Specific glutaryl-CoA dehydrogenating activity is deficient in cultured fibroblasts from glutaric aciduria patients.

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J Clin Invest. 1984;73(3):778-784. https://doi.org/10.1172/JCI111271.

Research Article

Patients with glutaric aciduria (GA) have greatly increased urinary excretion of glutarate. Their leukocyte and fibroblast sonicates have deficient ability to produce 14CO2 from [1,5-14C]glutaryl-CoA, an enzymatic process with two sequential reaction steps, dehydrogenation and decarboxylation. In normal individuals, it is not known whether these two reaction steps require one or two enzymes, and currently it is assumed that a single enzyme, glutaryl-CoA dehydrogenase (GDH), carries out these two reactions. Since GA patients also excrete increased amounts of 3-hydroxyglutarate and glutaconate in urine, it was thought that glutaryl-CoA in these patients may be dehydrogenated but not decarboxylated. We developed a new assay specific for glutaryl-CoA dehydrogenation which measures enzyme-catalyzed tritium release from [2,3,4-3H]glutaryl-CoA, and we studied the glutaryl-CoA dehydrogenating activity in cultured normal human fibroblasts and those from patients with GA. The Michaelis constant (Km) of normal human fibroblast GDH for [2,3,4-3H]glutaryl-CoA was 5.9 microM, and activity was severely inhibited by (methylenecyclopropyl)acetyl-CoA at low concentrations. Sonicates from all five GA fibroblast lines examined showed 2-9% of control glutaryl-CoA dehydrogenating activity, corresponding to the deficient 14CO2 releasing activity. These results indicate either that the conversion of glutaryl-CoA to crotonyl-CoA is accomplished by two enzymes, and patients with GA are deficient in the activity of the first component, or alternatively, that this process is carried out by a single enzyme which is […]

Specific Glutaryl-CoA Dehydrogenating Activity Is Deficient in Cultured Fibroblasts from Glutaric Aciduria Patients

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Abstract. Patients with glutaric aciduria (GA) have greatly increased urinary excretion of glutarate. Their leukocyte and fibroblast sonicates have deficient ability to produce $^{14}CO_2$ from [1,5-^{14}C]glutaryl-CoA, an enzymatic process with two sequential reaction steps, dehydrogenation and decarboxylation. In normal individuals, it is not known whether these two reaction steps require one or two enzymes, and currently it is assumed that a single enzyme, glutaryl-CoA dehydrogenase (GDH), carries out these two reactions. Since GA patients also excrete increased amounts of 3-hydroxylglutarate and glutaconate in urine, it was throught that glutaryl-CoA in these patients may be dehydrogenated but not decarboxylated. We developed a new assay specific for glutaryl-CoA dehydrogenation which measures enzyme-catalyzed tritium release from [2,3,4-^{3}H]glutaryl-CoA, and we studied the glutaryl-CoA dehydrogenating activity in cultured normal human fibroblasts and those from patients with GA. The Michaelis constant (K_m) of normal human fibroblast GDH for [2,3,4-^{3}H]glutaryl-CoA was 5.9 μM, and activity was severely inhibited by (methylenecyclopropyl)acetyl-CoA at low concentrations. Sonicates from all five GA fibroblast lines examined showed 2–9% of control glutaryl-CoA dehydrogenating activity, corresponding to the deficient $^{14}CO_2$ releasing activity. These results indicate either that the conversion of glutaryl-CoA to crotonyl-CoA is accomplished by two enzymes, and patients with GA are deficient in the activity of the first component, or alternatively, that this process is carried out by a single enzyme which is deficient in these patients. It is unlikely that urinary glutaconate and 3-hydroxyglutarate in GA patients are produced via GDH.

This work was presented in part at the Society for Pediatric Research Meeting, May 1982, Washington, DC.

Received for publication 20 July 1983 and in revised form 26 October 1983.

