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Threonine Dehydratase Deficiency: a Probable Cause of Non-ketotic Hyperglycinaemia

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A patient with classical symptoms of non-ketotic hyperglycinaemia (NKH) is presented. Threonine dehydratase was undetectable in a liver autopsy specimen, which was obtained within 1 h of death and immediately frozen at −70 °C. Activities of four marker enzymes were normal. This represents the first documentation of an inborn error of threonine metabolism and a new explanation of NKH.

Recently we made an observation in a patient with NKH (McKusick 23830) which suggested that a disorder of threonine metabolism is the cause of this disease. Since the manifestations of NKH have been attributed to high glycine concentrations in the central nervous system, (CNS), and since threonine is a precursor of glycine in CNS (Maher and Wurtman, 1980), we treated two infants with a threonine-free formula for 1 week (Krieger and Nigro, 1983). In order to provide minimum threonine requirements homogenized milk was added, but not until the seventh day. One of the two patients deteriorated suddenly on the seventh day, with clinical manifestations similar to those observed during the first week of life. Surprisingly, CFS glycine had not increased, but there was a 16 and 19 fold elevation of threonine in CSF and plasma, respectively. We hypothesized that the unbalanced diet caused excessive protein catabolism and that the endogenous threonine load was not metabolized because of an enzymatic block. A disorder of threonine metabolism was also suggested by the finding of slightly elevated threonine and serine concentrations in plasma and CSF on four of nine tests, performed prior to the dietary treatment.

Of the three known enzymes with activity toward threonine, one was previously measured in NKH and found to be normal (Tada *et al.*, 1969, 1974). It is serine transhydroxymethylase (STHM) (EC 2.1.2.1), which is active toward serine and threonine, also called threonine aldolase (EC 4.1.2.5) (Schirch and Gross, 1968). The other two enzymes are threonine dehydrogenase (EC 1.1.1.d) and threonine dehydratase (TD) (EC 4.2.1.16). We hypothesized (Figure 1) that deficiency of either one of these two enzymes could increase the metabolic load on STHM and that the resultant competition between threonine and serine for STHM might inhibit serine formation from glycine, causing hyperglycinaemia. The observed deficiency in glycine cleavage, which is rarely complete, thus could be a secondary phenomenon.

The original patient was no longer available for study. We therefore measured TD activity in the autopsy specimen of another patient, who is briefly presented. TD was measured because it appears to be the enzyme most responsive to increased metabolic threonine loads (White *et al.*, 1978) and its deficiency would thus have a more profound effect than deficiency of the dehydrogenase.

MATERIALS AND METHODS

The liver specimen was obtained within 1 h of death and stored at −70 °C for 4 months until the assays were performed. Three control specimens were stored for 1–7 days and obtained within 2–4 h of death. Two biopsy specimens were stored for 6 years. The control patients were four cases with congenital heart disease and one with lymphoangiomatous disease, ages 6 months to 4 years. The assay of TD was performed according to the method of Nishimura and Greenberg (1961), as modified by Yeung and Yeung (1972). The reaction product was measured by a method described by Goldstein *et al.* (1962). The following marker enzymes were measured: ornithine transcarbamylase (Prescott and Jones, 1969), (kindly performed by Dr Lynn Fleischer, Detroit), convertase (Mehler *et al.*, 1958), lactate dehydrogenase (Schwartz and Bodansky, 1960), and glutamate dehydrogenase (Fahien and Cohen, 1970), (kindly performed by Dr R. A. Mitchell, Detroit).

CASE DESCRIPTION

The patient had an uneventful delivery and early neonatal course, but paucity of movements and lack of interest in food gradually became apparent. At 6 months of age, he was investigated at the Winnipeg Children's Hospital because of failure to suck, progressive hypotonia and lethargy, causing inadequate ventilation, for which he required ventilatory assistance. Myoclonic jerks and seizure activity, consisting of increased extensor tone, were observed. An electroencephalogram showed burst suppression. There was no history of vomiting. Routine laboratory investigations were negative; notably, there was no evidence of ketoacidosis, either by history or laboratory tests. However, a urine organic acid screen was not done. Amino acid analysis by

