Comparison of Cytosolic and Mitochondrial Enzyme Alterations in the Livers of Propionic or Methylmalonic Acidemia: A Reduction of Cytochrome Oxidase Activity

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*Department of Pediatrics, Tohoku University School of Medicine Sendai 980, *Department of Neonatology, Japan Red Cross Hospital Center, Tokyo 150, †Department of Pediatrics, Tokyo Metropolitan Kiyose Children's Hospital, Tokyo 180-04 and ‡Department of Pediatrics, Toho University School of Medicine, Tokyo 143*

HAYASAKA, K., METOKI, K., SATOH, T., NARISAWA, K., TADA, K., KAWAKAMI, T., MATSUO, N. and AOKI, T. *Comparison of Cytosolic and Mitochondrial Enzyme Alterations in the Livers of Propionic or Methylmalonic Acidemia: A Reduction of Cytochrome Oxidase Activity.* Tohoku J. exp. Med., 1982, 137 (3), 329-334 — The activities of mitochondrial, cytosolic and microsomal enzymes in liver specimens obtained from three patients with propionic or methylmalonic acidemia were compared with those of control patients who had died from unrelated causes. Only the activity of cytochrome oxidase (mitochondrial enzyme) was significantly reduced in the patients of propionic acidemia and methylmalonic acidemia who were in the state of metabolic acidosis; in two patients the activity was less than 30% of that in controls, but in the other patient of propionic acidemia, who was under the treatment with a low protein diet (0.8 g/kg/day), the activity was 50% of that in controls. The metabolites of branched chain amino acids (tiglic acid, propionic acid, methylmalonic acid, succinic acid, tiglyl-CoA and propionyl CoA) exhibited no inhibitory effect on the cytochrome oxidase activity of the sonicated rat liver mitochondria. The reduction of cytochrome oxidase activity found in these organic acidemias may be caused secondarily by some unknown mechanism. — propionic acidemia; methylmalonic acidemia; cytochrome oxidase

Propionic and methylmalonic acidemias are rare inborn errors of metabolism. These disorders are often accompanied by hyperammonemia, lactic acidosis and fatty degeneration of the liver (Tanaka 1975; Rosenberg 1978), similar to those of Reye's syndrome. There is ample evidence that mitochondrial enzyme activities in the liver are reduced in Reye's syndrome (Snodgrass and DeLong 1976; Mitchell et al. 1980). In addition to these similarities to Reye's syndrome, the

Received for publication, December 2, 1981.

A part of the present findings have been published in preliminary form [Hayasaka et al. 1981].

activity of the glycine cleavage system in the liver mitochondria is significantly reduced in propionic or methylmalonic acidemia (Yoshida et al. 1969; Tada et al. 1974; Hayasaka et al. 1982). To examine in more detail the function of hepatic mitochondria in propionic or methylmalonic acidemia, the activities of cytosolic and mitochondrial enzymes in the livers of the patients were compared with those in control subjects.

MATERIALS AND METHODS

Chemicals

Cytochrome c, NAD⁺, propionyl CoA and tiglyl CoA were obtained from Sigma Chemical Company, St. Louis, MO. Reduced cytochrome c was prepared by reduction with ascorbic acid (Wharton and Tzagoloff 1967).

Patients

Patient K.H. (propionic acidemia) became progressively ill, showing muscular hypotonia and convulsions since age of 12 days. She had hyperglycinemia and hyperammonemia and died in the state of metabolic acidosis at the age of 53 days. After her death, a diagnosis of propionic acidemia was made by a marked excretion of propionic acid in urine and a deficiency of propionyl CoA carboxylase activity in the liver (Hayasaka et al. 1982).

Patient C.A. (propionic acidemia) suffered from frequent episodes of vomiting and lethargy associated with acidosis soon after birth. She had hyperglycinemia and hyperammonemia. Since the 65th day of age, she was put on a low protein diet (0.8 g/kg/day), and her clinical symptoms improved with the decrease of blood ammonia and plasma glycine. On the 118th day of age, the liver specimens were biopsied for enzymatic diagnosis. Then the defect of propionyl CoA carboxylase was demonstrated. Details were described in our previous report (Hayasaka et al. 1982).

