EXCRETION OF PROPYONYLGLYCINE IN PROPIONIC ACIDAEMIA


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SUMMARY

1. The urinary excretion of acylglycines has been studied in four patients with propionic acidaemia.
2. Propionylglycine was found in each of the patients.
3. This compound has not previously been detected in human body fluids. Propionylglycine was not present in the urine of control subjects or patients with non-ketotic hyperglycinaemia.
4. The excretion of propionylglycine was greatest in patients receiving the least dietary control.

Key words: propionylglycine, propionic acidaemia, acylglycines, ketotic hyperglycinaemia.

Patients with propionic acidaemia have a clinical syndrome of overwhelming illness early in life, originally described as ‘ketotic hyperglycinaemia’, and a defect in the enzyme propionyl-CoA carboxylase (Gompertz, Storrs, Bau, Peters & Hughes, 1970; Ando, Rasmussen, Nyhan, Donnell & Barnes, 1971b; Hsia, Scully & Rosenberg, 1970). The metabolic pathways involved are illustrated in Fig. 1. Large amounts of propionic acid have been found in the blood of patients studied during attacks of ketosis (Gompertz et al., 1970; Hommes, Kuipers, Elema, Jansen & Jonxis, 1968), but in patients studied in remission the values were only 10–20 times those of control subjects (Ando et al., 1971b). A similar pattern has been observed in isovaleric (3-methylbutanoic) acidaemia in which the concentrations of isovaleric acid in the plasma are not great during remission but rise dramatically during episodes of acute illness (Tanaka & Isselbacher, 1967; Tanaka, Budd, Efron & Isselbacher, 1966).

Large amounts of N-isovalerylglucose are excreted in the urine of patients with isovaleric acidaemia (Tanaka & Isselbacher, 1967; Ando, Klinberg, Ward, Rasmussen & Nyhan, 1971a).

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Conjugation with glycine has been thought to provide a mechanism for detoxification in isovaleric acidaemia. β-Methylcrotonylglycine (3-methylbut-2-enoylglycine) has been found in the urine of three patients with β-hydroxyisovaleric aciduria in whom a defect has been postulated in methylcrotonyl-CoA carboxylase (Eldjarn, Jellum, Stokke, Pande & Waaler, 1970; Gompertz, Draffan, Watts & Hull, 1971). Tiglylglycine (cis-2-methylbut-2-enoylglycine) was also found in the urine of the patient described by Gompertz et al. (1971).

To assess the generality of acylglycine formation after the accumulation of CoA derivatives of short-chain fatty acids, we set out to see if patients with propionic acidaemia excrete propionylglycine in the urine.

**MATERIALS AND METHODS**

Three of the patients with ketotic hyperglycinaemia have been reported (Ando et al., 1971b). V.B. was a 13-month-old boy, K.H. a 5-year-old girl, and C.E. a 4-5-year-old boy. J.B. was a 6-month-old boy with hyperglycinaemia who had multiple episodes of vomiting and dehydration from the first month of life. All of these patients had abnormally elevated concentrations of propionic acid in the plasma. The values ranged from 0.77 to 1.95 mg/100 ml, in contrast with a range of 0.05-0.22 mg/100 ml in a series of control subjects. Defective oxidation of propionate was documented in all four patients either in vivo or in cultured fibroblasts in vitro. They were all without ketosis at the time of study.

Urine specimens were also studied from two patients with non-ketotic hyperglycinaemia (Baumgartner, Ando & Nyhan, 1969) and from two control children. Urine specimens were collected on ice and stored frozen until analysed.

N-Propionyl-, N-butyryl-, N-isovaleryl- and N-valeryl-glycine were synthesized by the method of Bondi & Eissler (1910). N-Acetylglucose was purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. Trimethylsilylation was done using the reagents TRI-SIL and BSTFA [NO-bis(trimethylisilyl-trifluoroacetamide (Horning, Boucher, Moss & Horning, 1968)] obtained from Pierce Chemical Co., Rockford, Ill. TRI-SIL is a mixture of trimethylchlorosilane and hexamethyldisilazane in pyridine, based on that described by Sweeley, Bentley, Makita & Wells (1963).
Propionylglycine in propionic acidaemia

