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PRELIMINARY REPORT

Hepatic Induction of Mitochondrial and Cytosolic Acyl-Coenzyme A Hydrolases/Thioesterases in Rats Under Conditions of Diabetes and Fasting

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Acyl-coenzyme A (CoA) hydrolases/thioesterases are a group of enzymes that catalyze the hydrolysis of acyl-CoA thioesters to free fatty acids and CoA-SH. The potency of these enzymes may serve to modulate intracellular concentrations of acyl-CoAs, free fatty acids, and CoA to affect various cellular functions, including lipid metabolism. In this study, we investigated the effect of diabetes and fasting on the protein levels of mitochondrial (MTE-I) and cytosolic acyl-CoA thioesterases (CTE-I), multigene family members of this class of enzymes, in adult rat liver. Rats were treated with alloxan to induce diabetes or fasted for 72 hours. Western blot analysis with the liver homogenates revealed 2.8-fold and 3.8-fold increases in MTE-I and 8.5-fold and 9.2-fold increases in CTE-I under the diabetic and fasting conditions, respectively, compared with the control in which the level of MTE-I was 4.3-fold higher than CTE-I. Serum level of free fatty acids was elevated 5-fold and 2.5-fold in diabetic and fasted rats, respectively. These results confirm the adaptive induction of MTE-I and CTE-I in response to fatty acid overload in the liver, being consistent with their auxiliary role in fatty acid degradation.

LONG-CHAIN acyl-coenzyme A (CoA) hydrolases/thioesterases are a diverse group of enzymes that include microsomal carboxylesterases with multisubstrate specificities. Recently, the enzymes tentatively termed type-I acyl-CoA thioesterases were identified and have been characterized as highly specific to hydrolyzing acyl-CoAs (reviewed in Hunt and Alexson[1]). These enzymes are markedly induced in the liver of rats and mice treated with peroxisome proliferators, including the fibrate class of hypolipidemic agents, and are implicated in lipid metabolism. The type-I acyl-CoA thioesterases comprise 4 isoforms to form a highly homologous multigene family, with mitochondrial (MTE-I) and cytosolic isoforms (CTE-I) being the principal ones. Early studies demonstrated a slight, but significant, increase in cytosolic long-chain acyl-CoA hydrolase activity in the liver of diabetic rats,[2,3] although molecular identity of the induced activity was not determined. Fasting was also shown to induce both MTE-I and CTE-I mRNA, but the induction was confirmed at the protein level only for CTE-I in the mouse liver.[4] To obtain a further link between the type-I acyl-CoA thioesterases and lipid metabolism, we examined the effect of diabetes and fasting on the level of MTE-I and CTE-I proteins in the rat liver.

MATERIALS AND METHODS

Male Wistar rats weighing 200 ± 12 g (mean ± SD, n = 12) were used at 8 weeks of age. Rats were administered alloxan (Wako, Osaka, Japan) dissolved in saline at 200 mg/kg body weight, intraperitoneally (IP) once a day for 2 days, and killed by decapitation under anesthesia 48 hours after the last injection. Some rats were fasted for 72 hours under free access of water. Untreated animals were used as controls. The livers were excised, homogenized, and subjected to enzyme assay for palmitoyl-CoA hydrolase activity with the substrate at 20 μmol/L, as described previously.[5] Protein was determined using a Bio-Rad DC protein assay kit (Hercules, CA) with bovine serum albumin as the standard. For Western blotting, the liver proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), electroblotted to an Immun-Blot polyvinylidene fluoride (PVDF) membrane (Bio-Rad), and probed by rabbit anti-rat CTE-I (or ACH2)[6] or antirat catalase antibodies,[7] after which [^{125}I]-labeled protein A was