Patient S.H. (methylmalonic acidemia) had methylmalonic acidemia and deficiency of methylmalonyl CoA mutase activity in leukocytes. She was well controlled by dietary therapy until 10 months of age, as described in our previous report (Sato et al. 1981; Hayasaka et al. 1982). Since 20 months of age, she could not be controlled and died at 21 months in the state of metabolic acidosis.

Preparation of liver homogenates

The liver specimens of the patients were obtained at the time of autopsy, frozen and stored at -80°C within 4 hr after death.

For control studies, samples of livers were obtained at autopsy from 5-month-old female and 3-year-old male who died of congenital heart disease, from 53-year-old male who died of lung tumor, from 8-year-old female who died of sudden cardiac arrest of unknown cause, and from 48-year-old male who died of cerebral hemorrhage. All specimens of controls were frozen within 2-6 hr after death. Informed consent was obtained from the families of all subjects for this study.

Frozen tissues were thawed and homogenized with 9 volumes of 0.25 M sucrose containing 5 mM Tris-HCl buffer (pH 7.4). After centrifugation (600 × g for 10 min), the supernatant was used for enzyme assays.

Preparation of rat liver mitochondria

Wistar rats weighing 150-200 g were killed by decapitation, and the liver mitochondria were prepared according to the method of Schneider and Hogeboom (1950). Mitochondria were suspended in 0.25 M sucrose containing 5 mM Tris-HCl buffer (pH 7.4) and were subjected to sonic disintegration at 140 W for 5 min.

Assay of enzyme activities

The following enzymes were assayed by measuring the change in absorbance at 340 nm using a spectrophotometer: lactate dehydrogenase (EC. 1.1.1.27) (Schwartz and Bodansky 1966), alanine aminotransferase (EC. 2.6.1.2) (Segal and Matsuzawa 1970), glucose 6-phosphate dehydrogenase (EC. 1.1.1.49) (Langdon 1966), glutamate dehydrogenase (EC 1.4.1.3) (Beaufay et al. 1959) and NADP⁺-linked isocitrate dehydrogenase (EC. 1.1.1.42) (assay conditions, Sigma Technical Bulletin No. 153, March, 1977). The activity of aspartate aminotransferase (EC. 2.6.1.1) was determined by the rate of oxaloacetate formation according to the method of Bergmeyer and Bernt (1974). The activity of glucose 6-phosphatase (EC. 3.1.3.9) was measured by the rate of Pi formation according to the method of Nordlie and Arion (1966). Determination of monoamine oxidase (EC. 1.4.3.4) activity was performed by measuring increases in absorbance at 250 nm according to the method of Tabor et al. (1955). The activity of succinate dehydrogenase (EC. 1.3.99.1) was measured by the method of Prospero (1974). The assay of cytochrome oxidase (EC. 1.9.3.1) activity was determined by the rate of cyanide sensitive oxidation of reduced cytochrome c essentially according to the method of Wharton and Tzagoloff (1967).

Protein determination

This was done by the method of Lowry et al. (1951).

RESULTS

The activities of ten enzymes in the liver specimens obtained from the patients with propionic or methylmalonic acidemia were compared with those of control cases (Table 1). Only the activity of cytochrome oxidase located in the inner mitochondrial membrane was remarkably reduced in Patient K.H. (propionic acidemia)

TABLE 1. *Enzyme activities in the liver from propionic acidemia, methylmalonic acidemia or control cases*¹

Enzyme	Propionic acidemia		Methylmalonic acidemia	Controls
	Patient K.H.	Patient C.A.	Patient S.H.	
Glucose 6-phosphate dehydrogenase ³	57.8		38.6	31.1 (18.3— 58.8) ²
Lactate dehydrogenase ³	2776.3		2072.6	2504.5 (1426.7—3128.2)
Glucose 6-phosphatase ⁴	98.3		108.3	87.7 (85.0— 93.3)
Aspartate aminotransferase ^{3,7}	1356.6		1166.2	792.7 (612.0— 952.0)
Alanine aminotransferase ^{3,7}	554.3		641.1	339.3 (207.2— 515.7)
Isocitrate dehydrogenase ^{3,7}	670.0		689.3	791.4 (641.1— 775.0)
Monoamine oxidase ⁵	26.3	14.2	42.8	21.9 (13.4— 30.4)
Succinate dehydrogenase ⁶	26.9	28.9	18.9	32.9 (19.3— 41.4)
Cytochrome oxidase ⁶	8.6	20.7	9.5	45.5 (33.6— 59.9)
Glutamate dehydrogenase ⁷	70.0	57.8	67.0	34.8 (23.0— 47.2)

1) Enzyme activities are expressed as nmoles of product per min per mg protein except for monoamine oxidase for which the activity was expressed in terms of units per mg protein (one unit of enzyme is defined as the amount of enzyme which produces an initial rate of change in optical density at 250 nm 0.001 per min at 30°C).