Combined gas chromatography–mass spectrometry

Aliquots of urine and of acylglycine reference standards in aqueous solution (1, 10 and 100 mg/100 ml) were acidified with HCl, saturated with NaCl, and extracted three times each with ethyl acetate and with diethyl ether. The extracts were combined, dried over anhydrous Na₂SO₄, and evaporated in a current of N₂ at room temperature. Methylation was done with diazomethane in methanol–diethyl ether, 1:10, by the small-scale method of Schlenk & Gellerman (1960). Aliquots equivalent to 10 µg of creatinine from the patients with propionic acidaemia and 200 µg from the others were injected into an LKB 9000 mass spectrometer with a gas chromatographic inlet system and chromatographed at 155° on a glass column (183 cm × 4 mm) packed with 3% OV-25 on Chromosorb W (HP) 80–100 mesh. Fig. 2(a) illustrates the separations obtained for reference compounds. The relationship between the detector response and the sample size of the methyl ester of authentic propionylglycine was linear through zero over the range tested (0.5–5 µg/5 µl injected). The recovery of propionylglycine through the extraction procedure increased from 60% at 1 mg/100 ml to 103% at 100 mg/100 ml. In calculation of the quantities of propionylglycine in urine, a value of 80% for recovery was used.

N-Trimethylsilyl derivatives of the methyl esters were prepared by dissolving the esters in NO-bis(trimethylsilyl)-trifluoroacetamide with 2% trimethylchlorosilane added to catalyse the reaction. The derivatized reference compounds were separated by temperature programming from 50° at 3°/min on a glass column (183 cm × 4 mm) packed with 3% OV-1 on Chromosorb W (HP) 80–100 mesh. Methylene unit values were obtained by co-chromatography of the samples and standard alkanes of C₈ to C₂₂ (Dalgliesh, Horning, Horning, Knox & Yarger, 1966).

Silicic acid chromatography–automatic organic acid analyser

Samples (5 ml) of urine that had been freeze-dried and reference compounds were chromatographed on an automatic organic acid analyser constructed as described by Kesner & Muntwyler (1966). The peaks were collected in fractions and the organic solvents were evaporated at 70°. The residues were dissolved in TRI-SIL, and an equal volume of NO-bis-(trimethylsilyl)-trifluoroacetamide was added after 5 min.

RESULTS

Propionylglycine was found in the urine of each of the patients with propionic acidaemia. The compound was identified as propionylglycine in the following way.

The retention time of the methylated compound on the OV-25 column was 0.53 relative to that of N-valerylglycine methyl ester, and the identical relative retention time was found for methylated propionylglycine (Fig. 2). The methylated compound from urine gave an identical mass spectrum to that of authentic propionylglycine methyl ester (Fig. 3). The retention time of the methyl ester-N-trimethylsilyl derivative of the urinary compound on the OV-1 column was 12.89 methylene units, and an identical retention time was obtained using derivatized propionylglycine. Silicic acid chromatography of the same urine specimens revealed an unknown peak in the area between lactic and succinic acids. Authentic propionylglycine was eluted in the same area. The mass spectrum of the trimethylsilyl derivative of this peak compound was identical with that of NO-bis(trimethylsilyl)propionylglycine.

The amounts of propionylglycine excreted are shown in Table 1. This compound was not B
Fig. 2. Gas–liquid chromatography of methylated extracts of urine and of authentic acylglycines on 3% OV-25 at 155°. (a) Standard compounds; (b) urine sample from patient J.B. with propionic acidaemia; (c) urine sample from a control child. Abbreviations: solvent (S), acetylglycine (A), propionylglycine (B), butyrylglycine (C), isovalerylglycine (D), valerylglycine (E) and tiglylglycine (T).

Fig. 3. Mass spectrum of the authentic propionylglycine methyl ester (a), and of unknown gas chromatographic peak (b).
detected in urine specimens from control subjects or patients with non-ketotic hyperglycinaemia. The limit of detection of the method is 0.05 mg/100 ml. The patients with propionic acidaemia had a minimum of 0.4 mg/100 ml and as much as 14.8 mg/100 ml.

**TABLE 1. Excretion of propionylglycine in the urine of patients with propionic acidaemia**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Plasma glycine (mg/100 ml)</th>
<th>Plasma propionate (mg/100 ml)</th>
<th>Urinary propionylglycine (mg/24 h)</th>
<th>(mg/mg of creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.B.</td>
<td>12.4</td>
<td>1.95</td>
<td>10.0*</td>
<td>9.7*</td>
</tr>
<tr>
<td>C.E.</td>
<td>9.1</td>
<td>1.77</td>
<td>7.3</td>
<td>—†</td>
</tr>
<tr>
<td>V.B.</td>
<td>2.0</td>
<td>1.75</td>
<td>0.43</td>
<td>1.8</td>
</tr>
<tr>
<td>K.H.</td>
<td>3.2</td>
<td>0.77</td>
<td>0.43</td>
<td>3.1</td>
</tr>
</tbody>
</table>

* Mean of specimens from five consecutive days (range 3.8–14.8 mg/100 ml; 6.3–14.7 mg/24 h; 0.18–0.80 mg/mg creatinine). The results given for C.E., V.B. and K.H. were based on single collections of urine.

