Biochemical Pharmacology, Vol. 56, pp. 1485–1488, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S006-2952(98)00166-X

SHORT COMMUNICATION

Loss of Response of Carnitine Palmitoyltransferase I to Okadaic Acid in Transformed Hepatic Cells

Guillermo Velasco, Patricia Passilly,† Manuel Guzmán*‡ and Norbert Latruffe†*

*Department of Biochemistry and Molecular Biology I, Faculty of Biology, Complutense University, 28040 Madrid, Spain; and †Laboratoire de Biologie Moleculaire et Cellulaire, Faculte des Sciences Mirande, Universite de Bourgogne, BP 400, 21011 Dijon, France

ABSTRACT. The specific activity of carnitine palmitoyltransferase I (CPT-I) was similar in mitochondria isolated from rat Fao and human HepG2 hepatoma cells and from rat hepatocytes, but almost twofold higher in permeabilized hepatoma cells than in permeabilized hepatocytes. Short-term exposure to okadaic acid induced a *ca.* 80% stimulation of CPT-I in hepatocytes, whereas no significant response of the enzyme from hepatoma cells was evident. Thus, the high CPT-I activity displayed by hepatoma cells may be reached by hepatocytes upon challenge to okadaic acid. Reconstitution experiments with purified mitochondrial and cytoskeletal fractions showed that the cytoskeleton of hepatocytes produced a more remarkable inhibition of CPT-I than the cytoskeleton of Fao cells. The present data may be explained by a disruption of interactions between CPT-I and cytoskeletal components in tumor cells that may be involved in the okadaic acid-induced activation of hepatic CPT-I as previously suggested. BIOCHEM PHARMACOL **56**;11:1485–1488, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. carnitine palmitoyltransferase I; hepatocytes; hepatoma cells; okadaic acid; cytoskeleton; mitochondria

Mitochondrial fatty acid oxidation provides a major source of energy in heart, skeletal muscle and liver (reviewed in [1–3]). Hepatic fatty acid oxidation also supplies extrahepatic tissues with ketone bodies as a glucose-replacing fuel [1–3]. CPT-I,§ the outer mitochondrial membrane carnitine palmitoyltransferase, catalyzes the pace-setting step of long-chain fatty acid translocation into the mitochondrial matrix [1–3]. Recent determination of flux control coefficients of the enzymes involved in hepatic long-chain fatty acid oxidation shows that CPT-I plays a pivotal role in controlling the flux through this pathway under different substrate concentrations and pathophysiological states [4, 5]. It is well established that long-term changes in hepatic CPT-I activity occur in response to alterations in the nutritional and hormonal status of the animal [1–3]. In addition, CPT-I is subject to allosteric inhibition by malonyl-CoA [1–3].

In recent years, a novel mechanism of control of hepatic CPT-I activity has been put forward. Several studies using permeabilized hepatocytes have shown that various agents exert short-term effects on CPT-I activity in parallel with changes in the rate of long-chain fatty acid oxidation (reviewed in [1]). Thus, cyclic 3′:5′-adenosine monophosphate analogues (e.g. dibutyryl-cAMP), effectors which increase intracellular cyclic 3′:5′-adenosine monophosphate levels (e.g. glucagon, forskolin) and protein phosphatase inhibitors (e.g. okadaic acid) are able to stimulate hepatic CPT-I [1]. This short-term activation of CPT-I is assumed to be mediated by a malonyl-CoA-independent mechanism [6] that may involve the phosphorylation of cytoskeletal component(s) and the subsequent disruption of interactions between CPT-I and the cytoskeleton [7, 8]. However, the significance of this putative mechanism of control of CPT-I activity is as yet unknown. In the context of the aforementioned hypothesis, it is conceivable that the regulatory properties of CPT-I may change under pathophysiological situations in which the organization of the cytoskeleton is altered. Since it is well established that the cytoskeleton of transformed cells is disorganized, the present work was undertaken to study the regulation of CPT-I activity by okadaic acid in hepatoma cells compared to hepatocytes in primary cultures.