J. Clin. Invest.

0021-9738/84/03/0778/07 $1.00
Volume 73, March 1984, 778–784

Introduction

The inherited metabolic disorder, glutaric aciduria (GA),[1] was first identified by Goodman et al. (1) in two siblings with intermittent metabolic acidosis who had accumulation in blood and excessive urinary excretion of glutaric acid. These children, as well as others subsequently described (2–4), developed progressive dystonia and dyskinesia within the first few months of life. In these patients, the accumulation of glutarate is due to a deficient oxidizing activity for glutaryl-CoA, an intermediate in the metabolism of lysine, hydroxylysine, and tryptophan (Fig. 1) (5, 6). Further metabolism of glutaryl-CoA occurs via glutaryl-CoA dehydrogenase (GDH), a flavine adenine dinucleotide (FAD)-containing mitochondrial enzyme found mainly in the liver and kidney. It is believed that this enzyme catalyzes the conversion of glutaryl-CoA to crotonyl-CoA by two successive reactions, namely dehydrogenation of glutaryl-CoA to glutaconyl-CoA, and decarboxylation of the glutaconyl-CoA. It is not known at present whether these two reactions are catalyzed by a single enzyme or by two enzymes. Thus far, these two activities of the partially purified bovine and rat enzymes have been inseparable (7, 8).

In studying the nature of the enzyme in patients with GA, a problem arises in measuring the dehydrogenating activity. Pure and partially purified acyl-CoA dehydrogenases are usually assayed spectrophotometrically, measuring reduction of an electron-accepting dye, such as dichloroindophenol. By using crude cell homogenates as an enzyme source, nonspecific dye reduction occurs, and this makes accurate measurement of dehydrogenation impossible. Thus, previous assays of human GDH measure $^{14}CO_2$ release from [1,5-^{14}C]glutaryl-CoA, namely the

1. *Abbreviations used in this paper:* FAD, flavin adenine dinucleotide; GA, glutaric aciduria; GA II, glutaric aciduria type II; GDH, glutaryl-CoA dehydrogenase; IVA, isovaleric acidemia; IVDH, isovaleryl-CoA dehydrogenase; MCPA-CoA, (methylenecyclopropyl)acetyl-CoA.

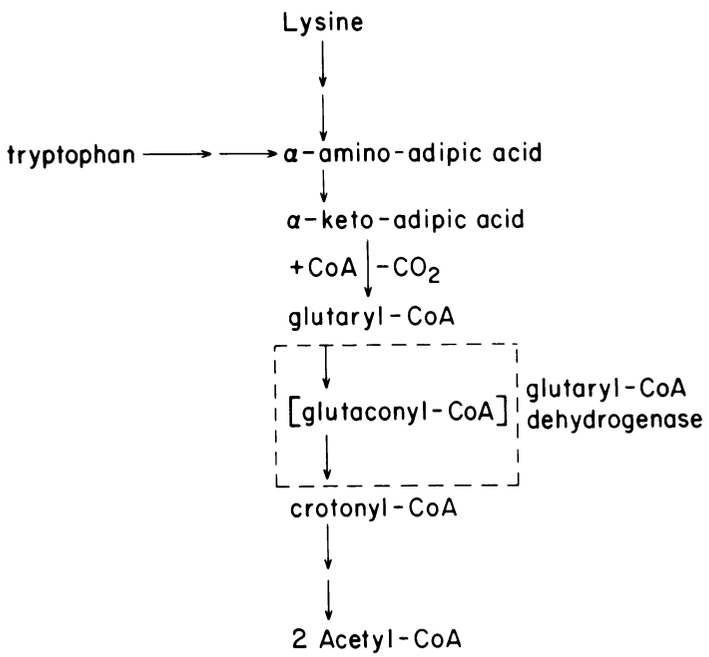

Figure 1. Major lysine degradation pathway.

