omental oxidase (H.O. production). Nevertheless, mitochondrial oxidation of sebacyl-carnitine could be recorded. This activity was 10–20% of that measured in the same mitochondrial preparation with palmitoyl-CoA (t-carnitine) or decanoyl-carnitine. Further, there was no indication of interference by sebacyl-CoA with carnitine palmitoyltransferase.

In conclusion, intravenously infused dodecanedioic acid is readily catabolized in control rats in which less than 30% of the infused dose is recovered in urine as shorter dicarboxylic acids. The administration of clofibrate, a drug which, among other effects, stimulates both mitochondrial and peroxisomal β-oxidation in rats, strongly decreases the excretion of dicarboxylates in urine.

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Introduction

During the last decade, several new inborn errors of mitochondrial and peroxisomal fatty acid oxidation have been described. These inborn errors of metabolism are likely to be much more common than has been recognized until now, and they may present in a variety of different ways. This review will be confined to the inborn errors of mitochondrial β-oxidation, and it will report the recent progress that has been made in the study of these disorders. Progress in understanding disorders of peroxisomal β-oxidation has been described elsewhere (Fahimi & Sies, 1987).

Investigations of defects of fatty acid oxidation

The most important initial investigations to be performed in patients with suspected defects of fatty acid oxidation are the determination of the circulating blood concentrations of intermediay metabolites and of urinary organic acids. Hypoglycaemias, when associated with low blood ketone body concentrations, normal or high non-esterified fatty acid concentrations, strongly suggests that a defect of fatty acid oxidation is likely to be present. A defect of mitochondrial β-oxidation is often associated with the excretion of abnormal metabolites in the urine, and these metabolites can be detected by analysing the urine by gas chromatography, gas chromatography–mass spectrometry and high performance liquid chromatography. Thus, in the presence of a defect of mitochondrial fatty acid oxidation, long-chain fatty acids may be directed to α-oxidation which is followed by peroxisomal β-oxidation to form dicarboxylic acids that are subsequently excreted (Van Hoof et al., 1988). Moreover, the accumulation of acyl-CoA esters in the mitochondrial matrix is associated with the increased urinary excretion of acylcarnitines and glycine conjugates owing to the action of carnitine acyltransferases and glycine-N-acylase, respectively. These metabolites can be of substantial diagnostic value, thus for example, the presence of medium-chain dicarboxylic acids, suberylglycine and octanoylcarnitine, are very suggestive of medium-chain acyl-CoA dehydrogenase deficiency (Bhuiyan et al., 1987). However, the urinary excretion of abnormal metabolites can be extremely variable, and some patients under non-stressed conditions may not excrete any abnormal metabolites. It is imperative, therefore, to obtain appropriate blood and urine samples when the patient has a spontaneous episode of illness. If this is not possible, then samples should be collected during metabolic stress induced by carefully controlled fasting in hospital. The starvation test is potentially very dangerous, particularly in children, and it should only be performed by individuals experienced in metabolic investigations. The next step in the investigation is to measure the flux through fatty acid oxidation either radiochemically or spectrophotometrically using cultured fibroblasts or tissues from the patient. If these investigations are abnormal, direct assay of the individual enzymes of β-oxidation is required.

Enzyme defects

There are 15 enzymes involved in the β-oxidation of saturated fatty acids and defects of seven of these have been described to date [see below]. No defects have yet been reported in patients of carnitine/acylcarnitine translocase, the enoyl-CoA hydratases, the hydroxyacyl-CoA dehydrogenases, the long-chain 3-oxoacyl-CoA thiolase or of the auxiliary enzymes required for the oxidation of polyunsaturated fatty acids. Carnitine deficiency has been discussed recently (Turnbull & Sherratt, 1985). The syndromes in which enzyme deficiencies have been found will now be discussed.
Carnitine palmitoyltransferase deficiency. Acyl-CoA esters cannot cross the inner mitochondrial membrane directly and are transported into the mitochondrial matrix by the concerted action of the enzymes carnitine palmitoyltransferase (CPT) I, carnitine/acylcarnitine translocase and CPT II. There is controversy as to the location of CPT I and II in the mitochondria and to whether CPT I and CPT II are the same enzyme protein (Bieber & Farrell, 1983; Murthy & Pandé, 1987; Ramsay et al., 1987).

Several patients with deficiencies of CPT have been described, although the conditions under which the enzyme has been assayed have been unsatisfactory in many cases (for discussion see Zierz & Engel, 1985; Sherratt et al., 1988). Few attempts have been made to determine whether CPT I and/or CPT II have been involved in these defects. The majority of patients present with exercise-induced muscle pain (DiMauro et al., 1980); the CPT deficiency being limited to skeletal muscle. However, one family has been described in which severe hypoglycaemia in infancy occurred (Bougnères et al., 1981). One person in this family was investigated and found to have no detectable CPT activity in a liver extract. It is not known whether there are different tissue isoforms of the enzyme which account for the different tissue specificities observed.

