EVIDENCE FOR THE ENZYMIC DEFECT IN β-METHYLCROTONYLGLYCINURIA

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1. Introduction

Three inborn errors of the leucine degradation pathway are known; maple syrup urine disease, isovaleric acidaemia and β-methylcrotonylglycinuria. Two children have been described who excreted large amounts of β-methylcrotonylglycine and β-hydroxyisovaleric acid in their urine [1, 2]. By analogy with the pathways of leucine degradation established in Mycobacterium spp. and Achromobacter spp. [3] and in chicken liver [4], it has been suggested that the defective enzyme in these children is β-methylcrotonyl CoA carboxylase [1]. The product of β-methylcrotonyl CoA carboxylation is β-methylglutaconyl CoA, which is then converted to β-hydroxy-β-methylglutaryl CoA. Assays for β-methylcrotonyl CoA carboxylase have depended on the measurement of either acetoacetate or acetyl CoA formed by the cleavage of the β-hydroxy-β-methyl glutaryl CoA produced [3, 4]. We have now established a $[^{14}C]CO_2$ fixation assay sufficiently sensitive to measure the carboxylation of β-methylcrotonyl CoA in human leucocyte preparations and have demonstrated a low activity of this enzyme in the case of β-methylcrotonylglycinuria we have previously described [2].

2. Materials and methods

Leucocytes were prepared from 20 ml venous blood [5] and were stored at −20° until assay. The leucocyte pellets were thawed and resuspended in 0.8–1.2 ml of distilled water and then broken further by freezing and thawing three times. Protein was measured by the Lowry method. β-Methylcrotonyl CoA was synthesised from β-methylcrotonic acid by the mixed anhydride method [6] and was measured using a hydroxamate assay [7].

2.1. *Assay of β-methylcrotonyl CoA carboxylase*

The reaction mixture contained 0.4 μmoles β-methylcrotonyl CoA, 1 μmole $KHCO_3$, 20 μCi $[^{14}C]NaHCO_3$, 1.33 μmoles ATP, 1.5 μmoles $MgCl_2$, 50 μmoles KCl, 1.25 μmoles mercaptoethanol, 33 μmoles Tris pH 8.0 and leucocyte protein up to 1.5 mg. The final volume of the reaction mixture was 0.5 ml. Incubations were performed in duplicate for 20 min at 37°. The reaction was stopped with 50 μl of 5 N perchloric acid at 4°. The incubation tubes were centrifuged at 1700 *g* for 15 min to precipitate protein; this also removed most of the unfixed $[^{14}C]CO_2$. 50 μl fractions of the supernatant were spotted on to 3 cm circles of Whatman 3 MM paper in triplicate and the paper hung in a fume cupboard overnight for the residual $[^{14}C]CO_2$ to exchange with atmospheric CO_2. The paper discs were cut out and counted in 15 ml of 0.6% butyl-PBD scintillator in toluene in a liquid scintillation counter.

The β-methylcrotonyl CoA-dependent fixation of $[^{14}C]CO_2$ showed linear kinetics up to 1.5 mg of leucocyte protein.

3. Results and discussion

A series of leucocyte preparations were made from 14 normal adults and assayed to establish the mean and the standard deviation for both the method and a normal adult population. The practical and ethical difficulties of obtaining 20 ml of venous blood from children only 2 years of age made it impossible to establish a normal range for a series of children the

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Table 1
β-Methylcrotonyl CoA-dependent fixation of $[^{14}C]CO_2$†.

Normal adults (14)	Age-matched control, 2 yr	Patient with β-methyl-crotonyl-glycinuria
Mean = 4686		
SD* = ± 1936	7568	913
n = 22		

† cpm/mg leucocyte protein/20 min.

* S.D. of a single estimation; obtained from the sum of between and within person components of variance.

the same age as the patient. We were able to obtain blood from one age-matched control. The results are presented above.

The result from the patient was almost two standard deviations below the adult mean; and was only 12% of the activity from the control child. The result from the age-matched control was above the adult mean but was within the adult range.

This patient was first diagnosed at 5 months of age and was then excreting vast amounts of β-methylcrotonylglycine and β-hydroxyisovaleric acid [2]. He was treated with large doses of biotin and these metabolites disappeared completely from his urine. He was discharged on a low maintenance dose of biotin which was reduced by his parents. At the time the leucocytes were collected for the enzyme assay, he was beginning to excrete β-hydroxyisovaleric acid and β-methylcrotonylglycine again. The low rate of β-methylcrotonyl CoA-dependent fixation of $[^{14}C]CO_2$ found in his leucocytes suggests that the excretion of these metabolites is associated with a defective β-methylcrotonyl CoA carboxylase activity. This is evidence that the pathway of leucine degradation in *man* is similar to that described in other species and appears to establish *in vitro* the enzymic basis of this inborn error of metabolism.

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