × *g* for 10 min, then at 100 000 × *g* for 1 h. The homogenates from sheep muscle, as well as human and sheep liver were centrifuged at 100 000 × *g* for 1 h. One 100 000 × *g* supernatant fraction from sheep muscle was dialyzed against saturated $(NH_4)_2SO_4$ for 15 h. The precipitate was dissolved in 50 mmol/l potassium phosphate buffer (pH 7.0) before enzyme assay.

2.4. *Enzyme assay*

γ-Butyrobetaine hydroxylase activity was assayed essentially as in [6]. The composition of the incubation mixture, unless otherwise specified, was: 2-oxoglutarate, 7.1 mmol/l; $FeSO_4$, 0.6 mol/l; sodium ascorbate, 14 mmol/l; catalase, 1.4 g/l; potassium phosphate buffer (pH 7.0), 14 mmol/l. The total volume was 0.7 ml. For amount of γ-[*methyl*-$^{14}C_3$]-butyrobetaine and protein in the experiments with the 100 000 × *g* supernatant fractions, see table 1. The same amounts of labeled γ-butyrobetaine and comparable volumes of tissue homogenate were used in the experiments with unfractionated homogenates or the 800 × *g* supernatant fractions. The incubations were carried out at 37°C for 30 min or 60 min and were terminated by the addition of 0.5 ml 10% (w/v) trichloroacetic acid. After cooling in ice for 1 h the suspension was centrifuged and the supernatant fraction was put onto a column of Dowex AG 50W X8 (1.5 × 7.5 cm, 13.2 ml), which was eluted with 1 mol/l HCl. The eluate was collected in fractions of 60 ml, 40 ml (containing carnitine), 3 × 10 ml and 120 ml. The amount of radioactivity was determined in 300 μl portions of the fractions using a liquid scintillation spectrometer. In the experiments with muscle tissue, the fraction containing carnitine was taken to dryness with a rotatory evaporator and redissolved in a small volume of water before measurement of radioactivity. The blank contained all components except for tissue extract and was carried through the whole procedure. The detection limit for enzymic formation of carnitine varied between 0.3% and 1.0% conversion of the substrate in different experiments. Protein was determined by the Lowry method with bovine albumin as standard and in the ammonium sulphate fractions by ultraviolet absorption [16].

Table 1
γ-[*methyl*-$^{14}C_3$]Butyrobetaine and protein content in the incubations with the 100 000 × *g* supernatant fractions of tissue homogenates

Samples	Labelled γ-butyrobetaine		Protein (mg)
	(mmol/l)	(mCi/l)	
Human liver	0.7	0.4	1.4–2.0
Human muscle	0.03	0.8	0.3–3.0
Sheep liver 1–3	0.7	0.8–0.9	2.5–2.9
Sheep liver 4–5[a]	0.7	0.8–0.9	4.0–5.0
Sheep muscle 1–3	0.7	0.8–0.9	0.2–0.7
Sheep muslce 4–5[a]	0.03 and 0.2	0.9	2.6–2.8
Sheep muscle 4–5[a,b]	0.03 and 0.2	0.9	2.9–4.9

[a] $FeSO_4$, 1 mmol/l, and KCl, 20 mmol/l, otherwise as specified in section 2
[b] Ammonium sulphate fractions

3. Results and discussion

The formation of carnitine from γ-butyrobetaine in the 100 000 × *g* supernatant fraction of sheep or human liver was linear with time during 60 min and with the concentration of liver tissue extract (fig.1). The specific activity of γ-butyrobetaine hydroxylase in human liver was of the same order of magnitude as in ovine liver (table 2). An activity of 4.2 nkat × (g protein)$^{-1}$ in ovine liver was reported [9]*, which is similar to that found by us. A liver hydroxylase activity was found in a 70 000 × *g* supernatant from two patients with carnitine deficiency of 2.4 and 5.4 nkat × (g protein)$^{-1}$ [17]*, respectively, whereas