MATERIALS AND METHODS

Cell Culture

The rat hepatoma cell line Fao and the human hepatoma cell line HepG2 were cultured as previously described [9]. They were transferred to their respective serum-free cultured media [9] supplemented with 1% (w/v) defatted and dialyzed BSA 24 hr prior to the experiments. Hepatocytes were isolated from male Wistar rats (250–300 g) which had free access to food and water by the collagenase perfusion

‡ Corresponding author: Dr. Manuel Guzmán, Department of Biochemistry and Molecular Biology I, School of Biology, Complutense University, 28040-Madrid, Spain. Tel. 34–913944668; FAX 34–913944672; E-mail: mgp@solea.quim.ucm.es

§ *Abbreviations:* CPT-I, carnitine palmitoyltransferase I; GDH, glutamate dehydrogenase.

Received 17 July 1997; accepted 9 April 1998.

method described in [6]. They were inoculated in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal calf serum (FCS). After cell attachment (*ca.* 6 hr), the medium was replaced with serum-free Dulbecco's modified Eagle's medium containing 10 nM dexamethasone and 1% (w/v) defatted and dialyzed BSA, and the hepatocytes were cultured for 14–18 hr before the experiments were performed.

CPT-I Assay

CPT-I activity was determined in digitonin-permeabilized cells as the tetradecylglycidate-sensitive incorporation of radiolabeled L-carnitine into palmitoylcarnitine. Briefly, attached cells (plated in P6 plates) or cells in suspension (scraped from F75 flasks), as indicated in every case, were preincubated for 45 min in the absence or presence of 20 μM tetradecylglycidate (kindly donated by Dr. J.M. Lowenstein, Brandeis University), a specific irreversible inhibitor of CPT-I (cf. [10]). Incubations were continued for an additional 45-min period in the presence or absence of varying concentrations of okadaic acid. Subsequently, CPT-I activity was monitored in cell monolayers [11] or suspensions [10].

For the determination of CPT-I activity in isolated mitochondria, the culture medium from 10–15 F75 flasks was aspirated, cells were washed in NaCl/P_i, scraped from the flasks, and homogenized in a medium containing 10 mM of Tris-HCl, pH 7.4, 0.25 M of sucrose, and 1 mM of EDTA. The resulting crude homogenates were directly used for isolation of mitochondria and determination of CPT-I and GDH activities exactly as described [6]. In some experiments, CPT-I activity in mitochondria was determined in the presence of a total cytoskeleton fraction that was isolated and characterized as described [12].

Statistical Analysis

Results shown represent the means ± SD of the number of experiments indicated in each case. Each experimental condition was always carried out at least in quadruplicate. Statistical analysis was performed by the Student's *t*-test.

RESULTS AND DISCUSSION

The properties of CPT-I were studied in two hepatoma cell lines, namely human HepG2 cells and rat Fao cells, which are commonly used as a model to study liver lipid metabolism (cf. [13–15]). As shown in Table 1, CPT-I specific activity was not significantly different in mitochondria isolated from hepatoma cells than in mitochondria isolated from hepatocytes in primary culture. Similar qualitative results were obtained when values of enzyme activity were referred to mass of mitochondrial protein and to activity units of GDH, a mitochondrial marker (Table 1). This indicates that no differences in mitochondrial mass are evident among the three types of cells.

TABLE 1. CPT-I activity in mitochondria isolated from hepatoma cells and primary hepatocytes

Cells	CPT-I Activity (nmol/min per mg protein)	CPT-I Activity (nmol/min per unit GDH activity)
Fao	1.87 ± 0.25	2.56 ± 0.79
HepG2	2.19 ± 0.34	1.87 ± 0.41
Hepatocytes	1.81 ± 0.22	2.34 ± 0.68

Values correspond to three separate experiments. See Materials and Methods for further details.

CPT-I activity was subsequently monitored in a permeabilized cell system. The use of this assay allows measurement of hepatocellular CPT-I activity in its physiological environment [1]. As shown in Fig. 1, CPT-I activity in primary hepatocytes was about half of that in hepatoma cells. This clearly indicates that CPT-I activity inside the hepatocyte is subject to certain constrictions that do not operate in hepatoma cells or in preparations of purified hepatic mitochondria. This is in line with the recent idea that interactions between the mitochondrial outer membrane and extra-mitochondrial cell components, most likely localized in the cytoskeleton, might be involved in the control of hepatic CPT-I activity [6, 7]. These interactions could be readily lost in preparations of purified mitochondria.