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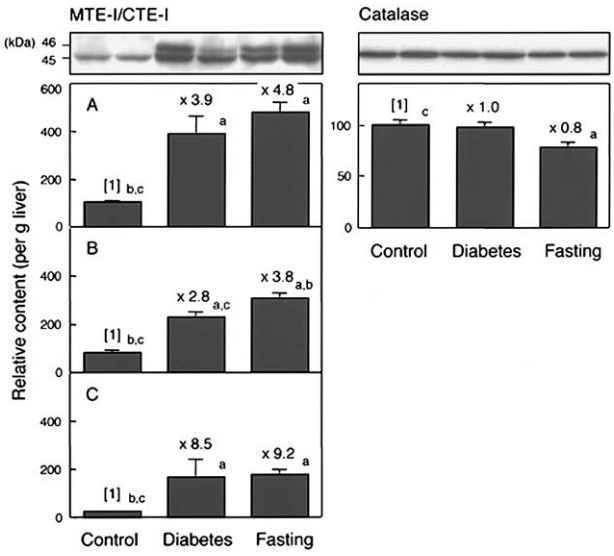

Fig 1. Effects of diabetes and fasting on the level of MTE-I and CTE-I in rat liver. The enzyme proteins were quantified by Western blotting of liver homogenates using an anti–rCTE-I antibody. The results are expressed as relative values, taking the one for controls in (A) as 100 (mean ± SD of 4 rats), with fold-induction over the control shown above each column. Lower-case letters, a, b, and c, represent significant differences ($P < .01$) from control, diabetes, and fasting groups, respectively. Blots (20 μg protein/lane) of 2 samples for each group are also shown. (A) Sum of MTE-I (45 kd) and CTE-I (46 kd); (B) MTE-I; and (C) CTE-I. For comparison, catalase was similarly examined as a control using an anti-catalase antibody.

used as the secondary antibody, and the radioactivity was measured by a BAS 2000 bioimaging analyzer (Fuji Photo Film, Tokyo, Japan).[5] The anti–rCTE-I antibody recognizes both MTE-I and CTE-I, whose amino acid sequences are 93% identical and molecular masses are quite similar.[8,9] However, clear separation of these enzymes was achieved by using a 10% polyacrylamide gel, enabling differential quantitative analysis (see Fig 1). The 45-kd and 46-kd polypeptides detected on the blots can be identified as MTE-I and CTE-I, respectively, based on previous Northern blot analysis demonstrating that the expression of CTE-I is hardly detected in the liver of untreated rats and becomes evident after induction, whereas MTE-I is expressed at a low, but significant, level even in the untreated rat liver[9] (see below). In previous work, Svensson et al[10] also separated and detected MTE-I and CTE-I as 45-kd and 46-kd polypeptides, respectively, on Western blots probed by an antirat MTE-I antibody. Serum glucose, triglyceride, and 3-hydroxybutyrate were determined by assay kits, Glucose-Test, Triglyceride G-Test, and AutoWako 3-HB (Wako, Osaka, Japan), respectively. Assays of free fatty acids and insulin were consigned to a clinical testing laboratory, SRL, (Tokyo, Japan). Statistical significance was examined by analysis of variance (ANOVA) followed by Fisher's protected least significant differences (PLSD) multiple comparison.

RESULTS

Alloxan-induced diabetes caused serious changes in energy metabolism in rats, as indicated by considerable loss of body weight and liver weight despite highly elevated levels of serum glucose and triglycerides (Table 1). Similarly, fasting for 72 hours resulted in marked loss of body and liver weight of rats; however, the serum glucose and triglyceride levels tended to decrease. In both cases, serum insulin level was very low, but the level of free fatty acids was 5-fold and 2.5-fold and that of 3-hydroxybutyrate, measured as a ketone body, was 39-fold and 26-fold higher than the controls in diabetic and fasting rats, respectively. Under these conditions, hepatic palmitoyl-CoA hydrolase activity was 1.8-fold increased in both cases compared with the control. As shown by Western blotting using an anti–rCTE-I antibody, hepatic contents of type-I acyl-CoA thioesterases were very low in control rats, in which CTE-I (46 kd) was difficult to detect, but MTE-I (45 kd) was at a significant level 4.3-fold higher than CTE-I (Fig 1). In diabetic and fasted rats, 2.8-fold and 3.8-fold increases in MTE-I and 8.5-fold and 9.2-fold increases in CTE-I were observed, respectively, compared with the control (Fig 1B and C). These increases corresponded to 3.9-fold and 4.8-fold induction of the type-I acyl-CoA thioesterases as the sum under diabetic and fasting conditions, respectively (Fig 1A). When examined using an anti-catalase antibody, no increase was observed in catalase, a housekeeping enzyme, used as a control (Fig 1, right panel), indicating specific induction of the type-I acyl-CoA thioesterases under these conditions.

