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Excretion Pattern of Branched-Chain Amino Acid Metabolites During the Course of Acute Infections in a Patient with Methylmalonic Acidaemia

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A 1-year-old boy with a typical B_{12}-responsive form of methylmalonic acidaemia was hospitalized twice due to acute bacterial infections. On both occasions, the child was lethargic with a severe ketoacidosis on admission. Intensive therapy with protein restriction, intravenous administration of electrolytes and antibiotics was effective within 4 days on both occasions. The urinary excretion of organic acids showed the same pattern on both occasions. There were rising excretion concentrations, reaching a peak value within the first 24-hour period, for the following compounds: 3-hydroxybutyric acid, 3-hydroxypropionic acid, 3-hydroxyisobutyric acid and 3-hydroxyisovaleric acid. Excretion concentrations of the following rose for 48 h: isobutyric acid, 2-methylbutyric acid, isovaleric acid, lactic acid and the 2-oxo-acids.

There was no increase until 12–24 h after the onset of severe illness in the excretion of propionic acid and methylmalonic acid. Propionic acid excretion was maximal at about 48 h, while peak excretion of methylmalonic acid was delayed until about 72 h after the onset of severe illness; at this time there was clinical improvement. The biochemical implications of this excretion pattern are discussed.

Methylmalonic acidaemia is a well known inborn error of metabolism. It can be caused by two different defects in the metabolism of methylmalonyl-CoA, namely either an L-methylmalonyl-CoA mutase deficiency (McKusick 25100) or a D-methylmalonyl-CoA racemase deficiency (McKusick 25112) (Morrow, 1974).

Clinically this disorder is characterized by episodes of severe ketoacidosis, usually triggered by catabolic situations such as infections and operations, with clinically quiet periods in between.

It has been suggested (Tan *et al.*, 1975) that the mechanism behind these ketoacidotic attacks is primarily a rising methylmalonic acid concentration in the patients due to increased pressure on the deficient enzyme, followed by a secondary ketoacidosis due to the fact that methylmalonic acid inhibits 3-hydroxybutyrate dehydrogenase.

In order to investigate this hypothesis further, we have examined the excretion pattern of branched-chain amino acid metabolites, 3-hydroxybutyrate and lactate during ketoacidotic attacks in a patient with methylmalonic acidaemia. Our results, which show the time-sequence in the urinary excretion pattern, make the explanation mentioned above highly unlikely.

CASE REPORT

The patient, a boy, has a typical B_{12}-responsive methylmalonic acidaemia. In the clinically quiet periods, he is unaffected and develops normally. Biochemical investigations in these periods show a constantly increased urinary concentration of methylmalonic acid. Apart from this, no other pronounced accumulations are indicated. No hyperglycinaemia is present.

The child has had two registered attacks of ketoacidosis at the ages of 18 months and 22 months, both times provoked by bacterial infections. On both occasions, the attacks resulted in lethargy within a few hours. They were treated intensively with protein restriction and intravenous administration of electrolytes and antibiotics. This treatment normalized the condition within 4–5 days on both occasions.

Twenty-four-hour urine samples were collected from the start of the attacks. In addition, a number of 24-hour urine samples were collected in clinically quiet periods in order to give reference values for the various metabolites prior to the onset of the attacks.

METHODS

Methylmalonic acid, 3-hydroxybutyric acid, lactic acid and 3-hydroxyisovaleric acid were measured gas chromatographically on a Hewlett–Packard 5830A gas chromatograph after ethylacetate extraction and trimethylsilyl derivatization with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS). Separation was obtained on a Dexsil 300 column by programming the oven at 4 °C/min from 60 °C. 2-Hydroxycaproic acid was used as the internal standard. Propionic acid, isobutyric acid, 2-methylbutyric acid and isovaleric acid were measured after alkaline hydrolysis of the urine samples for 24 h. The acids were isolated by vacuum distillation and separated on a 'Porapak' column at 170 °C. The acids were identified and quantified by a selected ion monitoring system (SIM). The instrument used was an AEI MS 30 mass spectrometer equipped with a five-channel multiple ion detector and a Pye–Unicam gas chromatograph. SIM-ions: propionic acid, isobutyric acid and 2-methylbutyric acid: 73.0281; isovaleric acid: 73.0281

