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DEFICIENCY OF ACYL-CoA:DIHYDROXYACETONE PHOSPHATE ACYLTRANSFERASE IN PATIENTS WITH ZELLWEGER (CEREBRO-HEPATO-RENAL) SYNDROME

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We have recently reported on plasmalogen deficiency in tissues and fibroblasts from patients with Zellweger syndrome. In this paper we have analyzed the activity of the first enzyme in the pathway leading to plasmalogen biosynthesis, *i.e.* acyl-CoA:dihydroxyacetone phosphate acyltransferase in liver, brain and cultured skin fibroblasts from Zellweger patients and controls. The results indicate a severe deficiency of this enzyme in Zellweger patients. Thus, the Zellweger syndrome constitutes the first inborn error of metabolism with a deficiency in an enzyme involved in phospholipid biosynthesis. Cultured amniotic fluid cells contained an enzymatic activity comparable to that of control fibroblasts. These findings suggest a method for prenatal diagnosis of this disease.

The cerebro-hepato-renal syndrome (Zellweger syndrome, ZS), is an autosomal recessive disorder, clinically characterized by hypotonia, craniofacial dysmorphia, disturbances in liver and kidney function and severe psychomotor retardation (1-6). Goldfischer (1) described a total absence of peroxisomes in hepatocytes and renal tubule cells of ZS patients in combination with structurally abnormal mitochondria which seemed defective in electron transport. These findings have been confirmed by several investigators (3,5,7,8). Recently, Hajra and coworkers (9,10) have shown that key enzymes involved in the initial steps of glycerol ether lipid biosynthesis are located in (micro)peroxisomes in rodent liver and brain. Guided by these results, we have found subsequently that the main end products of ether phospholipid biosynthesis, *i.e.* plasmalogens (1-O-alk-1'-enyl-2-acyl-

Abbreviations: DHAP-acyltransferase, dihydroxyacetone phosphate acyltransferase (EC 2.3.1.42); G3P-acyltransferase, *sn*-glycero-3-phosphate acyltransferase (EC 2.3.1.15); ZS, Zellweger syndrome

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phosphoglycerides) were virtually absent in liver, kidney, brain, muscle and heart of ZS patients (11,12). Also cultured skin fibroblasts from ZS patients contained significantly reduced ethanolamine plasmalogen levels (12), although the deficiency in these cells was less pronounced than in ZS tissues. These findings suggested that at least one of the enzymes involved in plasmalogen biosynthesis is deficient in ZS patients. Formation of the first lipidic intermediate in the biosynthetic route for plasmalogens (13), is catalyzed by acyl-CoA:dihydroxyacetone phosphate acyltransferase. We have assayed the activity of this enzyme in liver, brain and fibroblasts of ZS patients and controls and in amniotic fluid cells of controls.

MATERIALS AND METHODS

MATERIALS

L-[U-^{14}C]Glycerol 3-phosphate was obtained from The Radiochemical Centre, Amersham, England. Palmitoyl-CoA and fatty acid-poor bovine serum albumin were from Sigma, St. Louis, Mo., U.S.A. Pyruvate, β-NAD, L-glycerol 3-phosphate, L-glycerol 3-phosphate dehydrogenase and lactate dehydrogenase were purchased from Boehringer, Mannheim, F.R.G. Triethanolamine, silica gel HR and solvents were from Merck, Darmstadt, F.R.G.

METHODS

Enzyme sources. Fibroblasts and amniotic fluid cells were cultured and harvested according to standard procedures and stored at -70°C. Control and obligate heterozygote fibroblasts were obtained from skin biopsies of healthy volunteers and of the parents of ZS patients, respectively. Detailed clinical and biochemical findings concerning the ZS patients are presented elsewhere (12). Washed fibroblasts were resuspended in 5 mM Tris-HCl (pH 7.5) and 50 mM NaCl at a protein concentration of about 2 mg/ml. Homogenates were prepared by sonicating for three times 15 s at 80 W with 45 s intervals for cooling, followed by three cycles of freezing in liquid nitrogen and thawing.