Zierz & Engel (1985) have made an attempt to elucidate the nature of the enzyme defect by studying seven patients with exercise-induced muscle pain and proven CPT deficiency. The results suggested that the CPT deficiency in these seven cases was caused by the altered kinetic properties of a mutant enzyme.

Acyl-CoA dehydrogenase deficiency. There has been considerable recent interest in defects of the acyl-CoA dehydrogenases particularly because of the possibility that some of the defects may be important causes of the cot death syndrome (Howat et al., 1985). There are probably three straight-chain acyl-CoA dehydrogenases in human tissues (short-chain, medium-chain and long-chain) (Finocchiaro et al., 1987; I. M. Shepherd & D. M. Turnbull, unpublished work). The acyl-CoA dehydrogenases are located in the mitochondrial matrix, all are homotetramers and each subunit contains 1 mol of FAD per mol. The acyl-CoA dehydrogenases exhibit overlapping substrate specificities and the degree of overlap appears to vary considerably from species to species (Davidson & Schütz, 1982; Ikeda et al., 1985; Finocchiaro et al., 1987).

The definitive diagnosis of defects of acyl-CoA dehydrogenases depends on the assay of the enzyme activity in human tissues. Short-chain, medium-chain and long-chain acyl-CoA dehydrogenase activities are measured using n-butyryl-CoA (C4), octanoyl-CoA (C8) and palmitoyl-CoA (C16) respectively as substrates. An assay is satisfactory for mitochondrial fractions from human tissues (Turnbull et al., 1984), but an electron transfer flavoprotein (ETF)-linked assay, although technically demanding, is both more specific and sensitive (Freeman & Goodman, 1985a).

This latter assay has also been used with cell homogenates. Immunoprecipitation experiments using human tissue fractions (cultured fibroblasts, liver mitochondria and skeletal muscle mitochondria) have suggested that medium-chain acyl-CoA dehydrogenase is responsible for 50% of the activity involved in the dehydrogenation of butyryl-CoA (Hale et al., 1986; Amendt et al., 1987; I. M. Shepherd & D. M. Turnbull, unpublished work). These experiments used an ETF-linked assay (Freeman & Goodman, 1985a), but determination of acyl-CoA dehydrogenase activity using a dye-reduction assay with phenazine ethosulphate as an intermediate electron acceptor reveals different substrate specificities for the short-chain and medium-chain acyl-CoA dehydrogenases (I. M. Shepherd & D. M. Turnbull, unpublished work). It is important to be aware of these problems when making a diagnosis of short-chain acyl-CoA dehydrogenase deficiency.

Four patients with a deficiency of short-chain acyl-CoA dehydrogenase have been described. Turnbull et al. (1984) described a 46-year-old woman with proximal muscle weakness and muscle pain. There was a low activity of dehydrogenation of short-chain acyl-CoA esters in skeletal muscle mitochondrial extracts prepared from this patient. Short-chain acyl-CoA dehydrogenase activity in cultured skin fibroblasts from this patient was normal and there was a large ketotic response to fasting, indicating that the liver enzyme was also normal. Coates et al. (1986) described an infant who presented with failure to thrive, hypotonia, developmental delay, progressive muscle weakness and microcephaly. Short-chain acyl-CoA dehydrogenase activity was low in fibroblasts from this patient. Amendt et al. (1987) have recently described two children with short-chain acyl-CoA dehydrogenase deficiency. The first child presented with a severe metabolic acidosis. Following recovery from this initial episode she has grown and developed normally. The second child died on the sixth day of life with hypotonia, coma and a metabolic acidosis. The activity of short-chain acyl-CoA dehydrogenase was low in cultured skin fibroblasts from both of these patients, particularly after the medium-chain enzyme had been selectively immunoprecipitated.

Medium-chain acyl-CoA dehydrogenase deficiency presents in various ways including an acute fulminating illness (Duran et al., 1986), cot death (Howat et al., 1985), a Reye's syndrome-like picture (Taubman et al., 1987), unexplained hypoglycaemia (Coates et al., 1985) and systemic carnitine deficiency (Zierz et al., 1986). Since the defect was first characterized, at least 30 patients with this disease have been confirmed (Finocchiaro et al., 1987).

Investigations are now in progress in many laboratories to try to determine the molecular basis of these deficiencies, particularly the most frequently described medium-chain acyl-CoA dehydrogenase deficiency. We have studied the medium-chain acyl-CoA dehydrogenase in fibroblasts from 13 patients. While the residual activity ranged from 5 to 12% of the mean value of fibroblasts from normal controls, the variant medium-chain acyl-CoA dehydrogenase in the cells from these patients was indistinguishable from normal human medium-chain acyl-CoA dehydrogenase on the basis of molecular size. This suggests that in their patients the enzyme deficiency is probably due to a point mutation(s) in the medium-chain acyl-CoA dehydrogenase gene. Matsubara et al. (1986) have described the isolation of partial complementary DNAs (cDNA) encoding rat and human medium-chain acyl-CoA dehydrogenase. Using the cloned human cDNA as a probe, they assigned the gene for medium-chain acyl-CoA dehydrogenase to the short arm of human chromosome 1, band p31. In the future, cDNA cloning of medium-chain acyl-CoA dehydrogenase will provide a valuable tool for the molecular study of medium-chain acyl-CoA dehydrogenase and its genetic deficiency in man.