combined activity of dehydrogenation and decarboxylation. By using this method, impaired $^{14}CO_2$ production from [1,5-^{14}C]glutaryl-CoA has been demonstrated in leukocytes (1, 5), cultured fibroblasts (5, 6), and liver mitochondria (9) from GA patients. Defective decarboxylation of [1,5-^{14}C]glutaryl-CoA by fibroblast sonicates from GA patients is consistent with either a decarboxylation defect, a dehydrogenation defect, or both. However, specific dehydrogenation has not been measured. The report by Stokke et al. (10) that the two index patients with GA excreted increased amounts of glutaconic acid and 3-hydroxylglutaric acid was suggestive of the possibility that in those patients dehydrogenation of glutaryl-CoA occurred, but the glutaconyl-CoA was not decarboxylated. This would explain the increase in urinary glutaconate. Excess glutaconyl-CoA could be metabolized further via enoyl-CoA hydratase to form the 3-hydroxyglutarate, which is also present in the urine of those patients. To specifically assay the activity of glutaryl-CoA dehydrogenation in GA fibroblasts, measurement of $^{14}CO_2$ evolution from labeled glutarate is not adequate. Thus, we developed a sensitive, specific method which utilizes [2,3,4-^{3}H]glutaryl-CoA as substrate to assay glutaryl-CoA dehydrogenating activity in crude cell homogenates (tritium-release assay). By using this method, we measured dehydrogenating activity as well as release of $^{14}CO_2$ from [1,5-^{14}C]glutaryl-CoA in cultured fibroblasts from five GA patients to characterize the nature of the enzyme defect in this disorder. We also carried out kinetic studies on the normal enzymes, and we demonstrated that (methylenecyclopropyl)acetyl-CoA (MCPA-CoA), a toxic metabolite of hypoglycin, strongly inhibits GDH. The inhibition of GDH by MCPA-CoA had been suspected, from in vivo and liver slice experiments using [2-^{14}C]lysine, as being the cause of glutarate accumulation observed in patients with Jamaican vomiting sickness and in hypoglycin-treated rats (11, 12).

Methods

Materials. [1,5-^{14}C]Glutaric acid (21 mCi/mmol) was purchased from ICN Pharmaceuticals, Inc. (Irvine, CA). [2,3,4-^{3}H]Glutaric acid (10 mCi/mmol) and [2,3-^{3}H]isovaleric acid (10 mCi/mmole) were purchased from New England Nuclear (Boston, MA). The tritiated compounds were custom synthesized by catalytic tritiation of glutaconic and 3-methylcrotonic acid, respectively. Unlabeled glutaryl-CoA and CoA lithium salt were obtained from P-L Biochemicals, Inc. (Milwaukee, WI). [1,5-^{14}C]Glutaryl-CoA and [2,3,4-^{3}H]glutaryl-CoA were synthesized via glutaric anhydride by the method of Simon and Shemin (13). [2,3-^{3}H]Isovaleryl-CoA was synthesized as described by Rhead and Tanaka (14) via the mixed anhydride method. MCPA-CoA was similarly synthesized by using (methylenecyclopropyl)acetic acid prepared by treating hypoglycin A (L-2-amino-3-(methylenecyclopropyl)propionic acid) with L-amino acid oxidase (11). Purity of the CoA thioesters was confirmed by ascending paper chromatography on Whatman 3MM paper (Whatman Laboratory Products Inc., Clinton, NJ), using ethanol:0.1 M potassium acetate, pH 4.5 (1:1).

Tissue culture. Fibroblast cultures from the five GA patients (four lines kindly supplied by Dr. N. J. Brandt, Copenhagen, Denmark, and one line by Dr. L. Sweetman, San Diego, CA) and control cell lines were grown in Eagle's minimal essential media with nonessential amino acids and glutamine (Flow Laboratories, McLean, VA) containing 10% fetal calf serum, 0.1 g/liter kanamycin, and phenol red.