and 87.0417; 4-pentenoic acid (internal standard): 100.0511. 3-Hydroxypropionic acid and 3-hydroxyisobutyric acid were analysed after ethylacetate extraction and trimethylsilyl derivatization with BSTFA containing 1% TMCS at 60 °C for 30 min. Separation was performed on an OV-1 column by programming the oven from 100 °C at 10 °C/min. Detection was by SIM. SIM-ions: 3-hydroxypropionic acid: 177.0834; 3-hydroxyisobutyric acid: 177.0834; and 2-hydroxycaproic acid (internal standard): 261.1384. 2-Oxo-isovaleric acid, 2-oxo-3-methylvaleric acid and 2-oxo-isocaproic acid were analysed after hydroxylamine treatment under alkaline conditions for 30 min at 60 °C, followed by ethylacetate extraction and trimethylsilyl derivatization. Separation was performed on a Dexsil 300 column by programming the oven from 150 °C at 10 °C/min. Detection was done with SIM. SIM-ions: 2-oxo-isocaproic acid, 2-oxo-3-methylvaleric acid and 2-oxo-caproic acid (internal standard): 289.1529; 2-oxo-isovaleric acid: 275.1372.

Glycine was measured on a modified Contron amino acid analyser.

RESULTS

Figure 1 shows the urinary excretion of metabolites in the four 24-hour periods immediately following the onset of the first ketoacidotic attack (24–27 December 1976). The amounts are expressed relative to the maximal excretions found in that period. The first set of values represents the average values in clinically quiet periods, i.e. the presumed values on the day prior to the onset of the attack.

Figure 2 shows the corresponding values in the three 24-hour periods immediately following the onset of the second attack (4–5 April 1977).

The following facts can be extracted from the figures:

(1) Ketosis, represented by the large urinary excretion of 3-hydroxybutyric acid, was seen on the first day of the attack, whereas both lactic aciduria and methylmalonic aciduria were delayed by 1 or 2 days.

(2) The excretion pattern of the 3-hydroxyacids, i.e. 3-hydroxypropionic acid, 3-hydroxyisobutyric acid and 3-hydroxyisovaleric acid, parallels very closely that of 3-hydroxybutyric acid during both attacks.

(3) Taking into consideration that the urine collection intervals are located differently with respect to the onset of the attack for the two attacks, the peak excretion of total volatile acids (free and conjugated isobutyric, 2-methylbutyric and isovaleric acids) seems to be delayed compared with the excretion of 3-hydroxybutyric acid and the other 3-hydroxyacids.

(4) The peak excretion of the 2-oxo-acids, i.e. 2-oxo-3-methylvaleric acid, 2-oxo-isocaproic acid and 2-oxo-isovaleric acid, was also delayed in comparison to the 3-hydroxyacids.

(5) The propionic acid excretion parallels the excretion of the other short-chain monocarboxylic acids and was perhaps delayed compared with them. The