* Recalculated from the authors' data

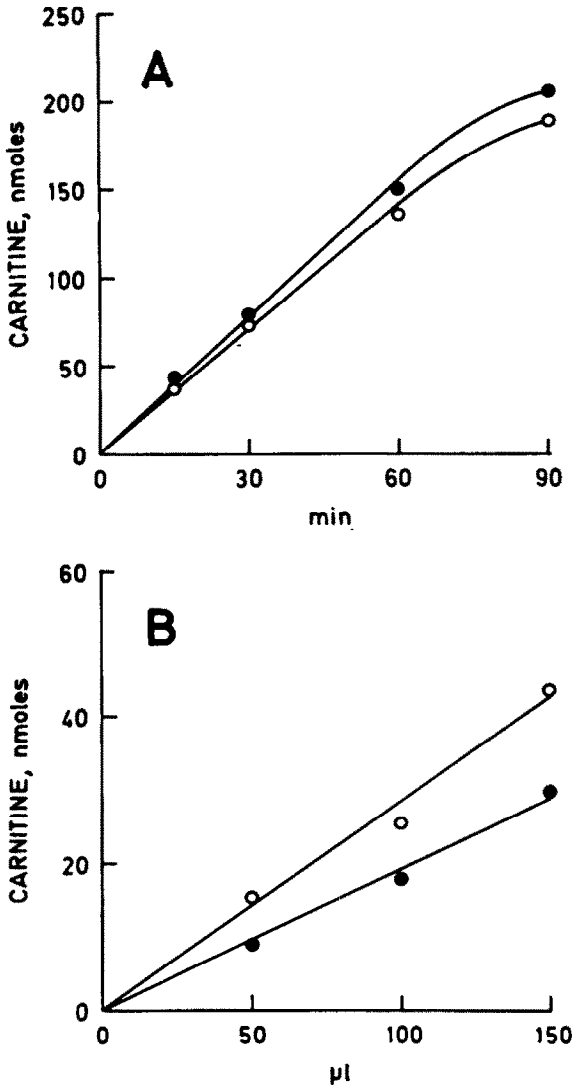

Fig.1. (A) Formation of carnitine from γ-butyrobetaine after different incubation times, using the 100 000 × *g* supernatant fraction of sheep liver homogenate containing 4 mg (○—○) or 5 mg (●—●) protein. (B) Formation of carnitine from γ-butyrobetaine in incubations with different amounts of sheep liver tissue extracts. The protein concentration in the 100 000 × *g* supernatants was 25 g/l (●—●) or 29 g/l (○—○), respectively.

the activity in liver tissue obtained from autopsy material in three cases was 2.2, 2.7 and 3.1 nkat × (g protein)$^{-1}$, respectively.

No γ-butyrobetaine hydroxylase activity (i.e., < 0.04 nkat × (g protein)$^{-1}$) could be demonstrated

Table 2
γ-Butyrobetaine hydroxylase activity in liver tissue extracts

Sample	Specific activity nkat × (g protein)$^{-1}$	
	Sheep	Human
1	3.5	5.7
2	4.5	11
3	5.7	12
4	8.3	
5	11	

Homogenates (33%) were prepared in 0.25 mol/l sucrose and centrifuged at 100 000 × *g* for 1 h. The supernatant fractions were assayed for enzyme activity, using γ-[*methyl*-$^{14}C_3$]-butyrobetaine as substrate

in homogenates from human skeletal muscle, neither in the 800 × *g* supernatant nor in the 100 000 × *g* supernatant fractions. This finding prompted us to assay the hydroxylase activity in sheep muscle. In the first experiments (sheep muscle, samples 1–3), no enzyme activity was found in the unfractionated homogenates or in the 100 000 × *g* supernatant fractions. In the next experiments (sheep muscle tissue 4–5), the assay was performed also with lower mass concentrations of γ-butyrobetaine with higher specific radioactivity and more tissue extract (table 1). In these experiments, we only tested the 100 000 × *g* supernatant fractions. In order to study the possible presence of an inhibitor, the extracts of liver and muscle were mixed and the assay was performed immediately and after 1 h preincubation (table 3). The liver hydroxylase activity was unaffected or only moderately decreased by the addition of muscle extract. In another experiment, the proteins of the 100 000 × *g* supernatant fraction of the muscle homogenate were concentrated with saturated ammonium sulphate before assay. In one of the duplicate assays the amount of radioactivity in the carnitine fraction was slightly higher (2.4% of incubated amount of substrate) than in the blank (1.0%). If this conversion reflects γ-butyrobetaine hydroxylase activity it would correspond to spec. act. 0.05 nkat × (g protein)$^{-1}$, i.e., $\lesssim$ 1% of the enzyme activity demonstrated in ovine liver tissue and < 5% of the activity reported in [9]. The reason for the discrepancy between our results and [9] is not readily

Table 3
Effect of the addition of sheep muscle extracts on the specific activity of γ-butyrobetaine hydroxylase activity in extracts of sheep liver

Liver sample	Addition	Preincubation (min)	Enzyme activity nkat × (g protein)$^{-1}$
	None	0	7.0
	Muscle extract	0	7.7
4	None	60	7.1
	Muscle extract	60	7.0
	None	60	7.8
	Muscle extract (ammonium sulphate precipitate)	60	6.7
	None	0	11
	Muscle extract	0	10
5	None	60	11
	Muscle extract	60	8.3
	None	60	11
	Muscle extract (ammonium sulphate extract)	60	7.8

apparent, since essentially the same incubation procedures were used. We analyzed the soluble fraction of the muscle homogenates as well as [9]; we also tested unfractionated homogenates. Carnitine was determined [9] with an enzymatic method, in which the formed CoASH was detected with 5,5′-dithiobis-2-nitrobenzoic acid [18]; the technique used here has a higher specificity.

Thus, we found no evidence for the presence of soluble or organelle-associated γ-butyrobetaine hydroxylase in muscle tissue. The results indicate that human skeletal muscle carnitine has its origin outside the muscle tissue and reaches the skeletal muscle by way of the blood stream.

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