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FEBS Letters 344 (1994) 239–241

FEBS 14019

FEBS LETTERS

Inhibition of carnitine palmitoyltransferase I by hepatocyte swelling

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Received 8 April 1994

Abstract

Incubation of hepatocytes under conditions known to increase their volume, i.e. with amino acids (glutamine, proline) or in hypo-osmotic medium, decreased carnitine palmitoyl-transferase I (CPT-I) activity. This effect of hepatocyte swelling was antagonized by okadaic acid and dibutyryl-cAMP. Physiological concentrations of glutamate inhibited CPT-I activity in digitonin-permeabilized hepatocytes but not in isolated mitochondria. Results suggest that the amino acid-induced inhibition of CPT-I shares a common mechanism with the amino acid-induced stimulation of acetyl-CoA carboxylase and glycogen synthase [(1993) Eur. J. Biochem. 217, 1083–1089].

Key words: Carnitine palmitoyltransferase I; Hepatocyte swelling; Glutamine; Ketogenesis

1. Introduction

Evidence is rapidly accumulating that changes in hepatocyte volume play an important role in the control of hepatocellular metabolic function [1,2]. Hepatocyte swelling induced by several amino acids, notably glutamine and proline, or by hypotonicity have a number of anabolic and anti-catabolic effects, such as stimulation of glycogen [3,4], lipid [4] and protein synthesis [5], or inhibition of glycogenolysis [6] and proteolysis [7]. Ketogenesis is also inhibited by hepatocyte swelling [8]. Although the mechanism responsible for this effect has not been described, amino acid-induced inhibition of hepatic ketogenesis was observed to be independent of changes in the concentration of malonyl-CoA [8], a physiological inhibitor of the key regulatory enzyme in the transport of long-chain fatty acids into the mitochondrial matrix, viz. carnitine palmitoyltransferase I (CPT-I) [9–12]. Changes in the kinetic characteristics of CPT-I that occur in parallel with, or independently of, intracellular malonyl-CoA concentration have been shown to be involved in a number of short- and long-term alterations of hepatic ketogenesis [9–12]. Therefore, the present work was undertaken to test whether changes in the intrinsic properties of CPT-I are involved in the amino acid-induced inhibition of hepatic ketogenesis.

2. Experimental

2.1. Hepatocyte isolation and incubation

Male Wistar rats (220–250 g) were used throughout this study. Hepatocytes were isolated as described in [13] and incubated in Krebs–Henseleit bicarbonate buffer supplemented with 10 mM glucose and 1% (w/v) defatted and dialysed bovine serum albumin. Incubations (4–6 mg of cellular protein/ml) were carried out in a total volume of 2 ml at 37° C under an atmosphere of O_2/CO_2 (19:1). The osmolarity of the medium (305 mOsm under iso-osmotic conditions) was varied to 225 mOsm (hypo-osmotic medium) or to 385 mOsm (hyper-osmotic medium) by changing NaCl concentration.

2.2. Rate of ketogenesis

The rate of ketogenesis was monitored in incubations containing 0.4 mM [1-^{14}C]palmitate (0.1 Ci/mol) bound to albumin. Reactions were stopped with 0.5 ml of 2 M perchloric acid and ketone bodies were extracted and quantified as described before [14,15]. Ketone bodies routinely accounted for 85–90% of total oxidation products.

2.3. CPT-I assay

CPT-I activity was determined as the tetradecylglycydate (TDGA)-sensitive incorporation of radiolabelled L-carnitine into palmitoylcarnitine by three different methods (A, B and C). TDGA is a potent, specific and irreversible inhibitor of CPT-I [14,16]. In brief, hepatocytes were incubated in the absence or in the presence of 5 μM TDGA. Aliquots were removed from both sets of incubations in order to monitor CPT activity. In methods A and B, CPT activity was measured in digitonin-permeabilized hepatocytes. Both methods were performed using the same detergent/cell protein ratio (ca. 40 μg digitonin/mg cell protein). In method A ('one-step assay'), 100 μl of hepatocyte suspension was added to 100 μl of prewarmed digitonin-containing assay medium exactly as described in [15], and so the cell permeabilization and enzyme assay were performed at the same time. In method B ('two-step assay'), hepatocytes were permeabilized and thorougly washed prior to determination of enzyme activity. Thus, 1.0 ml of hepatocyte suspension was added to 1.0 ml of prewarmed medium containing 0.20 mg digitonin, 5 mM Tris-HCl (pH 7.4), 150 mM KCl, 5 mM EDTA and 5 mM EGTA (Cl^- medium). The resulting mix was gently shaken for 5 s and rapidly diluted by transfer to tubes containing 40 ml of ice-cold Cl^- medium. Permeabilized cells were sedimented by centrifugation at 350 × *g* for 15 s, and pellets were resuspended in 1.0 ml of prewarmed digitonin-free Cl^- medium. The permeabilized-cell

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SSDI 0014-5793(94)00405-K

suspensions were incubated at 37°C for 5–15 min and then CPT activity was monitored. Finally, the third method (method C) measures enzyme activity in mitochondria isolated from hepatocyte suspensions as described in [17]. When the direct effect of glutamate on CPT-I activity was studied, iso-osmotic controls were always run in parallel by replacing potassium glutamate for identical concentrations of KCl.