To test this hypothesis, the effect of the phosphatase inhibitor okadaic acid on CPT-I activity was determined in

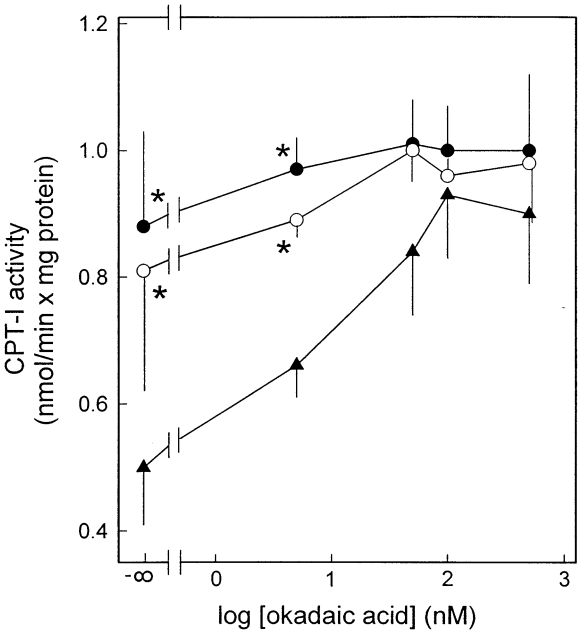

FIG. 1. Effect of okadaic acid on CPT-I activity in attached hepatoma cells and primary hepatocytes. Fao cells (●), HepG2 cells (○) and primary hepatocytes (▲) were incubated with varying concentrations of okadaic acid for 45 min, and CPT-I activity was subsequently determined with the permeabilized cell assay. Values correspond to 4 separate experiments. *Significantly different ($P < 0.01$) from primary hepatocytes.

TABLE 2. Comparative effect of okadaic acid on CPT-I activity in hepatoma cells and hepatocytes, both in suspension and in the attached state

Cells	Okadaic acid (500 mM)	CPT-I Relative Activity (%)	
		Attached cells	Cells in suspension
Fao	No	100 ± 17	100 ± 22
	Yes	112 ± 11 (5)	119 ± 10 (3)
HepG2	No	100 ± 23	n.d.
	Yes	120 ± 7 (4)	n.d.
Hepatocytes	No	100 ± 18	100 ± 12
	Yes	180 ± 12* (5)	171 ± 18* (4)

Values correspond to the number of expeiments indicated in parentheses. See Materials and Methods for further details. n.d. = not determined.

*Significantly different ($P < 0.01$) from the corresponding values with no okadaic acid.

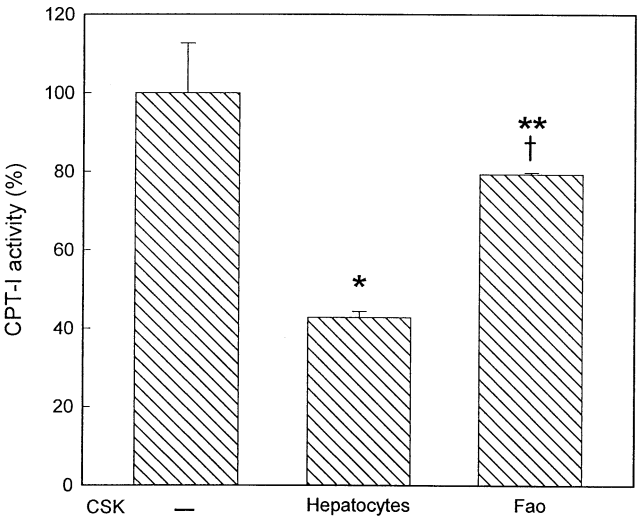

FIG. 2. Effect of a cytoskeletal fraction on CPT-I activity. Mitochondria from hepatocytes (1.5–2.0 mg protein) were incubated for 30 min in the absence (−) or presence of cytoskeleton (CSK; 0.15–0.20 mg protein) from hepatocytes or Fao cells, and CPT-I activity was subsequently determined as indicated in Materials and Methods. Values correspond to three separate experiments. Significantly different from incubations with no additions: *$P < 0.01$; **$P < 0.05$. $^{+}$Significantly different ($P < 0.01$) from incubations with cytoskeleton from hepatocytes.