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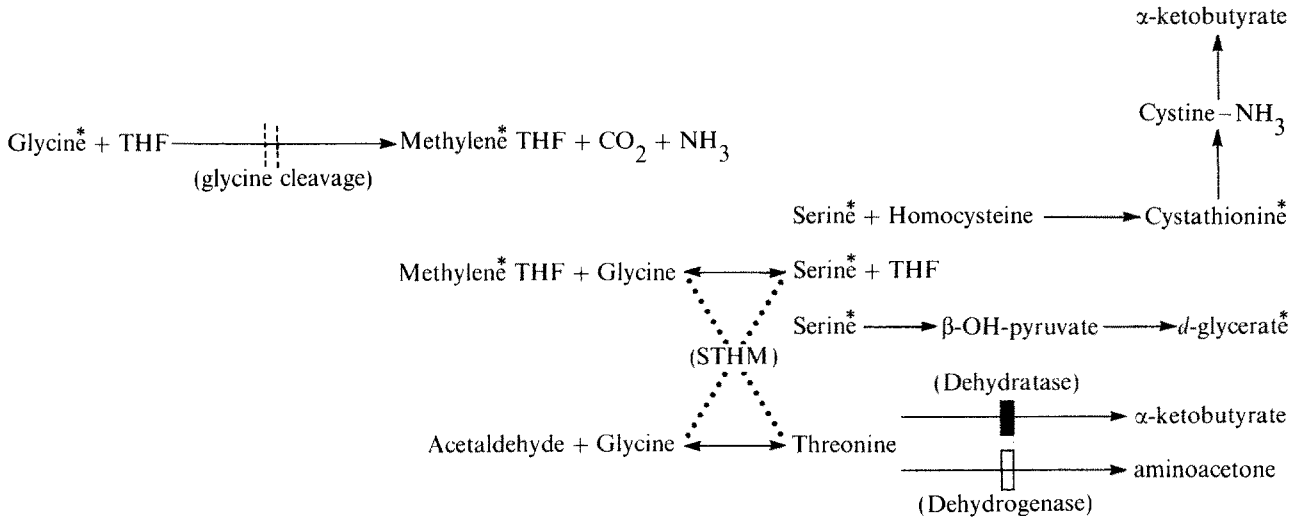

Figure 1 Non-ketotic hyperglycinaemia; proposed block and enzyme inhibition. The observed deficiency of threonine dehydratase (solid bar) or a deficiency of threonine dehydrogenase (open bar) could explain the partial deficiency of glycine cleavage (double dashed line), which has been reported in the literature, because threonine competes with glycine for serine transhydroxymethylase (dotted line). This hypothesis is compatible with observation in a previously reported case with NKH (Kølvraa *et al.*, 1980), where labelled glycine was administered and the label (asterisk) was observed in *d*-glycerate, with trace amounts in serine and cystathionine

column chromatography revealed elevation of plasma and cerebrospinal fluid glycine concentrations, 1920 and 370 μmol/l, respectively (normal 220 and 6.6 μmol/l). A diagnosis of NKH was made and he was placed on sodium benzoate and Clonazepam; strychnine was given in varying doses ranging from 0.5 to 1.5 mg kg^{-1} day^{-1}, depending on clinical condition. Within 1 week some improvement was noted, evidenced by increased muscle tone and spontaneous movements. Stopping treatment at 9 months of age was followed by increased seizure activity and failure to suck. Treatment was reinstituted, but lethargy increased 1 month later despite therapy. There was little spontaneous movement and the patient did not focus or follow. He was then placed on valium, folic acid, choline and sodium benzoate. One month later, at age 11 months, therapy was gradually discontinued, except for gavage feeding. His condition remained essentially unchanged. He withdrew to painful stimuli, but was otherwise unresponsive, with intermittent seizures consisting of flinging of the arms, eye rolling and stiffening. Sudden cardiorespiratory arrest occurred at 12½ months of age following a lumbar puncture which had not presented technical difficulties. Autopsy revealed petechial haemorrhages of the thyroid and pleura, and diffuse spongy changes of cerebral white matter.

