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DECREASE OF PALMITOYL-CoA ELONGATION IN PLATELETS AND LEUKOCYTES IN THE PATIENT OF HEREDITARY METHEMOGLOBINEMIA ASSOCIATED WITH MENTAL RETARDATION

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SUMMARY: Effect of the deficiency of NADH-cytochrome b_5 reductase on fatty acid elongation was studied in the platelets and leukocytes taken from a patient of hereditary methemoglonemia associated with mental retardation. The activity of fatty acid elongation was determined by measuring the incorporation of [2-^{14}C]malonyl-CoA into palmitoyl-CoA. The de novo biosynthesis of fatty acids was blocked by the addition of phosphotrans-acetylase, and the elongation system could be assayed in the homogenates separated from de novo biosynthesis. As compared to normal subjects approximately 40% decrease of fatty acid elongation was observed both in the platelets and leukocytes from the patient.

Hereditary methemoglobinemia (1) has been classified into two types; type I, in which cyanosis is the single clinical symptom and NADH-Cytochrome b_5 reductase (EC 1.6.2.2) deficiency is restricted to erythrocyte, and type II, in which cyanosis is associated with mental retardation, and the enzyme defect is not restricted to erythrocytes but is found in plateletes (2),

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leukocytes, (2,3) muscle, liver and cultured fibroblasts (3). The recent report (4) has indicated Type III deficiency in which the enzyme defect is shown to be in erythrocytes and leukocytes, but no neurological disorders are observed. The primary structures of the enzyme from human erythrocytes (5) and steer liver (6) were determined. It was also shown that there is no structural difference in the catalytic fragments of liver and erythrocyte enzyme of bovine (7) or human (8).

NADH-dependent microsomal electron transport system has been reported to be involved in microsomal fatty acid desaturation (9) and chain elongation in liver (10). Participation of NADH-cytochrome b_5 reductase in microsomal fatty acid chain elongation has also been demonstrated in brain (11,12). These facts prompted us to determine whether the defect in the reductase affect the fatty acid elongation in blood cells. In the present study we found the activity of palmitoyl-CoA elongation decreased in platelets and leukocytes from the patient with hereditary methemoglobinemia associated with mental retardation.

MATERIALS AND METHODS

Palmitoyl-CoA and malonyl-CoA were purchased from Sigma. NADH was from Boehringer. [2-^{14}C] Malonyl-CoA was from New England Nuclear. Normal blood samples were obtained from healthy adult humans and the blood sample of the patient with hereditary methemoglobinemia associated with mental retardation, described elsewhere (2,13), was obtained at the Yokohama Keiyuh Hospital. Platelets and leukocytes were prepared according to the previously described method (13). The enzyme preparation of each cell was prepared by repeated freezing and thawing, and homogenization as reported previously (2).

Enzyme preparations were assayed for their ability to incorporate [2-^{14}C] malonyl-CoA into palmicoyl-CoA. The standard assay mixture contained 100 mM of potassium phosphate buffer, pH 7.2, 50μM of NADH, 20μM of palmitoyl-CoA 0.2μg of phosphotransacetylase(EC 2.3.1.8), 50 μM of [2-^{14}C] malonyl-CoA (specific radioacivity 2Ci/mol), and 1-1.2mg protein of cell homogenate in a total volume of 0.5ml. The reaction was started by adding cell homogenate, incubated at 37^{o}C for 10 min, and was terminated with 0.5ml of 15% KOH/methanol. The methylation, identification of the products and determination of radioactivity were performed by the previously reported method (12). Protein was determined by the method of Lowry as modified by Bensadoun and Weinstein (14).

RESULTS

Phosphotransacetylase has been reported to depress the de novo fatty acid biosynthesis (15). As shown in Table I, the addition of phosphotransacetylase to the reaction mixture, incorporation of [2-^{14}C]malonyl-CoA into 16:0 decreased to a negligible rate by impeding de novo fatty acid biosynthesis, and uptake of radioactivity into 18:0 was slightly increased both in platelets and leukocytes. When leukocytes were separated into lymphocytes and granulocytes, the latter showed slightly higher specific activity (data not shown). Because of the paucity of specimens with the patient's leukocytes, further separation was not performed.