3. Results and discussion

Incubation of isolated hepatocytes in conditions known to increase their volume, i.e. with amino acids (10 mM glutamine or 10 mM proline) or in hypo-osmotic (225 mOsm) medium, decreased CPT-I activity, as measured in digitonin-permeabilized cells by method A (Table 1). This was accompanied by an inhibition of hepatic ketogenesis from palmitate in parallel cell incubations (Table 1). In contrast, a slight increase in CPT-I activity and in ketogenesis from palmitate was observed in hepatocytes incubated in hyper-osmotic (385 mOsm) medium (Table 1).

The inhibition of CPT-I and ketogenesis by hepatocyte swelling could result from an increase in the intracellular levels of malonyl-CoA, a physiological inhibitor of CPT-I [9–12]. However, Baquet et al. have shown that the amino acid-induced inhibition of hepatic ketogenesis can occur independently of increases in malonyl-CoA concentration [8]. In the present study, the simultaneous permeabilization of the plasma membrane and assay of CPT-I activity (method A) is assumed to have diluted intracellular malonyl-CoA [14,15]. Consequently, it is possible that the observed changes in CPT-I activity are due to stable post-translational modification of CPT-I, either directly [14,15,18] or indirectly [19]. Therefore, we tested whether factors that increase cell protein phosphorylation (e.g. dibutyryl-cAMP, okadaic acid) could prevent the observed effects of swelling. Table 1 shows that both agents were able to antagonize the effects of swelling on CPT-I activity.

In another set of experiments, hepatocytes were incubated with glutamine and/or okadaic acid, and CPT-I activity was determined in digitonin-permeabilized hepatocytes by method B (Fig. 1). The changes induced by these cellular effectors on CPT-I activity survived permeabilization of hepatocytes, extensive washing of the permeabilized cells, and subsequent incubation of the per-

Table 1
Inhibition of CPT-I activity and ketogenesis by hepatocyte swelling and its reversal by okadaic acid and dibutyryl-cAMP

Cell incubation	Percentage of incubations with no additions	
	CPT-I activity	Rate of ketogenesis
Hypo-osmotic medium (n = 6)	78.5 ± 5.4[a]	81.9 ± 7.0[a]
Hyper-osmotic medium (n = 4)	118.1 ± 3.7[a]	116.0 ± 4.5[a]
10 mM glutamine (n = 6)	74.7 ± 6.0[a]	78.4 ± 5.3[a]
10 mM proline (n = 4)	68.9 ± 8.3[a]	74.7 ± 8.1[a]
0.5 μM okadaic acid (n = 6)	154.6 ± 14.7[a]	148.6 ± 12.1[a]
50 μM dibutyryl-cAMP (n = 6)	137.2 ± 5.1[a]	132.4 ± 5.7[a]
Hypo-osmotic medium + 0.5 μM okadaic acid (n = 6)	149.6 ± 10.2[a]	140.7 ± 8.4[a]
Hypo-osmotic medium + 50 μM dibutyryl-cAMP (n = 4)	136.4 ± 5.7[a]	133.9 ± 3.2[a]
10 mM glutamine + 0.5 μM okadaic acid (n = 6)	142.0 ± 8.5[a]	143.4 ± 9.3[a]
10 mM glutamine + 50 μM dibutyryl-cAMP (n =4)	133.2 ± 5.0[a]	128.1 ± 4.4[a]
10 mM proline + 0.5 μM okadaic acid (n = 4)	145.9 ± 6.9[a]	139.5 ± 10.4[a]
10 mM proline + 50 μM dibutyryl-cAMP n = 4)	126.8 ± 4.0[a]	126.1 ± 3.7[a]

Hepatocytes were incubated for 30 min in the presence of the additions indicated. Then, part of the cells were used for measurement of CPT-I activity in digitonin-permeabilized hepatocytes by method A. The rest of the cells were used for determination of the rate of ketogenesis from [1-^{14}C]palmitate. Results represent the means ± S.D. of the number of cell preparations indicated in every case. 100% values of CPT-I activity and ketogenesis were 2.16 ± 0.43 nmol product/min × mg cell protein and 78.5 ± 8.0 nmol palmitate into product/h × mg cell protein, respectively. As determined by the Student's t-test. [a]Significantly different ($P < 0.01$) from incubations with no additions.

