DIFFERENT SUBCELLULAR LOCALIZATION OF PALMITOYL-L-CARNITINE HYDROLYSIS IN HUMAN AND RAT LIVER

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1. Introduction

In the course of studies of the effect of subcellular fractions of rat liver on the hydrolysis of palmitoyl-L-carnitine and palmitoyl-CoA, it was observed that the microsomal fraction hydrolyzed palmitoyl-L-carnitine [1], confirming the finding of Mahadevan and Sauer [2] and Hoppel and Tomec [3]. Palmitoyl-CoA hydrolase (EC 3.1.2.2) had a dual localization in rat liver, one in the mitochondria and one in the microsomal fraction [1]. Subcellular localization studies of brown adipose tissue from guinea pigs showed that both palmitoyl-CoA hydrolase and palmitoyl-L-carnitine hydrolase (EC 3.1.1.28) were localized in the mitochondria and possibly the soluble fraction [4].

No study has been devoted to the subcellular localization of palmitoyl-L-carnitine hydrolysis in human liver. In this report data are presented showing that the palmitoyl-L-carnitine hydrolysis is mainly associated with the mitochondria.

2. Materials and methods

Hepes and rotenone were obtained from Sigma Chemical Co., (St. Louis, MO, USA). Palmitoyl-L-carnitine chloride was purchased from Supelco Inc., (Bellefonte, PA, USA). [1-^{14}C]Palmitoyl-L-carnitine was purchased from New England Nuclear, (Boston, MA, USA.) The scintillation liquid (Scint-Hei 3) was from Koch-Light Lab., (Colnbrook, England). All other chemicals were of the highest purity commercially available.

2.1. *Preparation of subcellular fractions*

The subcellular fractions from rat liver and human liver (obtained from a biopsy and which appeared normal upon histological examination) were prepared essentially as in [5] with the modifications in [6].

2.2. *Enzyme assays and other analytical methods*

Palmitoyl-L-carnitine hydrolase activity was assayed by incubation samples from the subcellular fractions with [1-^{14}C]palmitoyl-L-carnitine (100 μM) at 37°C for increasing times up to 10 min. The incubation buffer was 20 mM Hepes (pH 7.4) containing 1 mM EDTA. The reaction was stopped by adding 2.0 ml of propan-2-ol/heptane/1 M H_2SO_4, 20/5/1 (v/v/v) [7] and unesterified ^{14}C-labelled palmitate was extracted as earlier described [1,8]. 0.7 ml of the heptane phase containing the unesterified ^{14}C-labelled palmitate was mixed with 10 ml Scint-Hei 3, and the radioactivity determined by liquid scintillation counting.

Malate dehydrogenase (EC 1.1.1.31) [9], rotenone insensitive NADPH-cytochrome *c* reductase (EC 1.6.2.4) [10], succinate-PMS-oxidoreductase (EC 1.3.99.1) [11,12], acid phosphatase (EC 3.1.3.2) [13] and lactate dehydrogenase (EC 1.1.1.27) [14] were assayed as described. All spectrophotometric measurements were performed with a Schimadzu recording spectrophotometer MPS 5000. Radioactivity was

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine *N'*-2-ethanesulfonic acid; PMS, phenazine methosulfate.

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counted in a Packard Tricarb Liquid Scintillation Spectrometer (Model 3385). Protein was determined using the Folin-Ciocalteu reagent [15].

3. Results and discussion

Palmitoyl-L-carnitine hydrolysis is dependent on substrate as well as protein concentration [1,4,16]. With 100 μM palmitoyl-L-carnitine and 0.3 mg protein/ml the rate of hydrolysis was constant for at least 10 min and proportional to added protein at 0.15–0.5 mg/ml (data not shown). In the present study all assays were performed within the linear range in terms of both substrate and enzyme concentrations.

Figure 1 shows the distribution of palmitoyl-L-carnitine hydrolysis compared with marker enzymes in fractions prepared from homogenates of human and rat liver. The recovery of enzymes and protein from both tissues was 90–104% (table 1). The marker enzymes for the mitochondrial fractions, succinate-PMS-oxidoreductase [11,12], light mitochondrial fraction, acid phosphatase [17], microsomal fraction, NADPH-cytochrome *c* reductase [17], and soluble fraction, lactate dehydrogenase [17], were distributed as expected. Malate dehydrogenase was localized both in the mitochondrial and the soluble fractions [3,17]. The contamination between fractions, calculated from marker enzymes, was low except for the presence of mitochondrial enzyme activity in the light mitochondrial fraction of human liver.