Long-chain acyl-CoA dehydrogenase deficiency has only been reported in a few cases. Hale et al. (1985) described three children who presented in early childhood with hypoglycaemia, hepatomegaly, cardiomyopathy and muscle weakness. Naylor et al. (1980) reported two siblings with intermittent hypoglycaemia, lethargy and coma, who were subsequently shown to have long-chain acyl-CoA dehydrogenase deficiency.

3-Oxoacyl-CoA thiolases. There are four 3-oxoacyl-CoA thiolases in mammalian cells which have different subcellular locations and substrate specificities (Middleton, 1973; Krühling & Tolbert, 1981). The cytoplasmic enzyme is involved in cholesterogenesis, is specific for acetoacetyl-CoA and is not activated by K⁺. The peroxisomal enzyme and one of the mitochondrial enzymes have a broad substrate speci-
ficity. The other mitochondrial thiolase is activated by K’ and is specific for acetoacetyl-CoA and methylacetoacetyl-CoA. The mitochondrial thiolase of broad substrate specificity is responsible for thiolysis of 3-oxoacyl-CoA esters generated during β-oxidation and no defects of this enzyme have been described. The other mitochondrial enzyme is primarily involved in ketone body metabolism and isocapnic degradation. Patients with a defect in this enzyme present with a metabolic acidosis and hyperglycaemia (Leonard et al. 1987). In the majority of patients the defect is associated with the urinary excretion of metabolites derived from isocapnic (2-methylacetacetate, 2-methyl-3-hydroxybutyrate and tiglylglycine) in addition to 3-hydroxybutyrate. The diagnosis is confirmed by measuring 3-oxoacyl-CoA thiolase activity using acetoacetyl-CoA as substrate in the presence and absence of K’. This latter group and 2-methylacetacetate-CoA as substrate (Middleton & Bartlett, 1983). It has been suggested that there is an hepatic and an extra-hepatic form of the enzyme, and that some of the variations in clinical presentation and urinary metabolite excretion are due to the involvement of the different isoenzymes (Leonard et al., 1987).

Glutaric aciduria type II. The enzyme defect in this disorder involves either ETF or electron transfer flavoprotein ubiquinone oxidoreductase (ETF-QO). ETF and ETF-QO mediate electron transfer to the ubiquinone pool from eight FAD-linked dehydrogenases (Frisman, 1988). The degradation of amino acids is inhibited in this condition, in addition to impaired fatty acid oxidation. Glutaric aciduria type II usually presents in one of three ways, each presentation being consistent within a family (Goodman & Frerman, 1984). Thus, there is a group of patients who have severe neonatal symptoms of hypoglycaemia and acidosis associated with congenital abnormalities. In the second group, the onset is also in the neonatal period, but there are no congenital abnormalities. The third group comprises patients who present later in life. However, the age of onset, the initial symptoms, the clinical findings and the tissues involved vary considerably in this latter group.

Frerman & Goodman (1985b) showed that cultured skin fibroblasts from three unrelated glutaric aciduria type II patients had no detectable ETF-QO catalytic activity and no immunologically detectable ETF-QO. Fibroblasts from the parents of two of these patients had ETF-QO activity intermediate between the control fibroblasts and the patient’s fibroblasts, indicating that the mode of inheritance was autosomal recessive. Fibroblasts from two other patients with severe glutaric aciduria type II had normal ETF-QO activities and antigen, but were severely deficient in immunoreactive ETF (Frerman & Goodman, 1985a,b). Ikek da et al. (1986) studied the biosynthesis of ETF in cultured skin fibroblasts from eight patients, and found a defect of the α-subunit of ETF in three of the cell lines. The defect in the α-subunit manifested itself in three different ways, suggesting that these defects result from three different variant alleles.

Conclusion

Defects of mitochondrial β-oxidation are a very important group of inborn errors of metabolism and significant advances in this field have been made during the last few years. It is likely that defects involving the other enzymes of β-oxidation will be described. However, the investigation of these patients requires a combination of clinical and biochemical skills which can be provided only in a few centres.

The treatment of defects of fatty acid oxidation includes the careful monitoring of patients, especially children, for the development of hypoglycaemia, and it is essential that the risks, prevention and management of hypoglycaemia are explained to the family. Children should not be starved and must be admitted for careful monitoring if they become unwell, particularly with a febrile illness with vomiting. A low fat diet with regular carbohydrate rich meals should help prevent hypoglycaemia. The administration of pharmacological doses of riboflavin has proved to be effective in some patients with glutaric aciduria type II (de Visser et al., 1986). Since the biochemical defect is expressed in amniocytes, the prenatal diagnosis of acyl-CoA dehydrogenase deficiency is possible (Bennett et al., 1987). Recent elucidation of the biochemical defect in glutaric aciduria type II suggests that prenatal diagnosis may also be possible in this disorder.

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