Infantile Form of Carnitine Palmitoyltransferase II Deficiency with Hepatomuscular Symptoms and Sudden Death

Physiopathological Approach to Carnitine Palmitoyltransferase II Deficiencies

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Abstract

Reported cases of carnitine palmitoyltransferase II (CPT II) deficiency are characterized only by a muscular symptomatology in young adults although the defect is expressed in extramuscular tissues as well as in skeletal muscle. We describe here a CPT II deficiency associating hypoketotic hypoglycemia, high plasma creatine kinase level, heart beat disorders, and sudden death in a 3-mo-old boy. CPT II defect (−90%) diagnosed in fibroblasts is qualitatively similar to that (−75%) of two "classical" CPT II-deficient patients previously studied: It resulted from a decreased amount of CPT II probably arising from its reduced biosynthesis. Consequences of CPT II deficiency studied in fibroblasts differed in both sets of patients. An impaired oxidation of long-chain fatty acids was found in the proband but not in patients with the "classical" form of the deficiency. The metabolic and clinical consequences of CPT II deficiency might depend, in part, on the magnitude of residual CPT II activity. With 25% residual activity CPT II would become rate limiting in skeletal muscle but not in liver, heart, and fibroblasts. As observed in the patient described herein, CPT II activity ought to be more reduced to induce an impaired oxidation of long-chain fatty acids in these tissues. (*J. Clin. Invest.* 1991.87:859–864.) Key words: hypoketotic hypoglycemia • ventricular arrythmia • human liver CPT II purification • fibroblasts • CPT II biosynthesis

Introduction

In man, long-chain fatty acids constitute a major source of energy, especially for the heart, the liver, and the skeletal muscle. In the myocardium, long-chain fatty acids are the preferred substrates when in resting state (1). In the liver, oxidation of long-chain fatty acids produces ketone bodies, enhances gluconeogenesis, and contributes therefore to the maintenance of normoglycemia during fasting (2). In the skeletal muscle, oxidation of long-chain fatty acids is essential during prolonged exercise (3). Therefore, a simultaneous dysfunction of the liver, the heart, and the skeletal muscle is usually observed in patients with defects in mitochondrial oxidation of long-chain fatty acids (4).

However, carnitine palmitoyltransferase (CPT)[1] deficiency is not known to cause simultaneous impairments in these tissues. Classically it results in two different clinical presentations. The muscular form is characterized by episodic muscle necrosis with paroxysmal myoglobinuria (5). The hepatic form (6) consists of fasting hypoglycemia with inappropriately low ketogenesis.

CPT is involved in the transport of long-chain fatty acids across mitochondrial membranes. It consists of two activities distributed in the outer (CPT I) and the inner (CPT II) mitochondrial membranes (7). Data obtained in animal tissues suggest that CPT I and CPT II are distinct proteins (8–10). The findings that the hepatic and muscular forms of the disease result from CPT I and CPT II deficiencies, respectively, (11) strengthen this hypothesis.

Tissual expression of the defect differs in both clinical forms. In the hepatic form, the defect is present in the liver but not in the skeletal muscle (12). This explains the lack of muscular injury in patients with CPT I deficiency. No informations are available about CPT I activity in these patients' heart. Data obtained in mouse and rat suggest the presence of distinct CPT I isoforms in the liver, the skeletal muscle, and the heart (13). Thus, the lack of cardiac symptoms in patients with CPT I deficiency would result from normal CPT I activity in this tissue.

In patients with the muscular form of CPT deficiency, a partial CPT II deficiency observed in all tissues tested (5), was shown to result from a defective protein bearing CPT II activity (14). No specific tissue isoforms of CPT II were shown to exist in human (14) or rat (13). The lack of cardiac and hepatic injury in patients with CPT II deficiency is therefore puzzling. Residual CPT II activity would be sufficient not to affect long-chain fatty acid oxidation in these tissues. Should this prove to be the case, cardiac and hepatic symptoms should be observed in patients with a more severe CPT II deficiency. We describe here such a patient.