From the data presented in fig.1 it is evident that palmitoyl-L-carnitine hydrolase activity in human and rat liver was differently localized. In rat liver it showed a microsomal distribution, confirming the finding earlier described [1–3]. The specific activity of the palmitoyl-L-carnitine hydrolysis in human and rat liver microsomes was 0.4 and 5.1 nmol/mg protein/min, respectively. In human liver the low activity in microsomes may, however, be due to contamination by mitochondria as some mitochondrial marker was found in the microsomal fraction (fig.1). Most of the palmitoyl-L-carnitine hydrolase activity was found in the mitochondrial fraction. The specific activity of palmitoyl-L-carnitine hydrolysis in human and rat liver mitochondria was 1.0 and 0.3 mmol .mg protein^{-1} .min^{-1}, respectively in the absence of

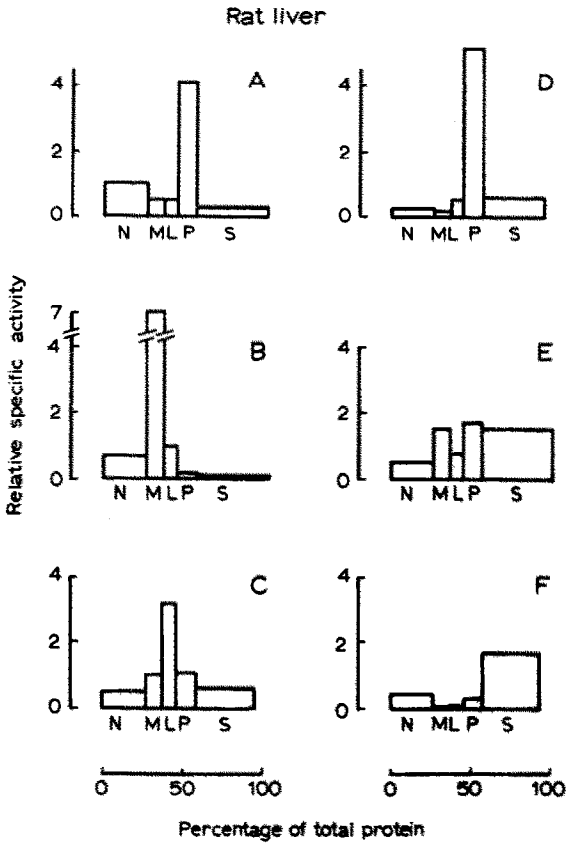

Fig.1. Distribution pattern of palmitoyl-L-carnitine hydrolase and some marker enzymes in fractions from human and rat liver. The relative specific activity (percentage of total activity/percentage of total protein) is plotted against the relative protein content in each fraction. N, the nuclear fraction; M, the mitochondrial fraction; L, the light mitochondrial fraction; P, the microsomal fraction; and S, the particle free supernatant. (A) Palmitoyl-L-carnitine hydrolase; (B) succinate-PMS-oxidoreductase; (C) acid phosphatase; (D) NADPH-cytochrome *c* reductase; (E) malate dehydrogenase; (F) lactate dehydrogenase.

exogenous CoA. Addition of 200 μM CoA (about 350 nmol/mg protein) increased the respective hydrolyzing activities to 9.8 to 14.5 nmol .mg protein^{-1} .min^{-1} in the mitochondrial fraction, but failed to result in any increased activity in the isolated microsomal fraction. As the endogenous, free CoA in liver mitochondria may be as high as 2.5 nmol/mg protein [18], it can not be excluded that hydrolysis of palmitoyl-L-carnitine in human liver mitochondria

Table 1
The absolute values (μmol .min^{-1}) of palmitoyl-L-carnitine hydrolase and some marker enzymes in the whole homogenate (i.e., cytoplasmic extract + nuclear fraction) of human and rat liver

	Absolute values and recovery %	
	Human liver	Rat liver
Palmitoyl-L-carnitine hydrolase	0.02 (104.1)	0.9 (97.4)
Succinate-PMS-oxidoreductase	0.7 (98.8)	60.5 (103.0)
Malate dehydrogenase	23.1 (89.6)	132.4 (102.5)
Acid phosphatase	2.8 (95.2)	7.8 (95.6)
NADPH-cytochrome *c* reductase	0.1 (101.1)	0.5 (95.4)
Lactate dehydrogenase	82.9 (91.4)	952 (93.7)
Protein	91.0 (102.2)	2067 (102.7)

Values in parentheses represent the recovery of protein and enzymic activities in the various fractions in fig.1, compared with the activity in cytoplasmic extract + nuclear fraction. Protein is given in mg

may be due to a combined action of carnitine palmitoyltransferase and palmitoyl-CoA hydrolase both of which are active in human liver mitochondria [19].

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