RESULTS

Activity of TD was not detectable in the liver specimen of the patient; control activity was 52–107 μmol h^{-1} g^{-1} tissue in the three autopsy specimens and 42–156 μmol h^{-1} g^{-1} in the biopsy specimen. Lactate dehydrogenase was 57% and glutamate dehydrogenase 83% of controls. Convertase activity was normal. Ornithine transcarbamylase was 3974 μmol h^{-1} g^{-1} tissue (normal: 3346 ± 782). The effect of handling was measured in rat liver. In two specimens, stored for 6 months at −70 °C, TD activity was 112 and 113 μmol h^{-1} g^{-1} tissue, compared to 105 and 115 μmol h^{-1} g^{-1} in two fresh specimens. Following storage at −70 °C for 6 years, activity was only 42 μmol h^{-1} g^{-1}. When a fresh specimen was exposed to room temperature for 2 and 4 h, activity decreased from 105 to 96 and 87 μmol h^{-1} g^{-1}, respectively. When the specimen that had been stored for 6 years at −70 °C was thawed and then exposed for 2 h at room temperature, activity decreased from 42 to 30 μmol h^{-1} g^{-1}.

DISCUSSION

A disorder of threonine metabolism has not been suspected before in NKH and only one poorly documented case of hyperthreoninaemia has ever been reported (Reddi, 1978). Since it is unlikely that mutations spare consistently one amino acid, we suggest that the rarity of hyperthreoninaemia is attributable to the availability of more than one metabolic pathway. Only an excessive metabolic load will, under these circumstances, reveal evidence of a metabolic defect. Whether the deficiency of threonine dehydratase, observed by us, is indeed the primary cause of NKH must now be proven on fresh liver biopsies, although the studies in rat liver indicate that our observation is not an artifact. Moreover, the finding of normal activity of ornithine transcarbamylase – known to be an unstable enzyme – also supports our belief that the patient's specimen was viable.

Variable deficiency of glycine cleavage is the only biochemical abnormality that has been recognized in NKH to date. Similar degrees of deficiency occur in ketotic hyperglycinaemias, including methylmalonic

and propionic acidaemia (Tada *et al.*, 1974; Ando *et al.*, 1972). These hyperglycinaemias are clinically and biochemically different than NKH. Deficiency of glycine cleavage has also been described in a patient with *d*-glyceric acidaemia (Kølvraa *et al.*, 1980) who had the clinical manifestations of NKH. Subsequently another patient with a typical clinical picture of NKH was described who also excreted large amounts of glyceric acid (Grandgeorge *et al.*, 1980). It is not certain whether deficiency of *d*-glycerate dehydrogenase or glycine cleavage was the primary defect in these cases.

Perry *et al.* (1977) demonstrated complete deficiency of glycine cleavage in frozen brain tissue of five patients dying with NKH. Only one patient with ketotic hyperglycinaemia was analysed and found to be normal, while a case with an unidentified form of hyperglycinaemia had partial deficiency. In view of the small number of cases and the use of autopsy specimens, it remains questionable whether involvement of brain by the enzyme defect indeed distinguishes NKH from the ketotic hyperglycinaemias. Partial deficiency of glycine cleavage in liver of cases with ketotic hyperglycinaemia is recognized as a secondary phenomenon. Deficiency of glycine cleavage thus cannot be used in the diagnosis of NKH. Elevation of CSF glycine and absence of ketoacidosis is currently the only distinctive feature of NKH. Prenatal diagnosis may be attempted by demonstrating glycine elevation in amniotic fluid (Garcia-Castro *et al.*, 1982), but the reliability of this approach remains to be proven. Demonstration of a specific enzyme defect in NKH thus may provide a more reliable method for prenatal diagnosis, if it can be shown that the enzyme is present in amniotic fibroblasts.