Methods

Case report

The boy was born at 3.4 kg after a full-term uncomplicated pregnancy to healthy parents with two living children and a stillborn. The parents were first cousins. No problems were noted until the age of 3 mo. At this time, after a 3-d history of fever, the infant was found to be lethar-

1. *Abbreviation used in this paper:* CPT, carnitine palmitoyltransferase.

gic with respiratory arrest. He was admitted to an intensive care unit. Partial then generalized seizures occurred and were ascribed to a deep hypoglycemia (0.2 mmol/liter). Physical examination found a slight hepatomegaly and a cardiomegaly (cardiothoracic index 0.57). Heart rate troubles (premature ventricular complexes, auriculo ventricular block, and ventricular tachycardia) occurred a few hours after admission. Laboratory data (normal values in parentheses) were: plasma glucose, 0.7 mmol/liter (3.5–6 mmol/liter); no plasma and urine ketone bodies; arterial pH, 7.42 (7.35–7.45); blood lactate, 2.5 mmol/liter (0.6–1.9 mmol/liter); plasma potassium, 6.5 mmol/liter (3.5–5.5 mmol/liter); aspartate aminotransferase, 167 IU/liter (5–25 IU/liter); alanine aminotransferase, 91 IU/liter (5–25 IU/liter); blood ammonia, 75 μM/liter (20–40 μM/liter); fibrinogen 1 g/liter (2–4 g/liter); creatine kinase, 1,140 IU/liter (15–100 IU/liter) with isozyme MM, 1,100 IU/liter (15–100 IU/liter) and isozyme MB 34 IU/liter (0–11 IU/liter); lactate dehydrogenase 870 IU/liter (138–276 IU/liter) with a normal profile of isozymes; total plasma carnitine level, 14 μmol/liter (28–71 μmol/liter) with esterified/total carnitine ratio 80% (< 30%). A complete recovery was obtained after 6 d of symptomatic therapy. L-Carnitine supply (50 mg · kg^{-1}/d) completely normalized plasma carnitine levels. Levels of total and free carnitine (expressed as mean±SD of five determinations) were 45±10 μmol/liter and 31.5±3.1 μmol/liter, respectively. Withdrawal of carnitine supply resulted in a fall in plasma carnitine level (16 μmol/liter) over 1 wk. An organic acid profile was determined in urine by gas chromatography-mass spectrometry after the supply of L-carnitine was stopped. It was normal and did not reveal the presence of increased amounts of adipic, sebacic, or suberic acids. Metabolic investigations were performed 1 mo after the acute episode (see Results). Long-chain acyl-CoA dehydrogenase assayed in fibroblasts was normal (Coates & Hale, Philadelphia, PA). The child presented normal growth and development. Symptomatology was restricted to a slight permanent hepatomegaly. At 17 mo of age, the child suddenly died after an overnight fast. Parents refused necropsy.

In vivo investigations

Oral challenges with triglycerides were undertaken under controlled circumstances after informed parental consent. They were clinically uneventful. The proband and control subjects (age range, 10–40 mo) were included in the protocol. All infants were on a normal diet. After an overnight fast children were loaded with medium-chain triglycerides (Mead Johnson, France) or long-chain triglycerides (olive oil) given at the dose of 1.5 g/kg. Acetoacetate and 3-hydroxybutyrate were assayed by standard enzymatic methods (15) in blood sampled immediately before the load and at indicated times for 120 min.