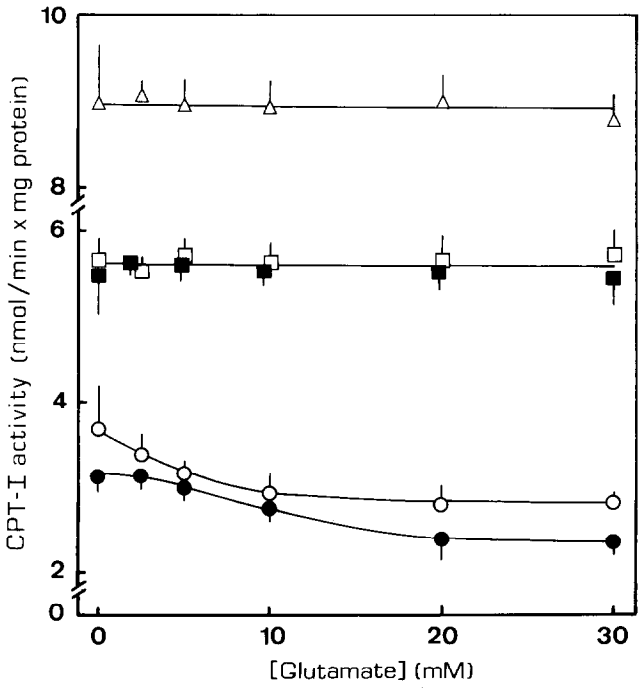

Fig. 1. Inhibition of CPT-I activity by glutamate in digitonin-permeabilized hepatocytes and its reversal by hepatocyte pretreatment with okadaic acid. Hepatocytes were incubated for 30 min with no additions (○) or in the presence of either 10 mM glutamine (●), 0.5 μM okadaic acid (□), or 10 mM glutamine plus 0.5 μM okadaic acid (■). Then, cells were permeabilized, washed in an excess of Cl^- medium, and incubated at 37°C for 5–15 min with increasing concentrations of glutamate. CPT-I activity was determined in those permeabilized cells by method B. Alternatively, CPT-I activity was determined by method C in mitochondria isolated from hepatocytes incubated with no additions (△). Results represent the means ± S.D. of 4 cell preparations.

meabilized cells at 37°C for 5–15 min, again indicating that the modulation of enzyme activity is stable. Therefore, although the inhibition of CPT-I by malonyl-CoA is a well-described property of the enzyme [9–12], other types of regulatory mechanisms could be involved in the control of hepatic CPT-I by hepatocyte swelling. Since the changes observed in CPT-I activity are stable in spite of the absence of fluoride in the medium, they are unlikely to be due to changes in the phosphorylation state of CPT-I.

A mechanism linking amino acid-induced hepatocyte swelling to stimulation of glycogen synthesis and lipogenesis has been recently suggested. Thus, the increase in the intracellular concentration of glutamate and (to a lesser extent) aspartate that is observed in swollen hepatocytes after incubation with glutamine or proline seem to be responsible, at least in part, for the stimulation of protein phosphatase(s) involved in the activation of glycogen synthase (and hence of glycogen synthesis) [20] and acetyl-CoA carboxylase (and hence of lipogenesis) [21]. Therefore, we wondered whether a similar mechanism could be responsible for the inhibition of CPT-I (and hence of ketogenesis independently of malonyl-CoA concentration) by hepatocyte swelling. From data presented in Fig. 1 it may be inferred that (i) concentrations of glutamate found in hepatocytes incubated with glutamine or proline [20] inhibited CPT-I when enzyme activity was assayed by method B in digitonin-permeabilized hepatocytes; (ii) preincubation of hepatocytes with glutamine induced a certain desensitization of the CPT-I enzyme toward glutamate; (iii) preincubation of hepatocytes with okadaic acid rendered CPT-I insensitive to the inhibitory effect of glutamate. Therefore, results suggest that the amino acid-induced inhibition of hepatic CPT-I may result from a glutamate-dependent mechanism related to that involved in the activation of glycogen synthase [20] and acetyl-CoA carboxylase [21].

It has recently been shown that the okadaic acid-induced stimulation of CPT-I is retained when mitochondria are still associated with other cellular fractions, e.g. in permeabilized cell ghosts and in crude cellular homogenates, but not when mitochondria are isolated for determination of enzyme activity [19]. Likewise, the inhibition of CPT-I by glutamate observed in permeabilized hepatocytes (Table 1 and Fig. 1) was not evident when enzyme activity was assayed by method C in mitochondria isolated from hepatocyte suspensions (Fig. 1). Okadaic acid is known to disrupt the cytoskeleton [22], and some effects of hepatocyte swelling are abolished by hepatocyte preincubation with colchicine, i.e. they seem to depend on the integrity of the cytoskeleton [23]. However, when we preincubated hepatocytes with colchicine or cytochalasin B, the effects of glutamine and okadaic acid on CPT-I activity were still evident (results not shown), suggesting that the integrity of the cytoskeleton is not necessary for the modulation of CPT-I activity by these cellular effectors. Our current research focuses on the characterization of the extra-mitochondrial cell components, presumably non-diffusible and possibly membranous, which seem to be required for the effects of glutamine or okadaic acid on CPT-I activity to be observed.

Acknowledgements: This work was supported by a grant (91/209) from the Fondo de Investigación Sanitaria del Ministerio de Sanidad y Consumo, Spain.

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