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DEFICIENCY OF CARNITINE PALMITOYLTRANSFERASE IN TRANSFORMED LYMPHOBLASTS FROM A PATIENT HAVING A DEFICIENCY OF CARNITINE PALMITOYLTRANSFERASE IN SKELETAL MUSCLE

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SUMMARY

Peripheral blood lymphocytes from a patient with a partial deficiency of carnitine palmitoyltransferase in skeletal muscle were isolated, transformed with Epstein-Barr virus and maintained in tissue culture. The patient's lymphoblasts have a partial deficiency of carnitine palmitoyltransferase activity representing 69 percent of that of control lymphoblasts. In mitochondria isolated from lymphoblast homogenates, kinetic studies reveal reduced activity in the patient's mitochondria which is particularly marked between 100-250 μM palmitoyl-CoA, representing 32 to 48 percent of the activity of controls. Lymphoblast mitochondria from the patient's father, who is clinically unaffected, have carnitine palmitoyltransferase activity which is intermediate between that of five normal subjects and the patient, suggesting that partial deficiency of carnitine palmitoyltransferase is an inherited metabolic disease.

INTRODUCTION

In 1975, Bank et al reported studies of two brothers who suffered from severe muscle cramps and myoglobinuria following exercise, particularly when the exercise followed fasting. Carnitine palmitoyltransferase activity was found to be nearly completely absent in their skeletal muscle biopsies (1). Since this publication, at least 10 additional patients have been reported having a nearly complete deficiency of carnitine palmitoyltransferase in skeletal muscle (2-6).

In contrast, we studied a young male having similar symptoms of recurrent muscle pain and myoglobinuria following exercise and found a partial deficiency of carnitine palmitoyltransferase in his skeletal muscle representing 46 percent of the activity in control muscle (7). Further studies indicate this to be due to a lack of carnitine palmitoyltransferase A (I) (8,9). Recently, Scholte and co-workers in Rotterdam reported a case with some clinical similarities but con-

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cluded that the partial deficiency of carnitine palmitoyltransferase in muscle and peripheral blood leukocytes was due to a lack of carnitine palmitoyltransferase B (II) (10). Layzer and co-workers have also reported a case with a partial deficiency of carnitine palmitoyltransferase in muscle, leukocytes, platelets and cultured fibroblasts (11,12).

Biochemical and genetic investigation of these patients and their relatives has been hampered by the lack of a readily available tissue having expression of the carnitine palmitoyltransferase deficiency. We recently established lymphoblast cell lines from our patient and his father by transformation of peripheral blood lymphocytes with Epstein-Barr virus (EBV). This paper reports the results of our studies of carnitine palmitoyltransferase activity in lymphoblasts from the patient, his unaffected parent and from control subjects.

MATERIALS AND METHODS

Peripheral blood lymphocytes were isolated from the patient, R.F. and his father, D.F., and transformed with Epstein-Barr virus as described by Sly et al (13). EBV-transformed lymphoblasts from five normal subjects were provided by Dr. J.E. Seegmiller of the University of California, San Diego. Lymphoblasts were grown in RPMI-1640 medium supplemented with 20 percent fetal calf serum and 1 percent L-glutamine. Cells were harvested by centrifugation and washed three times at 4° with phosphate-buffered saline. The cells were suspended in 0.25 M sucrose containing 5 mM Tris-HCl (pH 7.4) and disrupted with 12-15 strokes of a loose-fitting Dounce homogenizer. Mitochondrial fractions were obtained from the respective homogenates as follows: nuclei and cell debris were removed by centrifugation at 600 x g for 10 min and the resulting post-nuclear supernatant was centrifuged at 8,600 x g for 10 min. The pellet, representing the mitochondrial fraction, was resuspended with 0.25 M sucrose containing 5 mM Tris-HCl, pH 7.4. Protein was determined by the method of Lowry et al (14) and the respective fractions were stored frozen at -60° until use.