Enzyme assays. Activity of glutaryl-CoA dehydrogenation was determined with the tritium-release assay by using [2,3,4-^{3}H]glutaryl-CoA as substrate. This method is similar to the method used to assay isovaleryl-CoA dehydrogenase (IVDH) and butyryl-CoA dehydrogenase (14). In this assay, tritium is released as tritiated water from enzyme-catalyzed

2,3-dehydrogenation of the labeled thioester, when phenazine methosulfate is used as an intermediate electron acceptor (15). The assay was adapted for use with crude fibroblast sonicates as the enzyme source, and for most experiments, cells from one or two 750-cm^2 roller bottles were harvested with 0.5% trypsin/0.2% EDTA. The cells were sonicated in 1 ml of 25 mM potassium phosphate, pH 7.5, with 1 mM cysteine. This amount of homogenate was sufficient for 20–30 activity determinations. Several activity measurements could be made on cells from a 75-cm^2 flask. 10–50 μl of this fibroblast suspension containing 100–500 μg protein was incubated at 37°C for 15 min in a medium containing 40 mM potassium phosphate, pH 7.5, 1 mM phenazine methosulfate, 0.1 mM FAD, 75 μM [2,3,4-^{3}H]glutaryl-CoA. The final volume was 100 μl. The reaction was terminated by addition of 20 μl of cold 0.25 M HCl and placing the tubes in an ice bath. A 100-μl aliquot was pipetted onto a disposable pasteur pipette column containing 0.3 ml of AG 1-X8 anion-exchange resin in the acetate form (Bio-Rad Laboratories, Richmond, CA). The column was washed with 2.2 ml of deionized water, and the eluate was collected in a counting vial. The tritium in the eluate was quantitated by liquid scintillation with a Beckman LS-100 scintillation counter (Beckman Instruments, Inc., Fullerton, CA) using Biofluor (New England Nuclear) as the scintillant. Parallel reaction systems to which 0.5 mM MCPA-CoA was added were used to determine background radioactivity, and all assays were performed at least in duplicate. IVDH was measured with the tritium-release assay by using [2,3-^{3}H]isovaleryl-CoA under the identical reaction conditions.

Evolution of $^{14}CO_2$ from [1,5-^{14}C]glutaryl-CoA was determined according to the method described by Besrat et al. (7). The standard reaction mixture, in a 15 × 85-mm culture tube, contained 40 mM potassium phosphate, pH 7.5, 3 mM methylene blue, 0.1 mM FAD, 75 μM [1,5-^{14}C]glutaryl-CoA, and fibroblast sonicate (100–500 μg protein) in a final volume of 0.1 ml. Immediately after adding fibroblast sonicate to start the reaction, the tube was sealed by using a rubber septum fitted with a plastic centerwell containing 0.1 ml 1 M Hyamine hydroxide (J. T. Baker Chemical Co., Phillipsburg, NJ). After 15-min incubation with shaking at 37°C, the reaction was stopped by injection of 0.5 ml of 50% trichloroacetic acid, and shaking at 37°C was continued for 30 min to ensure maximal $^{14}CO_2$ trapping. The Hyamine-containing centerwell was transferred to a minivial containing 3 ml Atomlite scintillation fluid (New England Nuclear), and radioactivity was measured by liquid scintillation.

Results

Analysis of product of glutaryl-CoA dehydrogenation with the tritium-release assay. An analysis of the radioactive products produced in the tritium-release assay by measuring counts before and after lyophilization at basic pH is shown in Table I. Under these conditions, tritiated water will be lost, while macromolecular and other nonvolatile compounds will be retained. Acidic compounds, including substrate, although presumably removed by the ion-exchange step, would also remain as nonvolatile salts under these conditions.

A small amount of radioactivity was detected in the column eluate with no fibroblast sonicate added: nonlyophilizable radioactivity represents about 40% of that amount, and this may be due to a minor, nonanionic contaminant in the substrate preparation. These counts represent 0.06% of the total substrate radioactivity. The lyophilizable radioactivity that was present without the enzyme preparation most likely represents tritiated water formed by a slow, nonenzymatic proton-tritium exchange with the solvent during storage. Total radioactivity produced in the complete system (A) increased with increasing amounts of cell homogenate added. Most of the increase was in the lyophilizable fraction, while nonlyophilizable counts increased slightly with increasing amounts of fibroblast protein, possibly due to protein/substrate complexes which pass through the anion-exchange resin.