DHAP-acyltransferase assay. [U-^{14}C]DHAP was prepared from L-[U-^{14}C]G3P in a preincubation step in a mixture containing 50 mM triethanolamine-HCl buffer (pH 7.6), 0.6 mM ^{14}C-G3P (10 μCi), 5 mM pyruvate, 1 mM NAD^+ and 10 units of lactate dehydrogenase in a total volume of 1.0 ml. The reaction was started by addition of G3P-dehydrogenase (10 units) and allowed to continue for 1 h at 25°C. Lactate determinations indicated that the inclusion of the NAD^+ regenerating system caused a quantitative conversion of L-[U-^{14}C]G3P into [U-^{14}C]DHAP.

The standard DHAP-acyltransferase assay mixture contained 75 mM sodium acetate buffer (pH 5.4), 8 mM sodium fluoride, 8 mM $MgCl_2$, 0.4 mg bovine serum albumin, 0.15 mM palmitoyl-CoA, 0.1 mM DHAP and 20-40 μg homogenate protein in a total volume of 0.12 ml. After incubation for 2 h at 37°C the reaction was stopped by addition of 0.45 ml methanol/chloroform (2:1, v/v). After addition of 0.15 ml each of chloroform and a solution containing 2 M KCl and 0.2 M H_3PO_4 the contents of the tubes were thoroughly mixed on a Vortex and centrifuged for 2 min at 150 x g. The upper water/methanol layer was discarded and 0.2 ml of the chloroform layer, containing the labeled palmitoyl-DHAP, was spotted on Whatman 3MM filter disks. The filters were dried at room temperature for 30 min and then washed successively for 30 min each in 10%, 10%, 5% and 1% (w/v) ice-cold trichloroacetic acid

to remove traces of the radioactive precursor (14). The filters were air-dried overnight and transferred to scintillation vials containing Instagel for radioactivity determinations. Blanks without palmitoyl-CoA were run in parallel and were subtracted from the measured values. Control experiments showed that [^{14}C]DHAP was completely washed off the filters, whereas palmitoyl-[^{14}C]DHAP was fully retained on the disks in this procedure.

RESULTS

Table 1 shows the results of DHAP-acyltransferase activity measurements in human liver and brain. Although only a limited number of tissue samples from ZS patients and controls were available for analysis, it is obvious that DHAP-acyltransferase activity is strongly reduced in tissues of ZS patients as compared to controls.

The presence of DHAP-acyltransferase in cultured human skin fibroblasts has not been reported before. Preliminary experiments with fibroblast homogenates indicated the formation of a radioactive chloroform-soluble compound from [^{14}C]DHAP in the standard assay system to measure DHAP-acyltransferase. As indicated in Table 2 formation of this product is almost completely dependent on the presence of palmitoyl-CoA and fibroblast protein. The chloroform-soluble radioactivity migrated as a single spot upon thin-layer chromatography on silica gel HR plates with chloroform/methanol/acetic acid (90:10:10, v/v) as developing solvent mixture. Before and after reaction with phenylhydrazine the radioactivity coincided with synthetic palmitoyl-DHAP and its phenylhydrazone, respectively. These properties identify the chloroform-soluble lipid as palmitoyl-DHAP (15). When [^{14}C]G3P rather than [^{14}C]DHAP was incubated with fibroblast homogenate protein at pH 5.4, no labeled lipid was produced (Table 2). This indicates

TABLE 1
DIHYDROXYACETONE PHOSPHATE ACYLTRANSFERASE ACTIVITY IN TISSUES

Tissue	Dihydroxyacetone phosphate acyltransferase (nmol.2h^{-1}.mg protein^{-1})				
	Patients				Controls
	1	2	3	4	
Liver	0.01	0.24	0.01	0.06	1.8 - 3.4(n=4)
Brain	0.07	0.73	n.a.	n.a.	2.3 - 3.2(n=2)

n = number of controls; n.a. = not available.