The elongation of 16:0-CoA was linear within at least 10 min. The rate of reaction per protein was higher in leukocytes than platelets (Fig.1). As shown in Fig.2, the reactions were slightly off from linearity with enzyme concentration at low amount of homogenates both in platelets and leukocytes, for which no satisfactory explanation can yet be given.

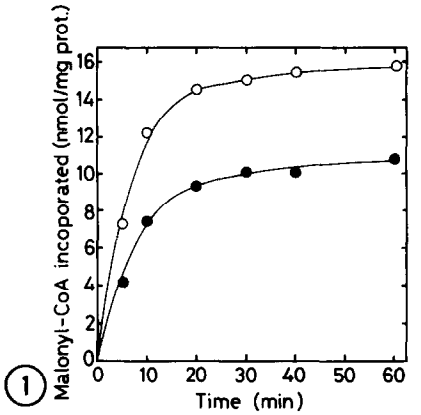
High concentration of palmitoyl-CoA resulted in a strong inhibition of elongation in these cells (Fig.3A). The apparent

Table I

Effect of phosphotransacetylase on fatty acid chain elongation by the human platelets and leukocytes

Human blood		Fatty acid formed (nmol/hr/mg prot.) 16:0	18:0
Platelet	-PTA[a]	5.3	6.2
	+PTA	N.D.[b]	7.2
Leukocyte	-PTA	4.8	11.5
	+PTA	N.D.	13.1

[a]PTA : phosphotransacetylase
[b]N.D.: not detected



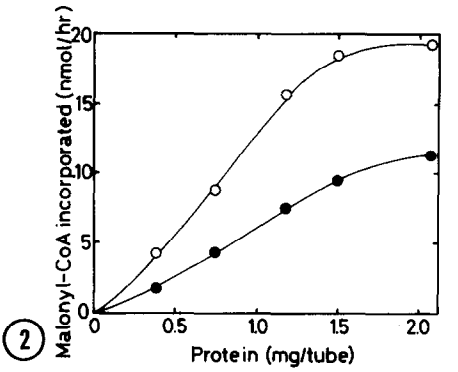


Fig. 1. Effect of duration of incubation on malonyl-CoA incorporation into palmitoyl-CoA by homogenates of human leukocytes and platelets. ○—○ , leukocytes; ●—●, platelets.

Fig. 2. Effect of varying the amount of homogenates of human platelets and leukocytes on the rate of malonyl-CoA incorporation into palmitoyl-CoA. ○—○ , leukocytes; ●—● , platelets.

Km values for palmitoyl-CoA were 1.1×10^{-4} M and 1.5×10^{-4} M, respectively with platelets and leukocytes. High concentration of malonyl-CoA also showed inhibition, but the degree of inhibition was relatively small both in plateletes and leukocytes (Fig. 3B). The apparent Km values for malonyl-CoA were 2.5×10^{-5} M with platelets and 2.8×10^{-5} M with leukocytes.

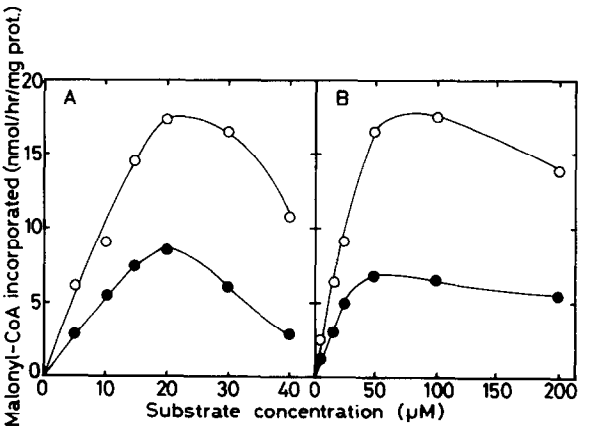


Fig. 3. Effect of substrate concentration on the rate of malonyl-CoA incorporation into palmitoyl-CoA by homogenates of human platelets and leukocytes. (A) Palmitoyl-CoA concentration; (B) Malonyl-CoA concentration: ○—○ , leukocytes; ●—● , platelets.