When 0.5 mM MCPA-CoA was added to reaction mixtures with increasing amounts of fibroblast protein, lyophilizable radioactivity in the eluate remained essentially identical to the no-enzyme values, indicating no dehydrogenation occurred. Nonlyophilizable radioactivity increased with increasing protein concentration to the same degree as in reactions without MCPA-CoA, giving further support for the assumption that this was due to protein/substrate complexing. When the values of the total radioactivity eluted in the MCPA-CoA inhibited system (B) were subtracted from the corresponding values obtained in

Table I. Analysis of Radioactive Products of Enzyme-catalyzed Tritium Release from [2,3,4-^{3}H]glutaryl-CoA

Cell homogenate added	Radioactivity eluted from column						Enzymatic tritium release ($A - B$)
	Complete system			Complete system with 0.5 mM MCPA-CoA			
	Total (A)	Lyophilizable	Nonlyophilizable	Total (B)	Lyophilizable	Nonlyophilizable	
μg protein	*cpm*	*cpm*	*cpm*	*cpm*	*cpm*	*cpm*	
0	252	162	90	257	163	94	−5
125	423	303	120	254	150	104	169
250	672	518	154	288	154	134	384
375	937	749	188	316	165	151	621
500	1110	902	208	361	186	175	749

The tritium-release assay was performed on a twice normal scale. From each reaction, a 1-ml aliquot of the column eluate was lyophilized, reconstituted in 1 ml water, and counted in a liquid scintillation counter. Another 1-ml aliquot was counted directly. Each determination was run in duplicate.

the complete system (A), the difference ($A - B$) linearly correlated with the amount of cell homogenate added. Accurate activity measurement of fibroblast glutaryl-CoA dehydrogenating activity was thus facilitated by using the parallel reactions containing MCPA-CoA, at each protein concentration, to determine background radioactivity.

Kinetic studies of glutaryl-CoA dehydrogenation in normal fibroblasts and its inhibition by MCPA-CoA. Kinetics of GDH were studied by using a normal cell line and cell lines from patients with isovaleric acidemia (IVA). Using the tritium-release assay, dehydrogenation of [^{3}H]glutaryl-CoA did not occur in the absence of fibroblast extract, and omission of FAD or phenazine methosulfate decreased activity to a variable degree (data not shown). Activity was linear with respect to time, from 5 to 20 min. Activity measured in a separate experiment was also linear with the amount of fibroblast protein, ranging from 65 to 480 μg (data not shown). Michaelis constant (K_m) values for [2,3,4-^{3}H]glutaryl-CoA in a normal cell line and an IVA cell line, as determined by double reciprocal plotting of substrate versus velocity, were 5.5 and 5.9 μM, respectively (Fig. 2).

MCPA-CoA strongly inhibited GDH activity. The activity of both GDH and IVDH were strongly inhibited by 10 μM MCPA-CoA and essentially completely inhibited by 200 μM MCPA-CoA. The inhibition of GDH appeared competitive, with an apparent inhibition constant (K_i) of 1 μM. Evolution of $^{14}CO_2$ from [1,5-^{14}C]glutaryl-CoA by normal cells was also inhibited by similar concentrations of MCPA-CoA.

Dehydrogenation defect in GA cell lines. The activities of tritium and $^{14}CO_2$ release from glutaryl-CoA are shown in Table II. In six normal cell lines, dehydrogenating activity was 14.04±0.42 pmol ^3H_2O produced/min per mg protein (mean±SD) by using the tritium-release assay, and 41.3±18.6 pmol $^{14}CO_2$ produced/min per mg protein by using the $^{14}CO_2$-release assay. The lower specific activity measured by the tritium-release assay may be due to the tritium isotope effect. In all five GA cell lines, dehydrogenation of [^{3}H]glutaryl-CoA was <10% of control, and in four of the lines, the activity was <5% of control, with activities ranging from 0.26±0.17 to 1.31±0.38 pmol ^3H_2O/min per mg protein. By using the $^{14}CO_2$-release assay, the GA cell lines had residual activity ranging from 0 to 5% of control. Very low levels of activity in GA fibroblasts were difficult to measure using the tritium-release assay because the background radioactivity became large relative to the radioactive product formation. Thus, kinetic studies on the mutant enzyme using the tritium-release method could not be performed.