In vitro investigations

Study of [1-^{14}C]fatty acid oxidation. The oxidation of [1-^{14}C]fatty acid oxidation in fibroblasts was measured with 100 μM [1-^{14}C]palmitate, 1 mM [1-^{14}C]octanoate, and 1 mM [1-^{14}C]butyrate as described (16). A similar methodology was used to study fatty acid oxidation in lymphocytes isolated from 15 ml of blood with Ficoll Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) according to manufacturer's recommendations. The final pellet of lymphocytes was suspended in 2–5 ml PBS. 10^6 cells in 0.5 ml PBS were incubated in 25-ml plastic culture flasks (Corning Glass Works, Corning, NY) with 500 μl of [1-^{14}C]fatty acid solution. Specific activities and final concentrations of palmitate, octanoate, and butyrate were 2.5 mCi/mmol, 0.1 mM; 1 mCi/mmol, 1.7 mM; 1 mCi/mmol, 1 mM, respectively. Octanoate and butyrate oxidation was estimated from the ^{14}CO$_2$ production. Palmitate oxidation was measured as the sum of produced ^{14}CO$_2$ and radioactive acid soluble compounds. Incubation time was 3 h. Production of radioactive compounds was linear with time during this period.

CPT assays. CPT I and CPT II activities were assayed as palmitoyl-L-[methyl-^{14}C]carnitine formed from L-[methyl-^{14}C]carnitine and palmitoyl-CoA. CPT I activity was measured as described (11) with 100 μM palmitoyl-CoA bound to 1% albumin, 500 μM L-carnitine and with or without 50 μM malonyl-CoA, in the 150 g supernatant of fibroblasts homogenized by sonication in 50 mM Tris, 150 mM KCl, pH

7.2. CPT II activity was assayed after solubilization of mitochondrial membranes by detergents. Fibroblasts were incubated at 4°C for 45 min in 0.5 M KCl 1% Tween 20, pH 7.2, before initiation of the assay. This assay was performed with 150 μl of fibroblast extract in a final volume of 500 μl. Complete incubation mixture contained 100 mM Tris pH 7.2, 2.5 mM reduced glutathione, 0.3% Tween 20, 0.15 M KCl, 100 μM palmitoyl-CoA, and 2.5 mM L-[methyl-^{14}C]carnitine (sp act, 0.5 mCi/mmol). Assay procedures were similar to those used for CPT I determination. Results of assays were expressed as nmol of palmitoyl-L-[methyl-^{14}C]carnitine. min^{-1}/mg protein. A more rapid spectrophotometric assay (17) was employed for screening enzymatic activity of CPT during its purification. Assay medium contained 115 mM Tris HCl, pH 8, 1.1 mM EDTA, 0.1% Triton X 100, 100 μM acyl-CoA, and 250 μM 5,5'-dithio-bis-(2-nitrobenzoic acid) and the sample. The reaction initiated by addition of 2 mM L-carnitine, was followed by monitoring the change in absorbance at 412 nm at 30°C. Proteins were assayed as described (18).

Purification of human liver CPT. CPT was purified from human liver obtained from kidney transplantation donors. Livers removed within 15 min after circulatory arrest were immediately frozen in solid CO$_2$. 80 g of frozen liver were homogenized in 800 ml of 0.25 M sucrose, 5 mM Hepes pH 7.5, 0.25 mM EDTA with an Ultra Turrax T25 (IKA Labor Technic, Germany), and then with a Potter-Elvejheim homogenizer. Mitochondrial membranes isolated from the homogenate as described by Clarke and Bieber (19) were suspended in 0.25 M sucrose, 0.25 mM EDTA, 2.5 mM Hepes, pH 7.5. Specific activity of cytochrome c oxidase (20), a marker of inner mitochondrial membrane was fivefold higher in final pellet than in total homogenate. Mitochondrial membranes mixed with an equal volume of 0.6 M KCl containing 2% Triton X 100 were incubated at 4°C for 30 min and then centrifuged at 100,000 g for 90 min. Supernatant was submitted to four chromatographic steps. The first three steps were essentially as described by Fiol and Bieber (21) except that the third column containing Cibacron Blue CL 6B was eluted using a gradient of 60 mM–1 M KCl, 0.1% Triton X 100; CPT was eluted at 850 mM KCl. Fractions containing CPT activity were pooled, equilibrated by dialysis with 10 mM KH$_2$PO$_4$, pH 7.8, 0.1% Triton X 100, and loaded onto a column of hydroxyapatite. The enzyme was eluted using a gradient of 10–300 mM KH$_2$PO$_4$, pH 6.8, 0.1% Triton X 100. Active fractions were pooled, concentrated, and analyzed for purity using SDS-PAGE (22).