It is possible that the described deficiency of TD is secondary to inhibition by glycine or by an unknown intermediary metabolite. However, the reverse is a more likely explanation since the TD defect was complete, whereas substantial glycine cleavage activity remained in most of the reported cases. The reverse explanation is compatible with current concepts of intermediary threonine metabolism. Since the affinity of STHM is greatest for serine and lowest for threonine, competition between glycine and threonine can be anticipated when there is a block of threonine catabolism due to deficiency of either, threonine dehydratase or threonine dehydrogenase (Figure 1). This explanation is also supported by the occasional finding of slightly elevated levels of plasma serine and threonine in our original patient. Using labelled glycine, Kølvraa *et al.* (1980) showed that, aside from *d*-glyceric acid, trace amounts of the label appear in serine and cystathionine. This observation is compatible with our hypothesis. Appearance of the label in cystathionine explains the elevation of α-aminobutyric acid, which was observed at the time of hyperthreoninaemia in the original case, although it was not included in the report (Krieger and Nigro, 1983). This metabolite is derived from α-ketobutyric acid. Since α-ketobutyrate derives from both, serine as well as threonine, it is possible that threonine dehydrogenase was defective in this original case.

Our hypothesis also explains the finding of *d*-glycerate in the patient of Kølvraa *et al.* (1976) who showed clinical manifestations of NKH but had, in addition, glycerate dehydrogenase activity in the heterozygous range. This amount of enzyme function should not cause accumulation of *d*-glycerate unless there is an excessive load of serine, which is not readily converted to glycine. We hypothesize that this occurs in NKH because of the competition between serine, threonine and glycine for STHM. Mutations appear to be common in man, most of which are silent because of heterozygosity of recessive traits or because compensatory pathways exist. One may therefore anticipate that heterozygous mutations can co-exist with other inborn errors of metabolism. Partial deficiency of glycerate dehydrogenase, as observed by Kølvraa *et al.* (1976), would be biochemically 'silent' unless the load is excessive. Co-existence of this defect thus may have been a chance occurrence.

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Case Report

PINK NAPKINS – PRESENTING FEATURE IN A CASE OF ALKAPTONURIA

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Pink staining of an infant's napkin is commonly attributed to uric acid crystals in urine whilst other, rarer causes include haemoglobinuria, dyes from sweets, anthocyanins from beetroot or berries, drugs, porphyria and contamination with *Serratia marcescens* (Cone, 1968). We report a case of alkaptonuria (McKusick 20350) presenting with pink staining of the napkin.

E.E. is a healthy, well-developed boy, the result of a normal pregnancy; his sister is 3 years older and is well. E.E. was referred at age 8 weeks after his mother reported pink-stained napkins to the Health Visitor. Discolorations had been noticed from age 1 week (washing both napkins and baby's buttocks produced a deep brown colour). Vitamin supplement administration coincided with disappearance of staining, which returned on discontinuing vitamins. Maternal vitamin C administration yielded unstained napkins but these became pink again when breast-feeding ceased. Additional vitamin C in E.E.'s diet keeps his napkins discoloration-free.

Urine samples were generally clear, of normal pH, and did not exhibit suspicious darkening. They were positive for reducing substances, negative for glucose, giving a transient blue-green colour with ferric chloride. Following alkalinization, urine darkened rapidly from the meniscus downwards, becoming very dark brown within hours. Alkaptonuria was confirmed as follows.

Spectroscopic examination of urine diluted in 0.2 mol/l phosphate buffer pH 6.5 revealed the presence of large quantities of a substance with an absorption maximum at 290.2 nm: the absorption maximum for homogentisic acid is 290 nm at pH 6.8. Under similar conditions uric acid has a maximum at 291 nm. However, derivative spectra, namely, $\frac{d^2A}{d\lambda^2}$ (Fell, 1981), from dilutions of E.E.'s urine were quite different from those of control urines. Furthermore, the maximum at 290.2 nm rapidly disappeared on treating E.E.'s urine with horse-radish peroxidase and glutathione (La Du and Zannoni, 1963), whereas uricase effected little change. By contrast, control urines with absorption maxima around 293 nm behaved in the converse manner. Finally, gas–liquid chromatography of E.E.'s urinary organic acids yielded a very large peak which co-chromatographed with authentic homogentisic acid (Dr. D. Isherwood, Alder Hey Hospital, Liverpool).

Aqueous homogentisic acid at pH 6 turns pink in daylight. In 1929 Garrod observed red-coloured urine in alkaptonuric subjects. Thus it is to be expected that alkaptonuria may reveal itself by pink staining of napkins and not exclusively by the traditional brown discoloration.

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