2) Mean and range are obtained from five control cases.

3) Cytosol.

4) Endoplasmic reticulum.

5) Outer mitochondrial membrane.

6) Inner mitochondrial membrane.

7) Mitochondrial matrix.

and Patient S.H. (methylmalonic acidemia); it was less than 30% of that in controls. On the other hand, the reduction of cytochrome oxidase activity in Patient C.A. (propionic acidemia) was not so remarkable in the liver, which was obtained at biopsy under the treatment with a low protein diet (0.8 g/kg/day); it was 50% of that in controls. The lower activity of cytochrome oxidase in the patients did not appear to be due to the presence of an inhibitor in the livers of the patients based on the results of the mixed experiments (data not shown). None of the activities of other mitochondrial, cytosolic and microsomal enzymes in the livers of the patients described in Table 1 was reduced as compared with those in controls; some were rather elevated, especially those of glucose 6-phosphatase, aspartate aminotransferase, alanine aminotransferase and glutamate dehydrogenase.

A number of metabolites of branched-chain amino acids were tested for their effects on cytochrome oxidase in the sonicated mitochondria of the rat liver. As shown in Table 2, no compound tested exhibited inhibitory effect on the cytochrome oxidase activity.

DISCUSSION

The present study revealed that the activity of cytochrome oxidase in the liver was significantly reduced in patients with propionic or methylmalonic acidemia, especially in the patients with metabolic acidosis, such as Patients K.H. and S.H. We demonstrated in the previous report (Hayasaka et al. 1982) that the glycine cleavage activity in the liver was reduced in Patients K.H. and S.H., but the activity was not reduced in Patient C.A. So, the observed reduction of cytochrome oxidase seems to have occurred also secondarily. Gregersen (1979) and Stumpf et al. (1980) reported that propionate and other short chain carboxylic acids inhibited oxygen consumption by rat liver mitochondria when α -ketoglutarate, pyruvate or succinate was substrate. In addition, Cathelineau et al. (1979) demonstrated that propionate acted to decrease the ATP content of rat liver mitochondria when succinate or glutamate was used as oxidizable substrate. These

TABLE 2. *Effects of metabolites of branched-chain amino acids on the activity of cytochrome oxidase in the rat liver mitochondria*

Additions	Activity (nmoles/min/mg protein)	% of control
None	1649.7	100
Propionic acid	1488.2	90
Methylmalonic acid	1649.7	100
Tiglic acid	1523.3	92
Succinic acid	1488.2	90
Propionyl CoA	1682.7	102
Tiglyl CoA	1699.2	103

All additions were 10 mM except for propionyl CoA and tiglyl CoA that were 5 mM.

Mitochondria were prepared and used after sonic disintegration.
Other conditions were described under Materials and Methods.

observations suggest the possibility that propionate and other short chain carboxylic acids may cause a reduction of cytochrome oxidase activity, resulting in the decrease of oxygen consumption and ATP content in the mitochondria. However, the cytochrome oxidase activity was not reduced when rat liver mitochondria were incubated at 37°C for 10 min with 10 mM propionate, methylmalonate or tiglic acid (data not shown). Furthermore, propionate, methylmalonate, tiglic acid, succinic acid, propionyl CoA or tiglyl CoA exhibited no inhibitory effect on the cytochrome oxidase activity of the sonicated rat liver mitochondria (Table 2). On the other hand, the decrease of cytochrome oxidase activity was not observed in the livers of the patients who died in the state of the metabolic acidosis due to other causes; pyruvate dehydrogenase complex deficiency and Leigh's encephalomyelopathy (Willems et al. 1977). Thus, the reduction of the cytochrome oxidase activity, as same as the disturbance of glycine cleavage system, would represent a dysfunction characteristic of the liver of the patients with propionic or methylmalonic acidemia, although the mechanism of the observed reduction remains unsolved.

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