Antibodies to purified CPT. Antibodies against CPT were produced (23) in rabbit by three injections of 50 μg purified CPT. Rabbits were killed 1 wk after the last injection. Preimmune serum was obtained before the first injection of the purified protein. The IgG fraction from preimmune and immune serum was obtained as described by Weatherill et al. (24).

Immunoprecipitation and electrophoresis. Immunoprecipitation experiments were performed (14) with preimmune and anti-CPT immune serum on the 100,000 g supernatant of fibroblasts solubilized in immunoprecipitation buffer (150 mM NaCl, 10 mM EDTA, 1% Triton X 100, 0.2% SDS, 10 mM PO$_4$H$_2$K, pH 7.4). Immunoprecipitated proteins were analyzed by SDS-PAGE (22) and electrotransferred to nitrocellulose sheet. Treatment and revelation of nitrocellulose sheet were performed as described (14).

Biosynthesis of CPT. Labeling of fibroblasts was performed similarly as described by Fenton et al. (25). Growth medium was removed from confluent monolayers of fibroblasts grown in 10-cm Petri dishes. Cell monolayers were washed with PBS and preincubated for 1 h at 37°C with 6 ml of methionine-free NEM modified Dulbecco's medium 10% FCS before incubation in 3 ml of the same medium containing 100 μCi of ^{35}S methionine. Medium was removed at the indicated time. Monolayers were disrupted by adding 1 ml of immunoprecipitation buffer (see above) to each dish. The resulting mixture was submitted to an immunoprecipitation with preimmune and anti-CPT immune serum (14). Immunoprecipitated labeled material was analyzed by SDS-PAGE (22). Gels were treated for fluorography with Amplify (Amersham Corp., Arlington Heights, IL), dried, and fluorographed according to supplier's direction.

Chemicals. Cibacron Blue CL6B and protein A Sepharose were from Pharmacia Fine Chemicals; L-carnitine was a gift from Sigma Tau (France); other chemicals were from Sigma Chemical Co. (St. Louis, MO); radioactive products were from Amersham International (Amersham, UK).

Results

The patient and controls were challenged with triglycerides. After long-chain triglycerides loading (Fig. 1 *A*) the patient's ketone bodies did not rise. The plasma ketone body course was similar in the patient and controls after medium-chain triglyceride loading (Fig. 1 *B*).

Oxidation of radiolabeled fatty acids was measured in the patient's lymphocytes and fibroblasts. As shown in Table I, palmitate oxidation was decreased by ~ 90%. It was not corrected by addition of 100 μM L-carnitine in the medium (data not shown). Oxidation of butyrate and octanoate which enter the mitochondria by a CPT-independent mechanism was within control range in lymphocytes and increased above the control values in fibroblasts.

CPT activities were assayed in fibroblasts (Table II). CPT I activity, identified by its suppressibility by malonyl-CoA (26) was measured in homogenate in which mitochondrial integrity was maintained (11). CPT was also assayed in homogenate preincubated with Tween 20. Inhibition of CPT I activity by detergents (27, 28) ensures that this assay only measures CPT II activity. When compared with control values, CPT I activity was normal, whereas CPT II activity was decreased by 90% in the patient's cells. CPT II assayed in lymphocytes was depressed by 40, 50, and 40% in the mother, the father and the sibling, respectively (results not shown).