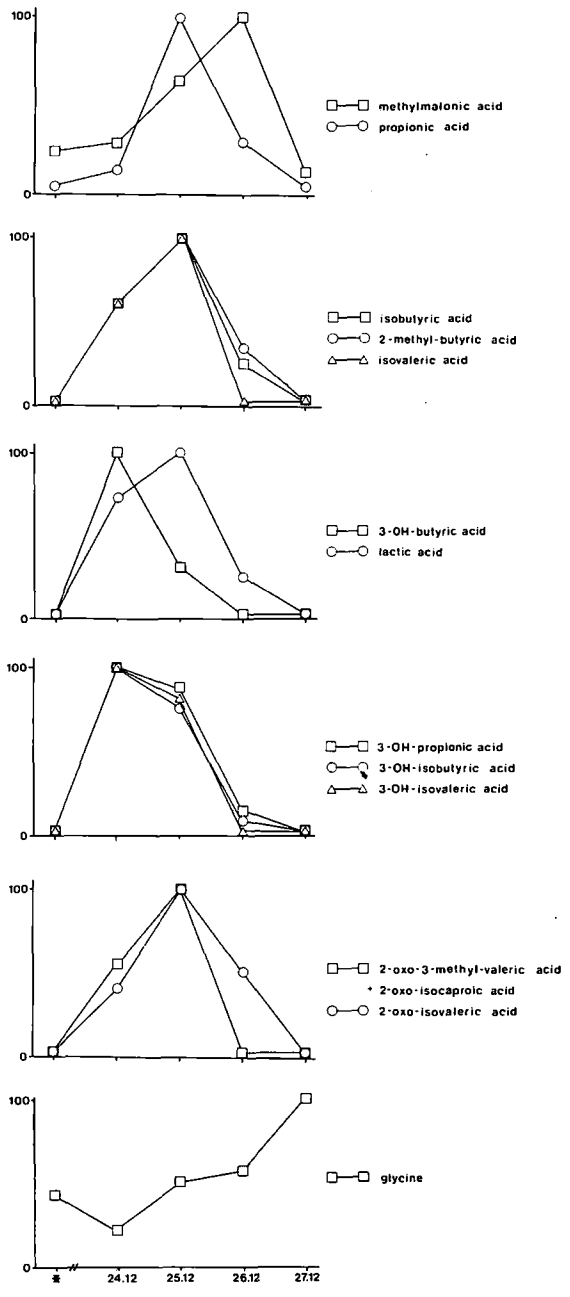

Figure 1 Time course of the excretion of metabolites in the period 24–27 December 1976. Values are expressed as percentages of the maximal values during this period. The asterisk indicates average values in clinically quiet periods. The maximal values are: methylmalonic acid: 13680 µg/mg creatinine; propionic acid: 769 µg/mg creatinine; isobutyric acid: 66 µg/mg creatinine; 2-methylbutyric acid: 45 µg/mg creatinine; isovaleric acid: 53 µg/mg creatinine; 3-hydroxybutyric acid: 6800 µg/mg creatinine; lactic acid: 1220 µg/mg creatinine; 3-hydroxypropionic acid: 726 µg/mg creatinine; 3-hydroxyisobutyric acid: 624 µg/mg creatinine; 3-hydroxyisovaleric acid: 1756 µg/mg creatinine; 2-oxo-3-methylvaleric acid + 2-oxo-isocaproic acid: 39 µg/mg creatinine; 2-oxo-isovaleric acid: 36 µg/mg creatinine; glycine: 787 µg/mg creatinine