Carnitine palmitoyltransferase (CPT-forward) was measured by a modification of the method described by Bremer (15) and Hoppel and Tomec (16). The incubation mixture contained 50 mM MOPS buffer, pH 7.0; 80 mM KCl; 4 mM dithiothreitol; 1 mM bovine serum albumin; 0.6 mM palmitoyl-CoA (P-L Chemicals, Milwaukee, WI) and 10 mM L-carnitine (Sigma, St. Louis, MO). [^{14}C-Me] D,L-carnitine (Amersham Inc., Arlington Heights, IL) was added to give a specific activity of [^{14}C]-L-carnitine of 0.24 mCi/mmol. The reaction was started by the addition of protein at a final concentration of 0.3 mg/ml. The volume was of the incubation mixture 0.200 ml. Duplicate samples were incubated at 37° for 20 min. Instead of assaying the n-butyl alcohol extract of the reaction mixture, 0.100 ml aliquots were pipetted directly onto 2.3 cm filter discs (3MM, Whatman Ltd., England) which had been pretreated with 10% trichloroacetic acid as described by Goldfine (17). The filter discs were washed four times with trichloroacetic acid solutions at 4° as previously described (18). The radioactive product, palmitoyl-L-carnitine, which remains on the filter paper, was analyzed by liquid scintillation counting (18). Determinations were carried out in duplicate and the results were calculated after correction for boiled protein blanks. The formation of palmitoyl-L-carnitine was linear to at least 20 min and to at least 0.3 mg mitochondrial protein/ml.

The radioactive product was further characterized by extracting duplicate filter discs with three 1.0 ml portions of chloroform/methanol, 2:1 by volume 100 μg of unlabeled palmitoyl-L-carnitine (P-L Biochemicals, Inc., Wilwaukee, WI) was added and the extract was evaporated to dryness with a nitrogen stream, taken up in a small volume of chloroform/methanol, 2/1, and applied to the origin of an 0.25 mm silica gel G plate (EM Reagents, Elmsford, NY). The 20 x 20 cm thin layer plate was developed to the top with chloroform/methanol/0.1M sodium acetate (4/4/1, by volume). Palmitoylcarnitine was visualized by staining with iodine vapors and the radioactive product was located by scanning for radioactivity with a Panax thin layer chromatography scanner (Panax Instruments, Ltd., Redhill, Surrey, England). Both the unlabeled carrier palmitoylcarnitine and the single radioactive peak had essentially identical Rf values, 0.41 versus 0.43, establishing the radioactive product as palmitoylcarnitine.

RESULTS AND DISCUSSION

As shown in Table, I, carnitine palmitoyltransferase activity in the homogenate of the patient's EBV-transformed lymphoblasts was 220 nmol mg^{-1} hr^{-1} compared with 319 ± 21 nmol mg^{-1} hr^{-1} in lymphoblasts from 5 normal subjects. The residual activity in the patient's lymphoblasts represents 69 percent of the activity found in five control lymphoblast homogenates. This is slightly greater than that reported earlier in homogenates of the patient's skeletal muscle using a different assay where the residual activity of the patient's muscle was 45 percent of that found in muscle from 6 control subjects (7).

A mitochondrial fraction was isolated from control and mutant lymphoblasts by differential centrifugation of the respective homogenates as described in the Methods. The respective mitochondrial fractions were incubated with saturating

Table I. Carnitine Palmitoyltransferase Activity in Skeletal Muscle and EBV-transformed Lymphoblasts from a Patient with a Recurrent Post-exercise Muscle Pain and Myoglobinuria.

Subject	Carnitine Palmitoyltransferase Activity	
	EBV-transformed Lymphoblast Homogenate*	Skeletal muscle Homogenate+
Controls	319 ± 21 (5)	2.71 ± 0.88 (6)
Patient, R.F.	220	1.23

+ - Results from Hostetler et al (7); μmol L-carnitine released/gm wet weight/min ± 1 std. deviation, (CPT-reverse).