Purification of human liver CPT was performed from mitochondrial membranes solubilized with Triton X 100 according to procedures described by Clarke and Bieber (19). CPT activity spectrophotometrically assayed with 100 μM palmitoyl-CoA as substrate was eluted as a single peak from each chro-
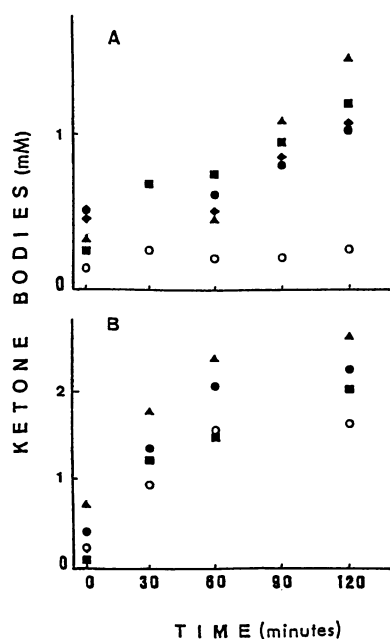
Figure 1. Time course of plasma ketone bodies level after triglyceride loadings. Long-chain (*A*) and medium-chain (*B*) triglyceride loadings were performed in the patient (o) and controls (●■▲◆) as described in Methods. Ketone bodies are expressed as the sum of 3-hydroxybutyrate and acetoacetate.

Table I. Radiolabeled Substrate Oxidation by Lymphocytes and Fibroblasts

	Substrates		
	[1-^{14}C]Palmitate	[1-^{14}C]Octanoate	[1-^{14}C]Butyrate
	nmol of radioactive product/10^6 cells per h		
Fibroblasts			
Patient	0.48; 0.64	6.05; 4.70	10.10; 8.10
Controls	3.84±1.16	2.04±0.75	5.50±2.44
Lymphocytes			
Patient	0.05; 0.03	0.16; 0.20	0.20; 0.24
Controls	0.53±0.12	0.23±0.04	0.30±0.07

Fatty acid oxidation was studied as described in Methods. Two independent experiments were performed in the patient's cells. Control values (mean±SD) were obtained from 11 independent experiments performed with different control cell lines.

matographic column. After the last chromatographic step, the protein with CPT activity was analyzed by SDS-PAGE. It migrated as a single band with an apparent molecular weight of 66 kD (Fig. 2). Enzymatic activity of this protein assayed with 100 μM acyl-CoA was negligible with short-chain acyl-CoA (C2-C6 derivatives). It was maximum with C12-CoA (19 μmol lauryl-CoA formed · min^{-1}/mg protein) and decreased with longer chain acyl-CoA. With palmitoyl-CoA, the activity was 6.5 μmol of formed palmitoylcarnitine · min^{-1}/mg protein. This enzymatic pattern was similar to that of CPT purified from animal tissues (19, 29). These proteins, unlike carnitine acetyl and octanoyltransferases (19, 30), do not show any significant activity with short-chain acyl-CoA as substrates.

Antibodies raised against human liver CPT were tested on carnitine acyltransferase activities assayed in solubilized mitochondrial membranes (Fig. 3). Immune IgG did not inhibit carnitine acyltransferase assayed with C2-C6-CoA derivatives but suppressed carnitine acyltransferase assayed with C12-C16-CoA derivatives by > 90%. Immunoprecipitation experiments were performed in fibroblasts (Fig. 4). As previously reported (14) anti-CPT immune serum recognized a single protein in control. The amount of this protein was barely detectable in the patient.

The biosynthesis of CPT was studied in fibroblasts. Cells were incubated with ^{35}S methionine. Then immunoprecipitation experiments followed by SDS-PAGE were performed with anti-CPT immune serum to analyze labeled products. A single

Table II. CPT Activities of Fibroblasts

	CPT II	CPT I	
	nmol/min per mg protein±SD		
Patient	0.17±0.04*	1.07±0.10	0.01±0.00
Controls	1.60±0.40	1.00±0.30	0.15±0.06

