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Tiglicaciduria in Propionicacidaemia

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Tiglic acid, which has not previously been found in human body fluids, was recently detected in the urine of two patients with propionicacidaemia. These patients had a documented defect in the oxidation of propionate. A competition between acrylyl-CoA and tiglyl-CoA for crotonase could explain the accumulation of tiglic acid.

Propionicacidaemia is an inborn error of organic acid metabolism in which there is a defect in propionyl-CoA carboxylase activity, and a clinical picture of overwhelming illness very early in life which was originally described as ketotic hyperglycinaemia (Childs *et al.*, 1961; Hommes *et al.*, 1968; Hsia *et al.*, 1970). These patients have large amounts of glycine in their blood and urine. They also have large amounts of propionic acid in body fluids (Ando *et al.*, 1971; Gompertz *et al.*, 1970). The excretion of ethyl methyl ketone (Menkes, 1966) is considered to result from the accumulation of propionyl-CoA (in experiments with intact fibroblasts we have demonstrated the conversion of [^{14}C]propionate into ethyl methyl ketone). The finding of tiglic acid (2-methylcrotonic acid) excretion in this condition provides additional definition of the abnormal internal biochemical environment in this disorder.

Materials and Methods

Analyses were performed on four patients with propionicacidaemia. Two patients were in complete clinical remission and were undergoing careful dietary control. C. E. was a classic case clinically and was in remission but not receiving dietary control (Ando *et al.*, 1971). A defect in propionyl-CoA carboxylase activity in this patient has been observed by assay of the enzyme from fibroblasts (T. Ando, J. D. Connor & W. L. Nyhan, unpublished work). J. B. was studied during an acute exacerbation without ketosis. Defective oxidation of [^{14}C]propionate was demonstrated *in vitro*, by using fibroblasts. Two patients with non-ketotic hyperglycinaemia and eight healthy subjects were chosen as controls. The concentrations of propionic acid in the plasma of C. E. and J. B. were 23.9 and 26.4μM, respectively. In the other patients they were 10.4 and 23.7μM. No patient excreted methylmalonic acid in a detectable amount.

Samples (5ml) of urine were acidified with 1ml of 15% (w/v) sulphosalicylic acid. The volatile fatty acids were distilled as previously described (Ando *et al.*, 1971) and separated on a Porapak QS 80–100-mesh column installed in a Hewlett–Packard model 402 gas chromatograph (Fig. 1). The detector-response–sample-size graphs for authentic acids were linear through zero over the range tested (0.5–10nmol). Recovery of tiglic acid added to urine specimens from a control individual ranged from 70 to 92% in duplicate analyses at 4–20μM concentration. In calculation of tiglic acid in urine, a value of 80% for recovery was used. Mass spectrometry was carried out in an LKB 9000 mass spectrometer with a gas-chromatographic inlet system equipped with a Porapak QS column.

Results

The chromatographic pattern obtained for C. E. is shown in Fig. 1. The largest peak of organic acid had a retention time of 1.36 relative to that of isovaleric acid. The other major peak was that of propionic acid. Neither compound was present in the urine of the control subject illustrated. The retention time of the unknown peak was identical with that of tiglic acid. Co-chromatography with authentic tiglic acid revealed a single peak. The mass spectrum of the compound was identical with that of authentic tiglic acid. There was a prominent molecular ion with a mass (*m/e*) of 100, which is the molecular weight of tiglic acid, and there was a peak at *m/e* 85, which is consistent with the loss of a methyl group.

The amounts of tiglic acid found in the urine of C. E. and J. B. are shown in Table 1. In each instance they exceeded the amounts of propionate in the urine by 2–3 times. The concentration of tiglic acid in the urine varied from 7 to 150μM. Excretion was at the rate of 4–20mg/g of creatinine. Tiglic acid was not detected in the urine of the other glycinaemic patients studied or of the control individuals. None of the