* - nmol palmitoyl-L-carnitine formed/mg protein/hr ± 1 std. deviation, (CPT-forward).

amounts of [^{14}C]-L-carnitine and increasing amounts of palmitoyl-CoA and the rate of formation of radioactive palmitoyl-L-carnitine was determined. The results are shown in Figure 1. Mitochondria from control lymphoblasts exhibit a bimodal substrate-velocity plot as previously described in mitochondria from heart and liver (19). Mitochondria from the patient's lymphoblasts exhibit a reduced rate of palmitoyl-L-carnitine formation particularly at palmitoyl-CoA concentrations greater than 100 μM. The residual activity of carnitine palmitoyltransferase in the patient's mitochondrial fraction represents about 40 percent of the activity in the controls. The differences are statistically significant at each palmitoyl-CoA concentration. The rate of palmitoyl-L-carnitine formation by the control lymphoblast mitochondria at saturating concentrations of palmitoyl-CoA is less than that previously reported in heart and liver mitochondria (19).

We recently isolated and transformed lymphocytes from the patient's father who is clinically unaffected. A mitochondrial fraction was isolated and the rate of palmitoyl-L-carnitine formation was determined at several concentrations of palmitoyl-CoA. Rates of carnitine palmitoyltransferase activity in the father's lymphoblast mitochondria were intermediate between that of 5 controls and the patient. At 75 μM palmitoyl-CoA, formation of palmitoyl-L-carnitine by control

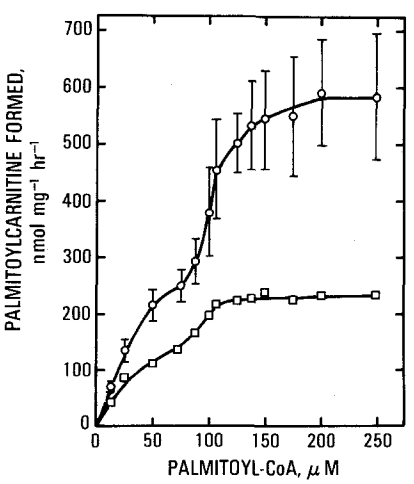

Figure 1. Palmitoyl-CoA dependence of mitochondrial carnitine palmitoyltransferase activity. Mean ± 1 std. deviation. Circles, controls (n = 5); squares, the patient.