CPT I and CPT II activities were assayed in fibroblast homogenates as described in Methods. CPT activities of the patient were assayed in three independent experiments. The number of determinations ranged from 1 to 3 for 10 control lines. * $P < 0.01$.
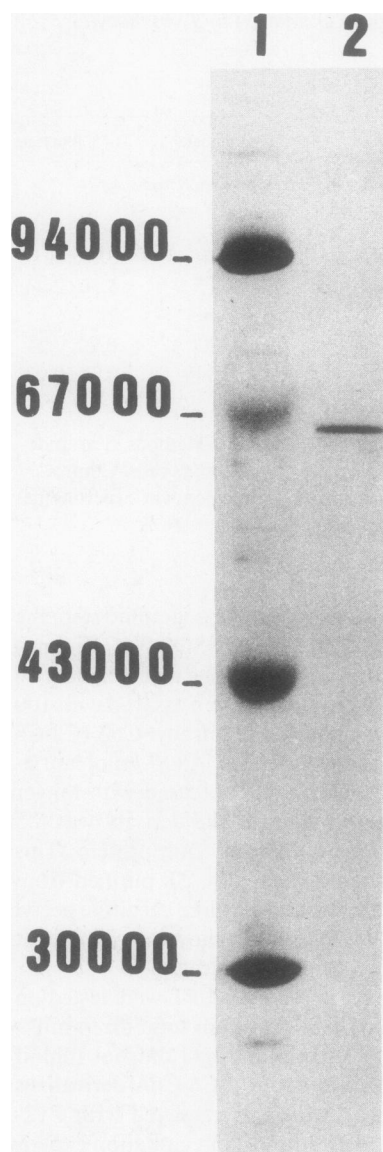
Figure 2. Analysis of purified human liver CPT by SDS-PAGE. Electrophoresis was performed as indicated in Methods. (Lane 1) Molecular weight standards (phosphorylase b, 94 kD; BSA, 67 kD; ovalbumin, 43 kD; carbonic anhydrase, 30 kD); (lane 2) 1.5 µg purified CPT.

Figure 3. Effect of anti-CPT immune serum on carnitine acyltransferases activities of human liver mitochondrial membranes. Solubilized mitochondrial membranes (150 µg protein) were preincubated with 50 µg IgG from preimmune serum (control experiment) or anti-CPT immune serum and protein A Sepharose. Then carnitine acyltransferase was spectrophotometrically assayed with 100 µM acyl-CoA of various chain length in the 500 g supernatant of incubation medium. Carnitine acyltransferase activity measured in the supernatant of incubation performed with anti-CPT immune serum was expressed as percentage of activity measured in control experiment. Carnitine acyltransferase activity (expressed as micromoles of acylcarnitine formed per minute) was 14.2, 15.0, 10.1, 10.3, 18.3, 16.2, 7.6, and 6.5, when assayed with $C_{2,4,6,8,10,12,14,16}$ acyl-CoA as substrate, respectively.

labeled band was observed (Fig. 5). Labeling of this band (Fig. 5 A, lanes 1, 3, 5) increased with incubation time with ^{35}S methionine. Its molecular weight was similar to the purified human liver CPTs. This band disappeared in competition experiments (Fig. 5 B, compare lane 1 with lane 2) in which an excess of CPT was added before addition of antiserum and was not detected if antiserum was replaced by nonimmune serum (Fig. 5 A, lanes 2, 4, 6). As compared with control, radiolabeling evidenced in the patient (Fig. 5 C, lane 2) a single protein of similar electrophoretic mobility but in decreased amount.

Discussion

We describe here an unusual form of CPT II deficiency associating hypoketotic hypoglycemia, heart beat disorders, high plasma creatine kinase levels, and sudden death in a 3-mo-old boy.

Study of plasma ketone body course after triglyceride challenge allows to evidence an impaired oxidation of fatty acids in the liver (31). Comparison of ketone body course after long (12
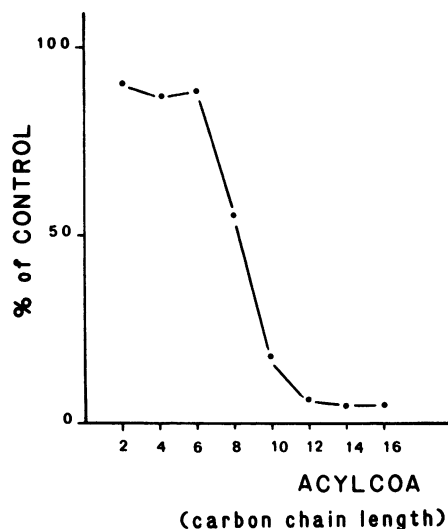
carbons or more) or medium (8–10 carbons)-chain triglyceride loadings roughly localizes the site of the metabolic block. The patient's plasma ketones rose after medium-chain triglyceride loading. However, they did not increase after long-chain triglyceride loading. This suggested the presence of an impaired oxidation of long-chain fatty acids in the liver. This metabolic block was found in lymphocytes and fibroblasts (Table I). Long-chain acyl-CoA dehydrogenase was found to be normal in fibroblasts. Plasma carnitine level (14 µmol/liter) ruled out a defect in carnitine transport, disease in which plasma carnitine level is close to zero (32).

