HYDROXYLATION OF γ-BUTYROBETAINE TO CARNITINE IN HUMAN AND MONKEY TISSUES

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1. Introduction

Carnitine serves as a carrier of acyl groups into and out of mitochondria and hence is considered to have a central function in the metabolic utilization and synthesis of fatty acids [1–4]. In the rat, the species most extensively studied, adipose tissue, heart, kidney and skeletal muscle catalyze the entire sequence of reactions from ε-*N*-trimethyl-L-lysine to γ-butyrobetaine; the same tissues, however, are reported not to hydroxylate the latter compound to carnitine [5–8]. Indeed, several studies suggested that the liver is the primary site of carnitine biosynthesis, and that the testis is a secondary site with much less capacity for such synthesis [6–9]. In fact it has been considered that extrahepatically synthesized γ-butyrobetaine is converted to carnitine exclusively in the liver, and that the carnitine so formed is rapidly transported to other organs [7,10].

That species differences may exist in tissue localization of carnitine synthesis was evidenced by the finding that, in the sheep, kidney and muscle tissues have significant capacity to hydroxylate γ-butyrobetaine, albeit relatively low as compared with liver (11). More recently, crude extracts from kidneys of cat, hamster, rabbit, and a single Rhesus monkey were shown to convert γ-butyrobetaine to carnitine [12,13]. In those species, the levels of γ-butyrobetaine-hydroxylating activity in kidney nearly equaled or exceeded that measured in the corresponding liver. In contrast, dog, guinea pig, mouse and rat kidney exhibited little or no capacity to hydroxylate γ-butyrobetaine.

In this study, in which additional primate species were examined, evidence is provided that the specific activity of γ-butyrobetaine hydroxylation in crude extracts of kidneys of Rhesus monkeys was significantly higher than that of extracts of Rhesus monkey livers. In contrast, the extracts of kidneys of Cebus monkeys, in comparison to Cebus monkey livers, exhibited considerably less capacity for γ-butyrobetaine hydroxylation. In the human, the specific activity of hydroxylase in kidney extracts was considerably higher than that of corresponding liver extracts; and skeletal muscle showed no detectable activity.

2. Experimental procedures

2.1. *Materials*

γ-[2,3-^{3}H]Butyrobetaine was synthesized as in [13] and had spec. act. 2.58×10^6 dpm/μmol.

2.2. *Tissue sources*

Nine monkeys (7 *Macaca mulata*, 2 *Cebus apella*) ranging in age from 3–5 years, were used. Kidneys and livers were removed from anesthetized animals (Nembutal, 50–70 mg/kg, i.p.) and immediately frozen in liquid nitrogen. Human tissue samples were obtained from randomly selected autopsies performed at the Albert Einstein College of Medicine Hospital between July and December 1978. The intervals between death and autopsy ranged from 2.5–12 h, and tissues were obtained within 15 min after the autopsy was started and immediately frozen in liquid nitrogen. In each case, samples were removed from organs at sites that grossly appeared to be most

nearly 'normal' or least unaffected; special care was taken, in this regard, in the sampling of livers from the two persons with metastatic carcinoma. The striated muscle sample was obtained from the intercostal region. In the notes accompanying table 1 are summarized the relevant gross and microscopic features of the tissues as given in the final autopsy report.

2.3. *Preparation of tissue extracts*

Weighed portions of each tissue were homogenized in exactly 2 vol. 20 mM Tris–HCl (pH 7.4) containing 2 mM monothioglycerol (and occasionally, as noted in table 1, also containing 1% Triton X-100) with a PT 10-35 Polytron homogenizer adapted with a PT 10ST sawtooth generator. The homogenates were centrifuged for 1 h at 105 000 × *g*; the resulting supernatants were decanted through glass wool to remove floating fat particles and stored on ice until assayed. Protein in the high speed supernates was determined by the Lowry method [14] with crystalline bovine serum albumin as a standard.

2.4. *Enzyme assays*

γ-Butyrobetaine hydroxylase activity was assayed as in [12,13]. The reaction mixtures (0.51 ml) contained: Tris–HCl (pH 7.7) 50 mM; $Fe(NH_4)_2(SO_4)_2$, 2 mM; sodium ascorbate, 15 mM; α-ketoglutarate, 2.50 mM; KCl, 20 mM; catalase, 1.5 mg ; 150–200 μl tissue extract; γ-[2,3-^{3}H]butyrobetaine, 1.18 mM (1.51×10^6 dpm/assay tube). Incubations were carried out at 37°C for 60 min, reactions being initiated by addition of substrate and terminated by addition of 50 μmol 2,2′-bipyridine–HCl. Water of the medium was collected by sublimation as in [13] for measurement of radioactivity. With the noted specific activity of the substrate used in this study and the known stereospecific displacement of only 1 out of the 4 labeled hydrogen atoms of the substrate on hydroxylation [15], and with a correction made for an observed isotope effect that underestimates the extent of hydroxylation by a factor of nearly 10-fold [13], the release into the medium of 645 dpm ^3H_2O/0.51 ml assay mixture is equivalent to formation of ~10 nmol carnitine. For purposes of comparison with other recently published data [16], γ-butyrobetaine hydroxylase activity in tissue extracts is expressed as specific activity in terms of nkat (nmol. S^{-1})/g protein in the 105 000 × *g* supernatants.