the three cells tested. One of the most remarkable effects elicited by okadaic acid in hepatocytes and other cell types is the hyperphosphorylation and subsequent disruption of the cytoskeleton (e.g. [16, 17]). Figure 1 shows that a remarkable 80% stimulation of CPT-I ensued upon exposure of primary hepatocytes to okadaic acid. Fifty percent activation of CPT-I occurred at *ca.* 10 nM okadaic acid, indicating that CPT-I stimulation is mediated by the inhibition of protein phosphatase 1 [7, 17]. This is in agreement with the observation that protein phosphatase 1 seems to be the main phosphatase involved in the regulation of the phosphorylation state of the cytoskeleton, and in turn in the control of cytoskeletal integrity [17]. In contrast to hepatocytes, a slight but not statistically significant stimulation of CPT-I to okadaic acid was evident in hepatoma cells (Fig. 1). Therefore, the high CPT-I activity displayed by hepatoma cells may be reached by hepatocytes upon challenge to okadaic acid.

It might be argued that the distinct behavior of primary hepatocytes and hepatoma cells may be a reflection of differences in their attachment to the substrate, that may in turn involve different configurations of the cytoskeleton. Hence, CPT-I activity was monitored in cell suspensions after cell scraping from the flasks. As shown in Table 2, okadaic acid produced a significant stimulation of CPT-I in hepatocyte suspensions but not in Fao cell suspensions.

To further support the notion that cytoskeletal components may inhibit CPT-I, reconstitution experiments were carried out with purified mitochondrial and cytoskeletal fractions. As shown in Fig. 2, the cytoskeletal fraction isolated from hepatocytes was able to markedly inhibit CPT-I. However, the decrease in CPT-I activity elicited by the cytoskeletal fraction prepared from Fao cells was much weaker, indicating that in hepatoma cells the cytoskeleton may adopt a configuration that is less effective in inhibiting CPT-I.

In conclusion, the present data support the notion that in hepatocytes okadaic acid liberates CPT-I from inhibitory constrictions imposed by cytoskeletal components that do not operate either in isolated mitochondria or in transformed liver cells. In this respect, Paumen *et al.* have recently put forward that cells that express high CPT-I activity are expected to withstand palmitate-induced apoptosis [18]. Whether liberation of CPT-I from the potential constrictions imposed by the cytoskeleton may help hepatoma cells to escape from apoptosis is currently under study in our laboratories. In addition, we have recently observed* that intermediate filaments are the components of the cytoskeleton most likely involved in the control of CPT-I activity. Interestingly, the interactions between intermediate filaments and mitochondria become disrupted in a stress situation such as heat shock [19]. Our results suggest that this might be the reason for the high CPT-I activity in hepatoma cells.

These investigations were supported by the Spanish Comisión Interministerial de Ciencia y Tecnología (SAF 96/0113), Fondo de Investigación Sanitaria (FIS 97/0039) and Comunidad Autónoma de Madrid (CAM-6648), as well as by the French ARC (Association pour la Recherche sur le Cancer) and the Ligue Bourguignone contre le Cancer.

References

1. Guzmán M and Geelen MJH, Regulation of fatty acid oxidation in mammalian liver. *Biochim Biophys Acta* **1167:** 227–241, 1993.

*Velasco G, Geelen MJH, Gómez del Pulgar T and Guzmán M, unpublished observations.