CPT activities were assayed in the patient's fibroblasts (Table II). CPT I was normal and CPT II was markedly reduced (10% of control values). The clinical symptoms of this CPT II–deficient patient strongly differed from the usual phenotype
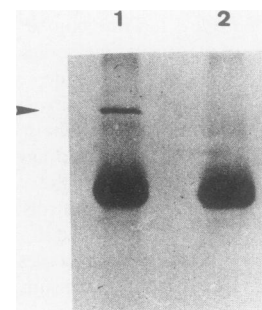

Figure 4. Analysis of cross-reacting material in fibroblasts from the patient with CPT II deficiency. Samples (1.5 µg protein) of solubilized fibroblasts from control and the CPT II–deficient patient were incubated with anti-CPT immune serum and protein A Sepharose. Proteins released from protein A Sepharose were analyzed by SDS-PAGE and immunoblotting. (Lane 1) control; (lane 2) patient.
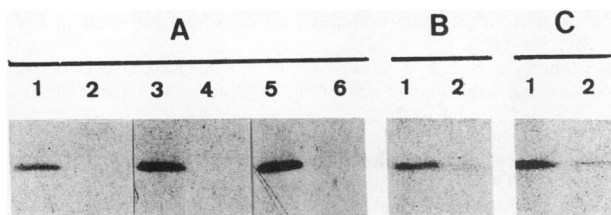
Figure 5. Biosynthesis of CPT II. Extracts of ^{35}S methionine-labeled fibroblasts were prepared as described in Methods. The confluent monolayer from one 10-cm Petri dish (~ 2 mg protein) was used for the extracts shown in each lane. Immunoprecipitation, electrophoresis, and fluorography were performed as indicated in Methods. (A) Monolayers from a control subject were incubated with ^{35}S methionine for 30 min (lanes 1 and 2), 60 min (lanes 3 and 4), and 120 min (lanes 5 and 6). Immunoprecipitation experiments were performed with 4 μl of anti-CPT immune serum (lanes 1, 3, 5) or with 4 μl of preimmune serum (lanes 2, 4, 6). (B) Lane 1, similar experiment as in A lane 5; lane 2, immunoprecipitation as in lane 1 except that 7 μg of unlabeled pure human liver CPT was added before the addition of antiserum. (C) Experimental conditions as in A lane 5. Lane 1, control fibroblasts; lane 2, fibroblasts from the CPT II-deficient patient.

of CPT II deficiency (5). Indeed, in the "classical" form of the defect, onset of the disease generally occurs in adolescence or adulthood. A first attack occuring in early infancy has never been reported. Patients suffer from recurrent episodes of rhabdomyolysis after prolonged exercise. CPT deficiency is observed in all tissues studied (skeletal muscle, liver, leukocytes, platelets, fibroblasts, etc.). Injury, however, is restricted to the skeletal muscle. During fast or challenges with long-chain triglycerides, patients display a normal or slightly impaired pattern of ketogenesis (mildly insufficient or delayed plasma ketones increase in about half the tested patients). Attacks of hypoketotic hypoglycemia or acute liver failure have never been reported. Cardiac dysfunction is not an usual feature of this phenotype. Mild ECG abnormalities (33, 34) and minor ventricle hyperthrophy (35) have been reported only in a few cases.