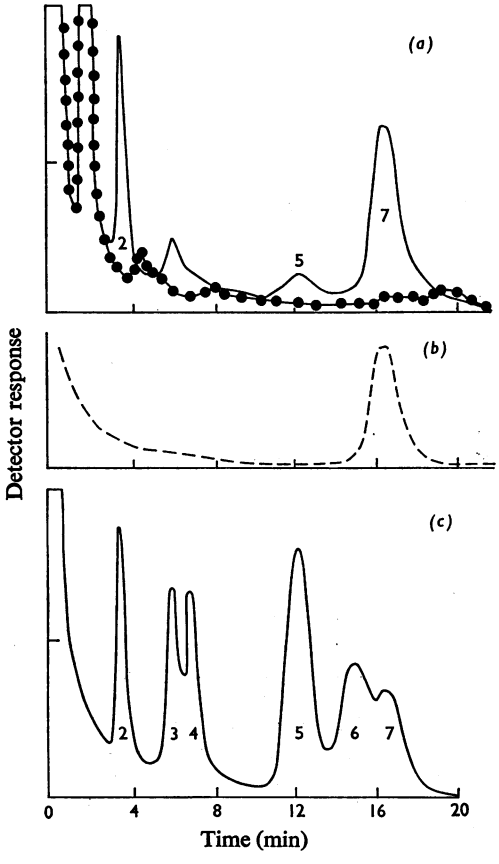

Fig. 1. *G.l.c. of the volatile acids of urine*

Chromatographic patterns of (*a*) acids from urine of two human subjects are shown. C. E. (——) was a patient with propionicacidaemia and ketotic hyperglycinaemia, and R. H. (●) was a control subject; (*b*) authentic tiglic acid; (*c*) a mixture of standards. For C. E. 0.08ml of urine equivalent (0.09mg of creatinine) was injected, and for R. H. 1.00ml of urine equivalent (0.15mg of creatinine). Column temperature was 200°C. Flow rate for carrier gas was 50ml of helium/min, and attenuation was 20×. The numbered peaks were (2) propionate, (3) isobutyrate, (4) butyrate, (5) α-methylbutyrate and isovalerate, (6) β-methylcrotonate and (7) tiglate.

subjects studied, whether patient or control, had detectable amounts of tiglic acid in the plasma.

Discussion

Tiglic acid is an intermediate of the metabolism of isoleucine. This amino acid is metabolized to 2-methylbutyryl-CoA by transamination and oxidative decarboxylation. Dehydrogenation of 2-methylbutyryl-CoA yields tiglyl-CoA. This compound is then normally hydrated to 3-hydroxy-2-methylbutyryl-CoA. After dehydrogenation to 2-methylacetoacetyl-CoA, the molecule is cleaved to form propionyl-CoA and acetyl-CoA.

Table 1. *Excretion of tiglic acid in urine*

Patient	Tiglic acid excreted (μM)	Tiglic acid excreted (mg/g of creatinine)
C. E.	150	14
J. B.		
(1)	43	20
(2)	7.4	4

The excretion of tiglic acid by patients with propionicacidaemia suggests the possibility of an abnormality in the metabolism of tiglyl-CoA, which is probably the source of urinary tiglic acid. Accumulation of tiglyl-CoA would result from an abnormality in the activity of crotonase (enoyl-CoA hydratase, EC 4.2.1.17), which catalyses the conversion into 3-hydroxy-2-methylbutyryl-CoA. This enzyme has a broad substrate specificity (Stern & del Campillo, 1956) and, therefore, a defect in its activity should be reflected in a number of metabolic pathways, e.g. accumulation of crotonyl-CoA, methacrylyl-CoA, and 3-methylcrotonyl-CoA would be expected together with tiglyl-CoA. Yet the acids corresponding to these CoA derivatives were not detected in the urine. However, methacrylyl-CoA may be hydrated non-enzymically (Stern & del Campillo, 1956), and 3-methylcrotonyl-CoA is also metabolized by a specific enzyme, 3-methylcrotonyl-CoA carboxylase (EC 6.4.1.4). Crotonyl-CoA, which is in the major pathway of fatty acid oxidation, may have a stronger affinity for the enzyme than tiglyl-CoA does. It is known that the substitution of a methyl group in the 2- or the 3-position of crotonyl-CoA results in a decreased reactivity of the thioester (Stern, 1961). Thus, the isolated excretion of tiglic acid is not incompatible with an abnormality in the activity of crotonase.

The mechanism of a defect in crotonase is not clear. It seems likely that the defect is secondary to a primary defect in propionyl-CoA carboxylase. This conclusion is reached because a complete block in propionate oxidation has been documented. The absence of tiglic acidaemia in the presence of propionicacidaemia probably supports the idea, although it could reflect very efficient renal excretion of tiglic acid.

The fact that acrylyl-CoA is a substrate for crotonase (Rendina & Coon, 1957) has led us to the

speculation that competition between acrylyl-CoA and tiglyl-CoA might be the mechanism of a decrease in tiglyl-CoA metabolism. Patients with propionicacidaemia metabolize intravenously injected [1-^{14}C]-propionate to 3-hydroxypropionate (T. Ando, K. Rasmussen & W. L. Nyhan, unpublished work). Metabolism of excessive quantities of propionyl-CoA via acrylyl-CoA to 3-hydroxypropionate might provide a pool of acrylyl-CoA to compete with tiglyl-CoA for hydration via crotonase and lead to accumulation of tiglyl-CoA.

A defect in two different enzymes could, of course, reflect a defect in the synthesis of a common cofactor, but this seems unlikely in the case of these two enzymes. Only two patients out of four with propionicacidaemia excreted tiglic acid. This could reflect genetic heterogeneity in this condition. In fact, we believe that heterogeneity is likely. However, the finding may be explained by the clinical condition of the patient. C. E. was receiving the least aggressive dietary therapy, and J. B. was acutely ill. The other two patients studied were in a condition of careful dietary control. It seems likely that excretion of tiglic acid indicates inadequate therapeutic control in this disease.

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