IVDH activity was assayed in the cell lines by a tritium-release assay under the same conditions used in the GDH assay. As expected, <5% of control activity (Table II) was found in cell lines from two patients with IVA, which has been shown to be due to a detect in IVDH (14). IVDH in the five GA cell lines was 30.9±11.6 to 54.1±28.2 pmol ^3H_2O/min per mg protein compared with 33.0±6.6 in a control cell line, demonstrating the absence of a general defect of acyl-CoA dehydrogenation in GA patients.

Discussion

Increased urinary excretion of glutaric acid occurs in at least two inherited metabolic diseases, GA and glutaric aciduria type II (GA II). For this study, we used cell lines from patients with GA. In these patients, the urinary organic acid pattern shows an elevation mainly of glutaric acid and its metabolites, 3-hydroxylglutaric and glutaconic acids, and this disease takes a distinctive neurodegenerative course. The urinary glutaric acid is presumably derived from glutaryl-CoA by enzymatic hydrolysis of its coenzyme A ester. Glutaryl-CoA, a common intermediate in the degradative pathways of lysine, hydroxylysine, and tryptophan, is normally converted to crotonyl-CoA by a mitochondrial enzyme or enzymes capable of dehydrogenation and decarboxylation. The precise mechanism of this reaction

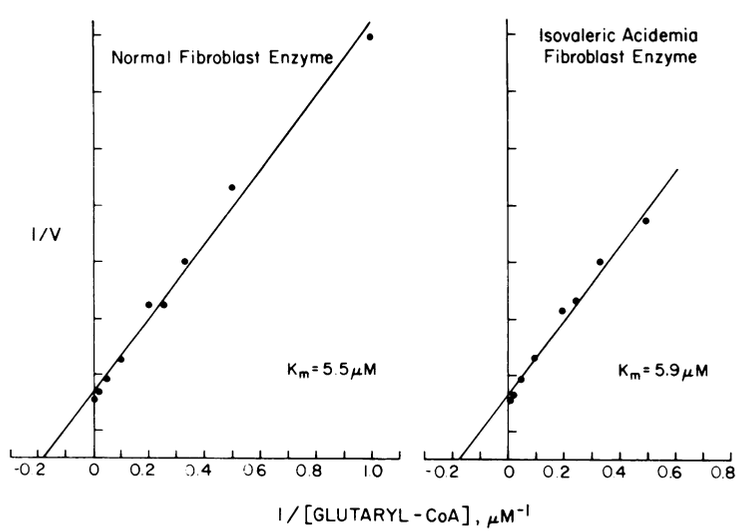

Figure 2. K_m determination for [^{3}H]glutaryl-CoA in enzyme obtained from a normal cell line and an IVA cell line. Enzyme assays were performed as described in the text. The K_m for each enzyme was calculated from a Michaelis-Menton plot.

Table II. GDH and IVDH Activity in Fibroblast Sonicates

Cell line	Type	Tritium release from [^{3}H]glutaryl-CoA		Release of $^{14}CO_2$ from [1,5-^{14}C]glutaryl-CoA		Tritium release from [^{3}H]isovaleryl-CoA	
		pmol ^3H_2O/mg protein/min		*pmol $^{14}CO_2$/ mg protein/min*		*pmol ^3H_2O/mg protein/min*	
810	GA	1.31±0.38	(4)	0.72	(1)	30.9±11.6	(3)
811	GA	0.26±0.17	(2)	2.14	(1)	40.2±1.4	(2)
812	GA	0.31±0.33	(3)	1.20	(1)	54.1±28.2	(3)
813	GA	0.71±0.73	(3)	0.91	(1)	39.9±11.5	(3)
989	GA	0.42±0.08	(2)	0	(1)	39.5±8.6	(2)
502	IVA	18.75±1.2	(2)	ND		0	(2)
747	IVA	18.07	(1)	ND		1.5	(1)
Control lines		14.04±0.42	(14)	41.3±18.6	(7)	33.0±6.6	(5)