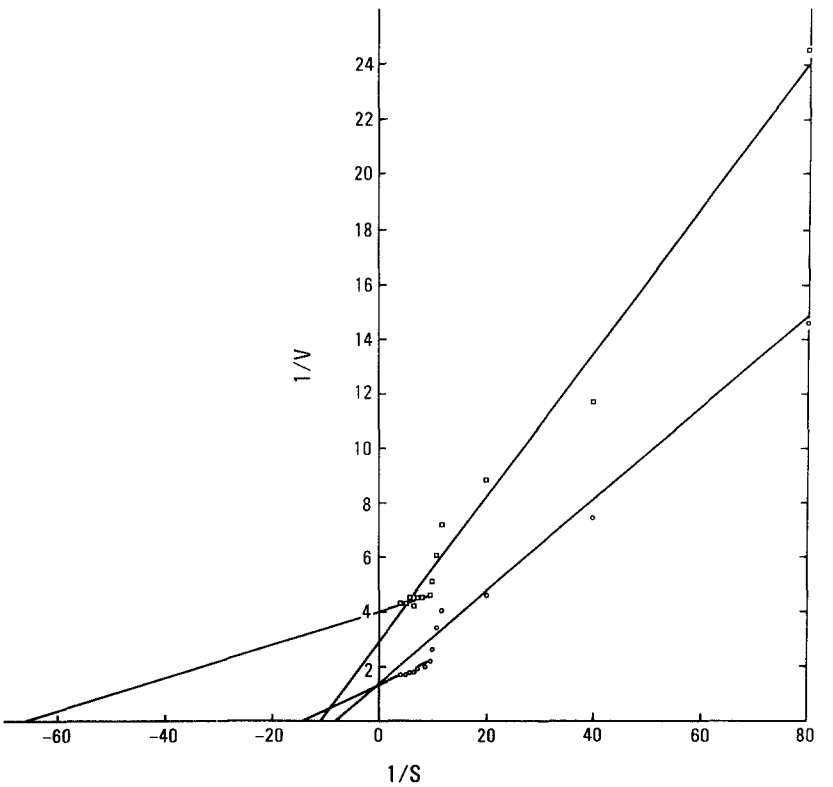

Figure 2. Double reciprocal plots of the palmitoyl-CoA dependence of carnitine palmitoyltransferase activity in lymphoblast mitochondria. Circles, controls; squares, the patient.

mitochondria was 4.2 ± 0.5 nmol mg^{-1} hr^{-1} versus 3.1 in the father ($p < 0.05$) and 2.3 in the patient ($p < 0.005$). At 200 μM palmitoyl-CoA, the following rates of palmitoyl-L-carnitine formation were obtained: controls, 9.9 ± 1.6; the father, 7.0 ($p < 0.01$) and the patient, 3.9 nmol mg^{-1} hr^{-1} ($p < 0.001$). These data strongly suggest that partial deficiency of carnitine palmitoyltransferase is an inherited metabolic disease. However, in order to provide a complete answer, studies of this type must be extended to EBV-transformed lymphoblasts from the patient's mother, three siblings and daughter, all of whom are clinically unaffected.

The data in Figure 1 were plotted as double reciprocals and the results are shown in Figure 2. The data points resolved into two lines reflecting the two portions of the curve shown in Figure 1. The two components represent palmitoyl-CoA concentrations of 12.5 to 100 μM (low) and 105-250 μM (high). The X and Y

Table II. Carnitine Palmitoyltransferase: Determination of Apparent Km for Palmitoyl-CoA and Vmax in Mitochondrial Fractions from Control Lymphoblasts and from the Patient's Lymphoblasts.

	Apparent Km (μM)		Vmax (nmol mg^{-1} hr^{-1})		R Value	
	Palmitoyl-CoA, μM		Palmitoyl-CoA, μM		Palmitoyl-CoA, μM	
	12.5-100	105-250	12.5-100	105-250	12.5-100	105-250
Controls (5)	120	71	733	790	0.99	0.94
Patient, R.F.	92	15	350	250	0.99	0.73

intercepts, representing the apparent Km and Vmax, were determined for the two portions of the curve. The position of the regression lines was determined by the method of least squares and the R values are shown in Table 2. The apparent Km for palmitoyl-CoA of the patient's lymphoblast mitochondria was similar to that of controls at low concentrations of palmitoyl-CoA, 92 μM versus 120 μM, respectively. However, at high concentrations, the apparent Km for palmitoyl-CoA of the patient's mitochondrial fraction was greatly reduced versus that of the five controls, 15 μM versus 71 μM. At low and high concentrations of palmitoyl-CoA, the Vmax for controls was 733 and 790 nmoles mg^{-1} hr^{-1} versus 350 and 250 nmol mg^{-1} hr^{-1} for the patient.

These experiments demonstrate that the partial deficiency of CPT previously demonstrated in the patient's skeletal muscle (7) is also present in his cultured EBV-transformed lymphoblasts; kinetic analysis of the dependency on palmitoyl-CoA of lymphoblast mitochondrial palmitoyl-L-carnitine synthesis appears to identify CPT-deficient lymphoblast lines with a high statistical degree of accuracy. Since EBV-transformed lymphoblast are permanent cell lines and grow to high cell densities, it is apparent that detailed biochemical studies can be made using lymphoblast mitochondria from CPT-deficient patients. This will also provide a method to study normal family members since it is difficult to justify muscle biopsy of unaffected relatives for scientific purposes alone.