Similarity between the patient described here and patients with the "classical" phenotype was restricted to an increased plasma creatine kinase level. The main clinical symptoms of this patient (hypoketotic hypoglycemia and severe heart beat disorders) are not observed in the "classical" form of CPT II deficiency.

These distinct functional consequences of CPT II deficiency led to investigate the protein carrying CPT II activity in CPT II-deficient patients. CPT was purified from human liver. The main characteristics of this protein and of anti-CPT immune serum were reported in Results. This protein with apparent molecular weight of 66 kD was enzymatically active in its solubilized form. Thus, it probably represents only CPT II activity (27, 28). The study of cross-reacting material with anti-CPT immune serum in fibroblasts from control and CPT-deficient subjects, strengthened this hypothesis. In control cells, anti-CPT immune serum recognized a single protein similar to purified human liver CPT. The amount of this protein was normal in two CPT I-deficient patients and decreased in two "classical" CPT II-deficient patients (14). In the proband, CPT II deficiency was also associated with a decreased amount of CPT II (Fig. 4).

Biosynthesis of fibroblast CPT II was studied with pulse labeling experiments with ^{35}S methionine (see Methods). The patient's cell line exhibited a strong decrease in newly synthesized CPT II with a normal electrophoretic mobility (Fig. 5). CPT II biosynthesis was similarly depressed (results not shown) in fibroblasts of one CPT II-deficient patient previously studied (14). These data suggest that in these two patients, CPT II deficiency resulted from similar mutations leading to a reduced CPT II biosynthesis. As observed in patients with the "classical" phenotype (5), CPT II deficiency although only documented in the patient's fibroblasts is probably ubiquitous. Indeed in human (14) as in rat (13), the protein bearing this activity was reported to be similar if not identical in liver, skeletal muscle, heart, and fibroblasts.

Depending on the magnitude of the residual activity the metabolic and clinical consequences of CPT II deficiency differ. A 50% defect was asymptomatic in the patient's heterozygous parents and one sibling. In previously studied patients with the "classical" form of CPT II deficiency (11, 14), a 25% residual activity led to an impaired long-chain fatty acid oxidation in the skeletal muscle. The lack of cardiac and hepatic symptoms, as well as a normal long-chain fatty acid oxidation in fibroblasts, suggest that a 25% residual CPT II activity does not induce a significant impairment of fatty acid oxidation in human liver, heart, and fibroblasts. CPT II would have to be more reduced to lead to metabolic consequences in these tissues. In the proband where a 10% residual activity was found, this condition appears to be realized. Indeed clinical symptomatology as well as metabolic studies in fibroblasts advocated an impaired long-chain fatty acid oxidation in the proband's liver, heart, skeletal muscle, and fibroblasts.

CPT I was previously shown to control oxidation of long-chain fatty acids in rat liver (26). It plays probably a similar role in human liver. Indeed liver CPT I deficiency was associated, in vivo, with hypoketotic hypoglycemia and, in vitro, with an impaired long-chain fatty acid oxidation in fibroblasts (11, 36).

In addition to the extent of the enzymatic defect, variable exposure to environmental factors (prolonged exercise, cold, fasting, etc.) could account for the phenotypic heterogeneity of CPT II deficiency. This hypothesis is supported by the description of CPT II deficiency in siblings, one asymptomatic and the other with the "classical" form of the disease (37).

In the patient described here, CPT II deficiency induced hepatic and muscular symptoms similar to those observed in most inherited defects of mitochondrial β oxidation. The cardiac expression of the defect has to be emphasized. The patient described here displayed severe heartbeat disorders, whereas hypertrophic or dilated myocardiopathy are the usual expression of heart injury in other disorders of fatty acid oxidation (32). Arrythmia could have caused sudden death in this patient. Severe CPT II deficiency could result in an accumulation of long-chain acylcarnitines, which was recently shown to promote arrythmogenesis in cat heart (38). Prevention of long-chain acylcarnitine accumulation by pharmacologic inhibition of CPT I could provide a promising approach for the prophylaxis of sudden cardiac death in such patients.

Acknowledgments

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