Table II

Comparison of palmitoyl-CoA elongation between blood corpuscles and effect of reduced nicotinamide nucleotides

	Incorporation of [2-^{14}C]malonyl-CoA into 16:0-CoA[a] (nmol/hr/mg protein)	
	NADH	NADPH[b]
Platelet	6.33 ± 0.95	5.54 ± 0.69
Leukocyte	9.72 ± 1.53	11.08 ± 1.39

[a]Mean ± SD (n=4)
[b]Assay conditions were as described in MATERIALS AND METHODS except 50 μM of NADPH were used in place of NADH.

There was an absolute requirement for the reduced nicotinamide nucleotides in palmitoyl-CoA elongation. Essentially no preference for either NADH or NADPH was observed both in platelets and leukocytes (Table II). As expected from the time course of the elongation reaction (Fig.1), leukocytes showed about twice the activity of platelets either with NADH or NADPH. Only the reseults with NADH were shown in Fig 1.

Palmitoyl-CoA elongation in platelets and leukocytes was determined in a patient of hereditary methemoglobinemia associated with mental retardation and the activity was compared with that of normal subjects. As shown in Table III, 38 and 39% decrease in the chain elongation activity was observed respectively in platelets and leukocytes of the patient.

DISCUSSION

In the present study, homogenates of platelets and leukocytes were used to allow the simple determination procedure for the small amount of specimens, and the suppression of de novo fatty acid synthesis by the addition of phosphotransacetylase enable us to determine the palmytoyl-CoA elongation, in which the NADH-cytochrome b_5 reductase system was involved. High

Table III

Palmitoyl-CoA elongation in platelets and leukocytes from a patient of hereditary methemoglobinemia with mental retardation

	Incorporation of [2-^{14}C]malonyl-CoA into 16:0-CoA		
	Control[a]	Patient[b]	
	(nmol/hr/mg protein)		(% decrease)
Platelets	6.3 ± 0.85	3.9	38
Leucocytes	13.3 ± 0.98	8.1	39

[a]Control values are mean ± SD (n=6)
[b]Patient values are mean of duplicate experiments

concentration of substrates showed inhibition in our experiments. The high concentration inhibition of fatty acyl-CoA was also observed in brain (16), and this was thought to be the detergent effect of the substrate. The slight inhibition of high malonyl-CoA concentration is not explained at present. The palmitoyl-CoA elongation system of plateletes and leukocytes did not exibit any preference for either NADH or NADPH. The same preference for NADH and NADPH have also been reported with rat brain microsomes (11,17).

The mechanism of microsomal fatty acid chain elongation has been shown to be the following 4 steps; (i) condensation, (ii) first reduction, (iii) hydration and (iv) second reduction. Cytochrome b_5 and NADH-cytochrome b_5 reductase have been reported to be involved in the reduction step of elongation (ii and/or iv) in liver (10) and brain (12). Condensation step (i) has been thought to be the rate-limiting step of elongation in liver (18) and brain (19), however, severe defect in NADH-cytochrome b_5 reductase might affect on the overall reaction rate of fatty acid elongation, in the blood corpscules. Thus the decrease in the activity of palmitoyl-CoA elongation in leukocytes and plateletes

of the patient may be the result of deficiency in NADH-cytochrome b_5 reductase.

Regulation of fatty acid chain elongation is important in connection with myelination, especially with the development of brain (11,19). On the other hand, decrease in the activity of fatty acid desaturation has been reported during brain development (20). The metabolic pattern might be different in the blood cells. Therefore it is of interest to test whether fatty acid desaturation is affected in blood cells of the methemoglobinemic patient.

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