3. Results and discussion

The results as presented in table 1 allow direct comparison of the specific activities of γ-butyrobetaine hydroxylase for all tissue extracts analyzed. For any particular tissue derived from the same individual, comparable specific activities were obtained for γ-butyrobetaine hydroxylase in extracts prepared with buffer containing only monothioglycerol, whether assayed in the absence or presence of 0.3% Triton X-100. Also, for different samples of the same organ extracted with or without 1% Triton X-100 in the extracting buffer or for different samples of the same tissue extracted after storage in the frozen state for varying number of days, comparable specific activities were determined.

A consistently relatively high activity was observed in all of the human kidney extracts, with an av. spec. act. 14.6 ± 4.5 nkat/g protein. In contrast, the average specific activity of the human liver extracts examined was 1.02 ± 0.48 nkat/g protein; the values for the 2 patients with liver metastatic carcinoma were in the lower range. Average specific activities recalculated from reported data for 3 autopsy liver samples [17] and for 3 surgical liver biopsy samples [16], gave values of 2.7 ± 0.5 and 9.6 ± 3.4 nkat/g protein, respectively. A major cause of the discrepancy in the results probably relates to differences in assay conditions. No detectable γ-butyrobetaine hydroxylase activity was found in human muscle, confirming a recent report [16].

For each of the 7 Rhesus monkeys examined in this study, the specific activity of γ-butyrobetaine hydroxylase in extracts of kidney tissue was significantly higher than that determined in the extracts of the corresponding livers; an average of 18.8 ± 6.2 nkat/g protein for the kidneys compared to 11.2 ± 5.3 nkat/g protein for the liver extracts. In contrast, the Cebus monkey kidney had very little capacity for γ-butyrobetaine hydroxylation compared to an av. spec. act. 3.92 ± 1.14 nkat/g protein for the Cebus liver extracts. The last value agrees with γ-butyrobetaine hydroxylase activities of 4.2 and 4.4 nkat/g

protein for ovine [11] and rat [12] liver, respectively.

The finding of γ-butyrobetaine hydroxylase activity in kidneys of Rhesus monkeys and humans that equals or exceeds that present in the corresponding livers, extends earlier results [12] that suggested species differences in tissue localization of carnitine synthesis. These finding strongly argue against the prevailing generalization, based on studies carried out almost exclusively with the rat, that the liver is the exclusive or primary site of carnitine synthesis.

Table 1
γ-Butyrobetaine hydroxylase activity in human and monkey tissue extracts

Animal species				Specific activity (nkat/g protein)		
				Kidney	Liver	Muscle
Human	1.	D.H.,	autopsied 12 h after death	13.6, 14.8[a]	1.09, 1.05[a]	–
				16.3[b]	1.02[b]	–
				13.7[b,c]	0.74[b,c]	–
	2.	M.M.,	autopsied 4.5 h after death	8.50	0.94	0.00
				10.2[b]	1.35[b]	0.00[b]
				11.8[b,d]	2.01[b,d]	0.00[b,d]
	3.	L.L.,	autopsied 2.5 h after death	22.3	0.62	–
				21.6[e]	–	–
	4.	M.C.,	autopsied 6 h after death	12.8	0.33	–
Cebus monkey (*Cebus apella*)	1.			1.00	3.11	–
	2.			0.77	4.72	–
Rhesus monkey (*Macaca mulatta*)	1.			26.6	13.5, 16.9[f]	–
	2.			9.60	3.40	–
	3.			24.4	19.7	–
	4.			17.4	6.65	–
	5.			14.2	9.21	–
	6.			15.8	9.62	–
	7.			23.3	10.4	–

[a] Assayed in presence of 0.3% Triton X-100, a concentration of detergent in the assay mixture equivalent to that present when tissue samples extracted in the presence of Triton X-100 were used
[b] Tissue samples extracted with buffer containing 1% Triton X-100
[c] Extracts freshly prepared from frozen-stored tissues 6 days later
[d] Extracts freshly prepared from frozen-stored tissues 5 days later
[e] Extracts freshly prepared from frozen-stored tissues 2 days later
[f] Extracts prepared from tissue sampled from another liver lobe

Case 1: D. H., autopsy report no. A78-54, 65 year old female. Cause of death: bronchopneumonia in a patient with disseminated breast carcinoma. Tissue pathological abnormalities: kidney, mild arteriolarnephrosclerosis; liver, fatty infiltration and microscopic foci of metastatic carcinoma subcapsularly
Case 2: M. M., autopsy report no. A78-77, 62 year old male. Cause of death: dissecting aneurysm of ascending aorta. Tissue pathological abnormalities: kidney, mild arteriolarnephorsclerosis; liver, none; striated muscle, none
Case 3: L. L., autopsy report no. A78-94, 82 year old male. Cause of death: perforation of carcinoma of stomach with acute peritonitis, massive liver metastases. Tissue pathological abnormalities: kidney, mild arteriolarnephrosclerosis; liver, extensive metastatic carcinoma
Case 4: M. C., autopsy report no. A78-115, 43 year old female. Cause of death: metastatic carcinoma distal and local (with genito-urinary obstructive disease) from primary origin in Bartholin's gland. Tissue pathological abnormalities: kidney, extensive acute pyelonephritis, papillary necrosis; liver, metastatic carcinoma mild focal fatty change. Because of the diffuse microscopic nature of the acute pyelonephritis in this individual, it is possible that the kidney sample contained considerable numbers of polymorphonuclear leukocytes and bacteria

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