2. Zammit VA, Regulation of ketone body metabolism. A cellular perspective. *Diabetes Rev* **2:** 132–155, 1994.
3. McGarry JD and Brown NF, The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem* **244:** 1–14, 1997.
4. Drynan L, Quant PA and Zammit VA, Flux control exerted by mitochondrial outer membrane carnitine palmitoyltransferase over β-oxidation, ketogenesis and tricarboxylic acid cycle activity in hepatocytes isolated from rats in different metabolic states. *Biochem J* **317:** 791–795, 1996.
5. Spurway T, Sherrat HSA, Pogson CI and Agius L, The flux control coefficient of carnitine palmitoyltransferase I on palmitate β-oxidation in rat hepatocyte cultures. *Biochem J* **323:** 119–122, 1997.
6. Guzmán M, Kolodziej MP, Caldwell A, Costorphine CG and Zammit VA, Evidence against direct involvement of phosphorylation in the activation of carnitine palmitoyltransferase by okadaic acid in rat hepatocytes. *Biochem J* **300:** 693–699, 1994.
7. Velasco G, Sánchez C, Geelen MJH and Guzmán M, Are cytoskeletal components involved in the control of hepatic carnitine palmitoyltransferase I activity? *Biochem Biophys Res Commun* **224:** 754–759, 1996.
8. Velasco G, Guzmán M, Zammit VA and Geelen MJH, Involvement of Ca^{2+}/calmodulin-dependent protein kinase II in the activation of carnitine palmitoyltransferase I by okadaic acid in rat hepatocytes. *Biochem J* **321:** 211–216, 1997.
9. Passilly P, Jannin B and Latruffe N, Influence of peroxisome proliferators on phosphoprotein levels in human and hepatic-derived cell lines. *Eur J Biochem* **230:** 316–321, 1995.
10. Guzmán M and Geelen MJH, Activity of carnitine palmitoyltransferase in mitochondrial outer membranes and peroxisomes in digitonin-permeabilized hepatocytes. Selective modulation of mitochondrial enzyme activity by okadaic acid. *Biochem J* **287:** 487–492, 1992.
11. Spurway TD, Agius L, Sherrat HSA and Pogson CI, Measurement of carnitine palmitoyltransferase I in hepatocyte monolayers. *Biochem Soc Trans* **22:** 118S, 1994.
12. van Bergen en Henegouwen PMP, den Hartigh JC, Romeyn P, Verkleij AJ and Boonstra J, The epidermal growth factor receptor is associated with actin microfilaments. *Exp Cell Res* **199:** 90–97, 1992.
13. Prip-Buus C, Bouthillier-Voisin AC, Kohl C, Demaugre F, Girard J and Pegorier JP, Evidence for an impaired long-chain fatty acid oxidation and ketogenesis in Fao hepatoma cells. *Eur J Biochem* **209:** 291–298, 1992.
14. Dixon JL and Ginsberg HN, Regulation of hepatic secretion of apolipoprotein B-containing lipoproteins: Information obtained from cultured liver cells. *J Lipid Res* **34:** 167–179, 1993.
15. Duclos S, Bride J, Ramirez LC and Bournot P, Peroxisome proliferation and β-oxidation in Fao and MH1C1 rat hepatoma cells, HepG2 human hepatoblastoma cells and cultured human hepatocytes: Effect of cipofibrate. *Eur J Cell Biol* **72:** 314–323, 1997.
16. Holen I, Gordon PB and Seglen PO, Protein kinase-dependent effects of okadaic acid on hepatocytic autophagy and cytoskeletal integrity. *Biochem J* **284:** 633–636, 1992.
17. Gurland G and Gundersen GG, Protein phosphatase inhibitors induce the selective breakdown of stable microtubules in fibroblasts and epithelial cells. *Proc Natl Acad Sci USA* **90:** 8827–8831, 1993.
18. Paumen MP, Ishida Y, Muramatsu M, Yamamoto M and Honjo J, Inhibition of carnitine palmitoyltransferase I augments sphingolipid synthesis and palmitate-induced apoptosis. *J Biol Chem* **272:** 3324–3329, 1997.
19. Collier NC, Sheetz MP and Schlesinger MJ, Concomitant changes in mitochondria and intermediate filaments during heat shock and recovery of chicken embryo fibroblasts. *J Cell Biochem* **52:** 297–307, 1993.