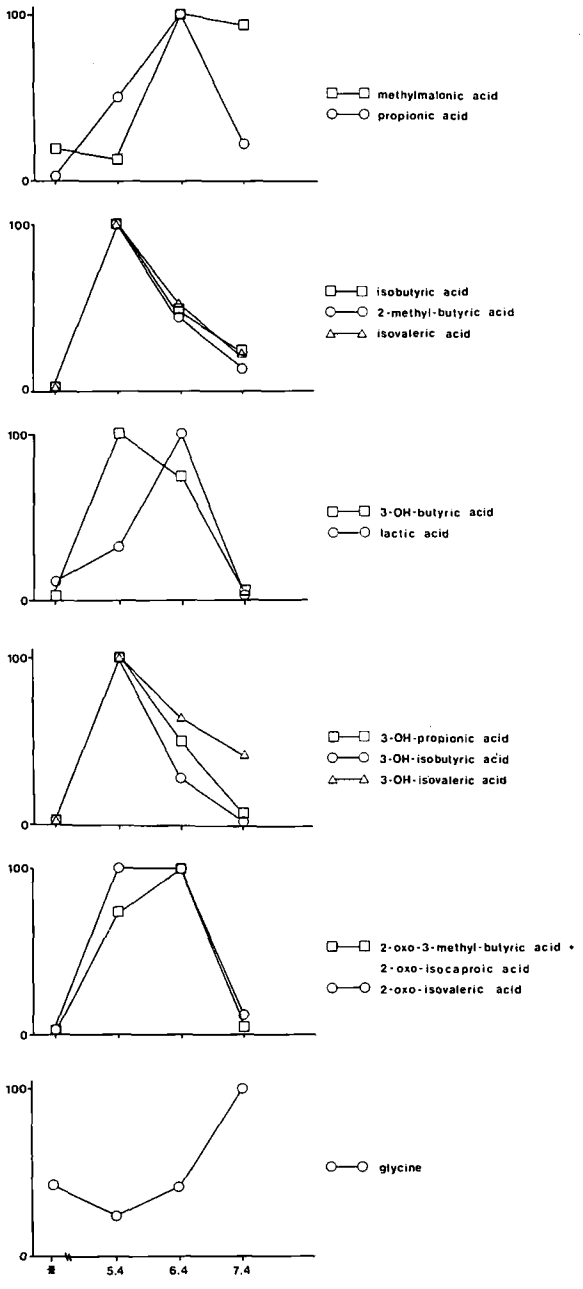

Figure 2 Time course of the excretion of metabolites in the period 4–7 April 1977. Values are expressed as percentages of the maximal values during this period. The asterisk indicates average values in clinically quiet periods. The maximal values are: methylmalonic acid: 10180 µg/mg creatinine; propionic acid: 178 µg/mg creatinine; isobutyric acid: 18 µg/mg creatinine; 2-methylbutyric acid: 26 µg/mg creatinine; isovaleric acid: 19 µg/mg creatinine; 3-hydroxybutyric acid: 4360 µg/mg creatinine; lactic acid: 620 µg/mg creatinine; 3-hydroxypropionic acid: 887 µg/mg creatinine; 3-hydroxyisobutyric acid: 808 µg/mg creatinine; 3-hydroxyisovaleric acid: 967 µg/mg creatinine; 2-oxo-3-methylvaleric acid + 2-oxo-isocaproic acid: 13 µg/mg creatinine; 2-oxo-isovaleric acid: 8 µg/mg creatinine; glycine: 743 µg/mg creatinine

propionic acid excretion and lactic acid excretion parallel each other closely. The peak excretion of propionic acid occurs 12–24 h prior to the peak excretion of methylmalonic acid.

(6) The excretion of glycine increased during both attacks.

DISCUSSION

Like a number of other organic acidaemias, i.e. propionyl-CoA carboxylase deficiency (Nyhan, 1974), isovaleryl-CoA dehydrogenase deficiency (Tanaka, 1975) and glutaryl-CoA dehydrogenase deficiency (Gregersen and Brandt, 1979), methylmalonic acidaemia is characterized by recurrent episodes of ketoacidosis (Tanaka, 1975). It is characteristic that these attacks are often precipitated by infections, during which protein and lipid catabolism is enhanced. This was also the case during the two ketotic episodes which are investigated in the present communication. The enhanced excretion of the 2-oxo-acids, which are early intermediates in the branched-chain amino acid metabolism, and the excretion of 3-hydroxybutyric acid serves to indicate this.

The underlying mechanism causing the excessive amounts of ketone bodies has never been understood fully. The mechanism proposed by Tan (Tan *et al.*, 1975) that methylmalonic acid inhibits 3-hydroxybutyric acid dehydrogenase and thereby causes an accumulation of 3-hydroxybutyric acid, is made very unlikely by the present results because the accumulation and increased excretion of methylmalonic acid is delayed until very late in the course of the attack.

The data presented here does not give the answer to the question of what triggers the ketoacidotic attacks. However, it documents an unusual excretion pattern during these attacks. This pattern can serve as a basis for formulating a series of hypotheses concerning the cause of the different elements in the metabolic derangements.