TABLE 2

REQUIREMENTS FOR DIHYDROXYACETONE PHOSPHATE ACYLATION IN FIBROBLAST HOMOGENATES

Omission from standard (pre)incubation mixture	Product formed	
	Counts/min	Per cent of complete mixture
None	6590	100.0
MgCl	6844	103.9
NaF	5842	88.6
Bovine serum albumin	390	5.9
Palmitoyl-CoA	192	2.9
Fibroblast protein	178	2.7
G3P-dehydrogenase	160	2.4

that at pH 5.4 a specific DHAP-acyltransferase, rather than a G3P-acyltransferase with affinity for both G3P and DHAP, is responsible for the acylation of DHAP in human fibroblasts. In this respect as well as in the pH-dependency of G3P and DHAP acylation (data not shown), human fibroblasts exhibit a similar behaviour as reported previously for rat brain (16) and guinea pig liver (10).

Table 3 compares the activities of DHAP-acyltransferase in fibroblasts of controls, heterozygotes and ZS patients. Whereas enzymatic activity in heterozygotes is in the range of control values, the data for ZS patients clearly indicate a deficiency of DHAP-acyltransferase in these cells. The radioactive lipid formed by ZS-fibroblast homogenates was identified as palmitoyl-DHAP by the same criteria as described above for the product isolated from control fibroblasts. Its synthesis was not diminished by addition of G3P to the incubation mixtures. These results indicate therefore, that a residual DHAP-acyltransferase activity is present in ZS-fibroblasts. Interestingly, cultured amniotic fluid cells from controls exhibited DHAP-acyltransferase activities at least comparable to those of control fibroblasts (Table 3).

DISCUSSION

Mammalian cells contain two different DHAP-acyltransferase activities, *i.e.* a microsomal activity identical with G3P-acyltransferase that utilizes

TABLE 3
DIHYDROXYACETONE PHOSPHATE ACYLTRANSFERASE ACTIVITY IN CULTURED CELLS

Cells	Number of cell lines	Dihydroxyacetone phosphate acyltransferase ($nmol.2h^{-1}.mg\ protein^{-1}$) Mean ± S.D.	Range
FIBROBLASTS			
Controls	27	9.80 ± 2.10	5.36 - 19.84
Heterozygotes	6	9.54 ± 3.74	5.90 - 15.42
Zellweger	9	0.66 ± 0.50	0.02 - 1.62
AMNION CELLS			
Controls	11	13.40 ± 3.94	7.72 - 18.48

G3P and DHAP and a peroxisomal activity that is specific for DHAP (17). The peroxisomal DHAP-acyltransferase has been implicated in glycerol ether lipid biosynthesis (13). The results presented in this paper show that cultured human skin fibroblasts contain this specific DHAP-acyltransferase. The fact that one of the key enzymes of the DHAP-pathway of ether lipid biosynthesis is present in cultured fibroblasts suggests also that fibroblasts are capable of *de novo* ether lipid synthesis. This suggestion was confirmed by the incorporation of {^{14}C}hexadecanol in the alkenyl chain of ethanolamine plasmalogen from fibroblasts (unpublished experiments). (Micro)peroxisomes have not yet been described for fibroblasts from human origin, but the detection in these cells of enzymes that are specifically located in (micro)peroxisomes) in liver (10,13) and brain (13,16), suggests that such organelles will be discovered in human fibroblasts as well.

Fibroblasts of ZS patients had less than 10 per cent of the DHAP-acyltransferase activity found in controls. It remains to be established whether the decrease in activity as well as the residual activity in ZS-fibroblasts is caused by the presence of less enzyme or by a cytosolic (pre)form of the enzyme which is less active because it is not properly assembled into a functional (micro)peroxisome. Although enzyme deficiencies in phospholipid catabolism are well known, this is, to our knowledge, the first report on a deficiency in human cells of an enzyme involved in phospholipid biosynthesis. The fact that DHAP-acyltransferase deficiency can

be measured in fibroblasts adds a new method for postnatal diagnosis of Zellweger syndrome. More importantly, the observed deficiency in ZS fibroblasts in combination with the finding that DHAP-acyltransferase is fully active in cultured amniotic fluid cells of controls, suggests that also prenatal diagnosis of ZS, based on DHAP-acyltransferase measurements in cultured amniotic fluid cells, should now be possible.

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