Values are expressed as mean±SD. The numbers in parenthesis indicate the numbers of experiments performed. Abbreviations are as follows: GA, glutaric aciduria fibroblasts; IVA, isovaleric acidemia fibroblasts; ND, not determined.

or reactions is currently unknown. GA may provide a useful experimental model for studying this aspect of lysine degradation, since cell homogenates and liver mitochondria from patients with GA lack the ability to convert glutaryl-CoA to crotonyl-CoA (1, 5, 6, 9). However, it could not be determined from these studies which step, dehydrogenation or decarboxylation, was defective.

In contrast, patients with GA II, first described by Przyrembel et al. (16), have increased urinary excretion of numerous organic acids in addition to glutaric acid. This seems to be caused by a defect in the degradation of fatty acids and of several amino acids at the level of acyl-CoA dehydrogenation (16). Unlike in GA, oxidation of [1,5-^{14}C]glutaryl-CoA to crotonyl-CoA is normal in cell homogenates from GA type II patients (17). Rather, the dysfunction of the several acyl-CoA dehydrogenases has been suggested to be due to defective electron-transfer flavoprotein (17), a cofactor required for these dehydrogenases, or electron-transfer flavoprotein dehydrogenase (18).

The tritium-release assay that we developed directly measures glutaryl-CoA dehydrogenating activity of crude cell homogenates. In contrast, measuring the $^{14}CO_2$ release from [1,5-^{14}C]glutaryl-CoA estimates the dehydrogenase activity indirectly. The $^{14}CO_2$-release assay, exclusively, had been used in human studies of GDH, since crude homogenates of liver and fibroblasts cannot be assayed directly for dehydrogenation by a dye-reduction assay. Conventional dye-reduction assays show nonspecific dye reduction when used to assay crude cell homogenates, due mainly to free CoA-SH generated from acyl-CoA esters by the action of thioesterases. The tritium-release assay, which is adaptable to several acyl-CoA dehydrogenases, eliminates this problem and can quantitate activity in disrupted fibroblasts. However, the original procedure of Rhead and Tanaka (14) and Rhead et al. (15) required isolating fibroblast mitochondria, and the separation of sufficient mitochondria from fibroblasts required a large amount of cells. In the present study, we used parallel reactions in which acyl-CoA dehydrogenation is inhibited by MCPA-CoA to adjust for the increase in background radioactivity which occurred with increased protein concentration in the reaction mixture. With the subtraction of this background value, reliable activity measurements can be made on crude cell homogenates, and activity measurements could be determined on cells from a single tissue culture flask.

Our data demonstrate that in five GA cell lines from three unrelated pedigrees, there is defective dehydrogenation as well as decarboxylation of glutaryl-CoA. IVDH activity was maintained at normal levels in these cell lines, confirming the specificity of the metabolic derangement in GA. Since the enzymes involved in the conversion of glutaryl-CoA to crotonyl-CoA have not been fully characterized or purified, the exact nature of this deficiency is not known. At present, there are two possible interpretations of this data: one is that dehydrogenation of glutaryl-CoA and subsequent decarboxylation of glutaconyl-CoA are catalyzed by two separate enzymes and GA patients are deficient in the former activity. The second possibility is that these two reactions are carried out by a single enzyme which is deficient in patients with GA. Gomes et al. (19) have suggested a coordinated reaction mechanism for the bacterial enzyme, which apparently is a single protein. No similar mechanistic studies have been accomplished by using a mammalian enzyme source, but evidence strongly supporting a single enzyme hypothesis has been obtained from studies on bovine (7) and rat (8) liver mitochondria: dehydrogenase and decarboxylase activities copurify with each of several successive purification steps. In humans, the apparent K_m, as measured by the tritium-release assay, for glutaryl-CoA dehydrogenation is 5.9 μM, and a K_m of 15.7 μM has been reported by Christensen and Brandt (6) for the dehydrogenation/decarboxylation reaction. Since two sequential reactions are involved in the latter determination and