Skin fibroblasts have recently been demonstrated to be deficient in carnitine palmitoyltransferase in patients with muscle deficiency of the enzyme (4,12). Thus,

fibroblasts also offer an alternative way to study this disease. However, the usefulness of fibroblasts would appear to be limited by their slow growth rates and tendency to become senescent. EBV-transformed lymphoblast cell lines appear to be superior in these respects.

In conclusion, these studies show a partial deficiency of carnitine palmitoyltransferase in EBV-transformed lymphoblasts from a patient with a partial deficiency of this enzyme in skeletal muscle. Intermediate levels of carnitine palmitoyltransferase activity in a lymphoblast mitochondrial fraction from the patient's unaffected father strongly suggest that this is an inherited metabolic disease. These studies do not indicate whether the decrease in carnitine palmitoyltransferase activity is due to a generalized decrease in both components of the carnitine palmitoyltransferase system (A and B, or I and II) or whether a specific decrease in one of these components is present. However, other approaches may be taken to elucidate this problem using EBV-transformed lymphoblasts.

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REFERENCES

1. Bank, W.J., Dimauro, S., Bonilla, E., Capuzzi, D.M. and Rowland, L.P. (1975) New Eng. J. Med., 292, 443-449.
2. Cumming, W.J.K., Hardy, M., Hudgson, P. and Walls, J. (1976) J. Neurol. Sci. 30, 247-258.
3. Herman, J. and Nadler, H.L. (1977) J. Ped., 91, 247-250.
4. Di Donato, S., Cornelio, F., Pacini, L., Peluchetti, D., Rimoldi, M. and Spreafico, S. (1978) Ann. Neurol., 4, 465-467.
5. Reza, M.J., Kar, N.C., Pearson, C.M. and Kark, R.A.P. (1978) Ann. Int. Med., 88, 610-615.
6. Patten, B.M., Wood, J.M., Harati, Y. and Howell, R.R. (1979) Am. J. Med., 67, 167-171.
7. Hostetler, K.Y., Hoppel, C.L., Romine, J.S., Sipe, J.C., Gross, S. and Higginbottom, P. (1978) New Eng. J. Med., 298, 553-557.
8. Hostetler, K.Y., Hoppel, C.L., Romine, J.S., Sipe, J.C., Gross, S. and Higginbottom, P. (1977) Clin. Res., 25, 125A.
9. Hostetler, K.Y., Hoppel, C.L., Romine, J.S., Sipe, J.C., Gross, S. and Higginbottom, P. (1977) Clin. Res., 25, 496A.
10. Scholte, H.R., Jennekens, F.G.I. and Bouvy, J.J.B.J. (1979) J. Neurol. Sci., 40, 39-51.
11. Layzer, R.B., Havel, R.J., Becker, N. and McIlroy, M.B. (1977) Neurol., 27, 379-380.

12. Layzer, R.B., Havel, R.J. and McIlroy, M.B. (1980) Neurol., in press.
13. Sly, W.S., Sekhon, G.S., Kennet, R., Bodmer, W.F. and Bodmer, J. (1976) Tiss. Antigens, 7, 165-172.
14. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem., 193, 265-275.
15. Bremer, J. (1963) J. Biol. Chem., 238, 2774-2779.
16. Hoppel, C.L. and Tomec, R.J. (1972) J. Biol. Chem., 247, 832-841.
17. Goldfine, H. (1966) J. Lipid Res., 7, 146-149.
18. Hostetler, K.Y. and van den Bosch, H. (1972) Biochim. Biophys. Acta, 260, 380-386.
19. Tomec, R.J. and Hoppel, C.L. (1975) Arch. Biochem. Biophys., 170, 716-723.