DISCUSSION

As the enzyme activity of MTE-I and CTE-I represents only a fraction of the total activity in the liver, the observed increase in hepatic palmitoyl-CoA hydrolase activity was only modest in diabetic and fasted rats. However, the data obtained in this study confirmed the adaptive induction of MTE-I and CTE-I in response to fatty acid overload in the liver.

Under the conditions of diabetes and fasting, reduction of insulin level results in stimulated fatty acid mobilization from adipocytes to the circulation, leading to an increased

Table 1. Effects of Diabetes and Fasting on Nutritional and Biochemical Parameters and Hepatic Acyl-CoA Hydrolase Activity

	Control	Diabetes	Fasting
Body weight gain (g/3 d)	34 ± 2†‡	−30 ± 18*	−43 ± 4*
Liver weight (g)	9.9 ± 0.3†‡	7.6 ± 1.2*‡	4.7 ± 0.3*†
Liver protein (mg/g liver)	233 ± 12†‡	263 ± 9*‡	302 ± 13*†
Palmitoyl-CoA hydrolase activity (μmol/min/g liver)	2.6 ± 0.5†‡	4.8 ± 0.8*	4.6 ± 0.7*
Serum levels of			
Glucose (mg/dL)	138 ± 9†	838 ± 201*‡	72 ± 11†
Triglyceride (mg/dl)	82 ± 14†	815 ± 335*‡	47 ± 15†
Free fatty acids (mEq/L)	0.4 ± 0.1†‡	2.0 ± 0.2*‡	1.0 ± 0.2*†
3-Hydroxybutyrate (μmol/L)	162 ± 38†‡	6,231 ± 2,355*	4,269 ± 343*
Insulin (ng/mL)	1.0 ± 0.7	0.2 or < 0.1§	< 0.1§

NOTE. Results are expressed as mean ± SD of 4 rats.

*†‡Significantly different ($P < .01$) from control, diabetes, and fasting groups, respectively.

§Serum insulin levels of diabetic and fasted rats were less than 0.1 ng/mL, except 2 diabetic rats with 0.2 ng/mL.

flux of fatty acids into the liver, which in turn, elevates hepatocellular concentration of long-chain acyl-CoAs.[11] These acyl-CoAs may undergo fatty acid oxidation and reesterification into triglycerides. In this study, consistent with these situations, the serum levels of free fatty acids and 3-hydroxybutyrate (ketone body) were elevated both in the diabetic and fasted rats, and the type-I acyl-CoA thioesterases were markedly induced to similar extents in the liver in both. As indicated by the serum triglyceride level, hepatic fatty acid reesterification was also enhanced in the diabetic rats that had even higher levels of free fatty acids. However, it was not in the fasted rats, suggesting that the induction of type-I acyl-CoA thioesterases is more associated with enhanced fatty acid oxidation rather than reesterification. In a situation of enhanced fatty acid oxidation, MTE-I may serve to maintain optimal conditions for mitochondrial β-oxidation by hydrolyzing excessive acyl-CoAs taken up into this organelle to supply sufficient CoA to β-oxidation enzymes.[1] In the cytosol, CTE-I may support ω-oxidation by hydrolyzing acyl-CoAs to free fatty acids that can be ω-hydroxylated by microsomal CYP4A enzymes and subsequently oxidized to dicarboxylic acids.[1] Therefore, these type-I acyl-CoA thioesterases could play an auxiliary role in fatty acid degradation. This may also be the case in hepatic adaptation of lipid metabolism after feeding on a high-fat diet.

Our data presented in this report further support the link between type-I acyl-CoA thioesterases and lipid metabolism. To constitute an effective pathway of fatty acid catabolism, gene expression of these enzymes seems to be regulated by peroxisome proliferator-activated receptor α in a manner synchronized with those involved in β-oxidation and ω-oxidation.[1,3,4,6-8]

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