since the K_m for the decarboxylation reaction alone has not been determined, no further conclusions about the enzyme mechanism are possible from the K_m data.

Even with relatively low specific activity sources for GDH and IVDH, such as fibroblasts, we were able to show clear differences in enzyme activities between mutant and normal cells using the tritium-release assay. Unfortunately, fibroblasts from obligate heterozygotes were not available for testing. Additionally, the sensitivity of the assay was insufficient to allow performing kinetic analysis on the residual activity of the mutant enzymes obtained from cultured fibroblasts. To do that, liver mitochondrial sonicates, which have roughly 20-fold higher specific activity for GDH than crude fibroblast sonicates, would be the better enzyme source.

Because the neurodegenerative picture in GA may be related to the toxicity of glutarate metabolites, understanding the mechanism of formation of these metabolites may be important. The origin of glutaconate and 3-hydroxylglutarate in urine of GA patients is not known, but a likely route of 3-hydroxylglutarate formation is via hydration of glutaconyl-CoA. If GDH were a single enzyme catalyzing two reactions, and the mutant enzyme was virtually totally defective in decarboxylation activity and only 90–98% deficient in dehydrogenating activity, this could result in small amounts of glutaconyl-CoA being formed. Alternatively, glutaconate could be produced via dehydrogenation of free glutaric acid. Free glutaric acid does not appear to be dehydrogenated by liver mitochondrial homogenates (7), but it is interesting to note that [1,5-^{14}C]glutaric acid is oxidized at the same, albeit slow, rate in intact leukocytes from normal subjects as well as GA patients (1, 2). This appears to suggest the existence of an alternate pathway of free glutarate metabolism in whole cells, which is not impaired in GA patients. Perhaps, since patients with GA have free glutaric acid in high concentration in the blood, glutaconate and 3-hydroxylglutarate may be produced by this route. As a third possibility, free glutaric acid in the gut lumen could be metabolized by intestinal bacteria to form glutaconate and 3-hydroxyglutarate. This hypothesis has not yet been directly addressed.

Hypoglycin intoxication (Jamaican Vomiting Sickness) induces massive GA, and the inhibition of GDH by MCPA-CoA, a metabolite of hypoglycin, was hypothesized as the cause (11, 12). Several other acyl-CoA dehydrogenases in addition to GDH are severely inhibited in hypoglycin poisoning, and the urinary metabolic pattern in hypoglycin poisoning is virtually identical to that of GA II. It is interesting that glutaconate and 3-hydroxylglutarate do not appear in urine of patients with either GA II or in urine of hypoglycin-treated rats (D. Hine and K. Tanaka, unpublished observation). In this report, we demonstrated strong inhibition of GDH by micromolar concentrations of MCPA-CoA. MCPA-CoA has previously been shown to be a "suicide" inhibitor of purified general acyl-CoA dehydrogenase (20). In our assay system in which glutaryl-CoA and MCPA-CoA were simultaneously added to cell homogenates, the inhibition appeared competitive, with an apparent K_i of 1 μM. This is probably due to the MCPA-CoA competing with the substrate for the initial binding to GDH, which is necessary before inactivation of the enzyme occurs.

Acknowledgments

This work was supported by grants from the National Institutes of Health (AM 17453) and the March-of-Dimes (1-378). Dr. Hyman was also supported by fellowships from the National Institutes of Health (GM 07439 and AM 06988) and a James Hudson Brown-Alexander Coxe fellowship.

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