† A complete 24 h collection was not obtained from this patient.

The amounts of propionylglycine excreted correlated with the clinical conditions of the patients. J.B., in whom the greatest amounts were found, was a young infant who died shortly after these specimens were obtained. C.E. was a boy in whom retardation was advanced at the time of diagnosis and close dietary control was not pursued, while in V.B. and K.H. very careful dietary management had been in effect for long periods of time. The amounts of propionylglycine in the urine also varied directly with the concentrations of glycine in the blood.

**DISCUSSION**

Propionyl-CoA is a product of the metabolism of isoleucine, valine, threonine and methionine. It is normally metabolized via the tricarboxylic acid cycle after CO₂ fixation to form methylmalonyl-CoA and isomerization to succinyl-CoA (Fig. 1). The activity of propionyl-CoA carboxylase is deficient in patients with propionic acidaemia and ketotic hyperglycinaemia (Hsia et al., 1970; Gompertz et al., 1970; T. Ando, J. D. Connor & W. L. Nyhan, unpublished observations). Although this metabolic block is virtually complete, relatively low concentrations of propionate are found in the blood, at least during remission. Thus, it is likely that there are alternative pathways for the metabolism of propionyl-CoA. The formation of propionylglycine could theoretically provide an alternative pathway and a mechanism for detoxification.

The excretion of isovalerylglycine in isovaleric acidaemia is an analogous finding. In this condition formation of the acylglucose is a major pathway amounting to 330–2800 mg of isovalerylglycine excreted/day (Tanaka & Isselbacher, 1967; Ando et al., 1971a). Similarly, about 100 mg of β-methylcrotonylglycine were found in the urine of methylcrotonyl-CoA carboxylase deficiency (Eldjarn et al., 1970). In contrast the amounts of propionylglycine excreted in the urine of patients with propionic acidaemia were small. It does not appear likely that the formation and excretion of propionylglycine is an important mechanism for the elimination of excessive propionyl-CoA.

These observations are consistent with the findings of Schachter & Taggart (1954) that
mammalian glycine N-acylase has increasing affinity as the carbon chain increases in a series of acetyl-CoA derivatives. For this reason one might expect not to produce acetylglycine which would be of value in avoiding a depletion of acetyl-CoA. To our knowledge studies have not been published on human glycine N-acylase using propionyl-CoA as the acyl donor.

The larger excretion of propionylglycine in two of the four patients might reflect higher amounts of propionyl-CoA in the tissues, although the plasma amounts of propionate were in the same range in all four patients (Table 1). Certainly it correlated with the clinical condition. It has been proposed (Gompertz, 1971) that the development of hyperglycinaemia in disorders in which the CoA derivatives of organic acids accumulate reflects an attempt by the body to stimulate an acylglycine method of detoxification. In this sense conditions in which acylglycine formation are efficient, like isovaleric acidemia and β-methylcrotonylglycinuria, would be expected not to have significant hyperglycinaemia, while conditions such as methylmalonic acidemia and propionic acidemia, in which acylglycine formation is not efficient, would be expected to have prominent elevation of glycine concentrations. This fits at least superficially with the facts. However, in this series of patients with propionic acidemia the amounts of propionylglycine varied directly with the degree of hyperglycinaemia, whereas the theory would appear to call for an inverse relationship. The presence or absence of acylation with glycine and its degree correlate well with the affinity of the purified enzyme which catalyses the conversion. It appears possible that a similar molecular understanding of the hyperglycinaemia may one day be elucidated.

Propionic acidemia may well be two or more conditions. Possible support for this suggestion may be the finding that two of these patients (J.B. and C.E.) excreted tiglic acid (cis-2-methylbut-2-enoic acid) in the urine (Nyhan, Ando, Rasmussen, Wadlington, Kilroy, Cottom & Hull, 1972). These patients, who excreted the higher quantities of propionylglycine, also excreted tiglylglycine in the urine (K. Rasmussen, T. Ando & W. L. Nyhan, unpublished observations). Their excretion of propionylglycine and tiglylglycine provides further evidence of the generality of the mechanism of acylglycine formation in the presence of accumulated CoA derivatives of organic acids.

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REFERENCES


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