The initial rise in 3-hydroxybutyric acid and the 2-oxo-acids can, as previously mentioned, be explained by the mobilization of fat and protein which occurs during the infections.

The rise in short-chain fatty acids is more difficult to explain since this phenomenon, in contrast to 3-hydroxybutyric acid accumulation (Landaas, 1977) and 2-oxo-acid accumulation (personal observation), has not been seen in normal persons during similar periods of metabolic stress. Since comparison between the quantities of short-chain fatty acids in unhydrolysed and hydrolysed urine samples (data for unhydrolysed urines not shown) has demonstrated that the short-chain fatty acids are primarily excreted as conjugates, we are of the opinion that the short-chain fatty acids in urine represent intracellular accumulation of the corresponding acyl-CoA's. If these accumulations can be interpreted as an indication that the branched-chain acyl-CoA dehydrogenases are rate-limiting, then the situation can be further aggravated by the fact that ketone bodies, especially acetoacetate, stimulate the branched-chain 2-oxo-acid-dehydrogenases, thereby increasing the pressure on the branched-chain acyl-CoA dehydrogenases (Paul and Adibi, 1978).

Landaas (Landaas, 1977) has discussed the occurrence of 3-hydroxyisobutyric acid, 2-methyl-3-hydroxybutyric acid and 3-hydroxyisovaleric acid in patients with ketosis but without inborn errors of metabolism. He proposed that the accumulation of at least 3-hydroxyisobutyric acid and 2-methyl-3-hydroxybutyric acid is caused by a high NADH:NAD ratio inhibiting the 3-hydroxyacid dehydrogenases in the isoleucine and valine pathways. Another possibility, however, is that they are formed by unspecific β-oxidation from accumulated short-chain acyl-CoA's. Support for this hypothesis is offered firstly by the fact that the excretion curve for the 3-hydroxyacids seems to mimic both the curve for 3-hydroxybutyric acid (which probably can be taken as a measurement for the induced β-oxidation) and the curve for the short-chain fatty acids. Secondly, support is given by the fact that 3-hydroxypropionic acid shows the same excretion pattern. It is generally agreed that this compound can only be formed by such an unspecific β-oxidation (Andro *et al.*, 1972). 3-Hydroxyisovaleric acid may be synthesized by ω-1-oxidation as proposed for the 3-hydroxyisovaleric acid in isovaleric acidaemia (Tanaka, 1975).

The occurrence of rising propionic acid concentration before rising methylmalonic acid concentration makes it unlikely that the propionic acid accumulation is due to a simple shift of the equilibrium of the propionyl-CoA carboxylase catalysed reaction towards propionyl-CoA induced by increased methylmalonyl-CoA concentration.

An indication of the mechanism behind the rising urinary propionic acid concentration is perhaps given by the fact that the time course of propionic acid excretion resembles the profile for excretion of the branched-chain fatty acids and the branched-chain 3-hydroxyacids. It may, therefore, be suggested that the propionic acid (propionyl-CoA) accumulation is due to enzyme inhibition of the propionyl-CoA carboxylase either by one of the branched-chain fatty acids or one of the branched-chain 3-hydroxyacids (eventually as their CoA-derivatives). Such a hypothesis would also explain why rising urinary methylmalonic acid concentrations only occurred when the concentrations of branched-chain fatty acids and branched-chain 3-hydroxyacids were falling, thereby opening the pathway to methylmalonic acid.

In vitro experiments have shown that isovaleric and propionic acids inhibit the metabolism of pyruvate in rat liver mitochondria (Gregersen, 1979). Such an inhibition in the present patient may at least partly explain how the rising rate of lactic acid excretion parallels the excretion of isovaleric acid and especially that of propionic acid.

The rising glycine excretion rate can in the same way, at least partly, be explained by an inhibition of the glycine cleavage system by the accumulated isobutyric and 2-methylbutyric acids, because studies on an isolated glycine cleavage system from rat liver show that isobutyryl-CoA and 2-methylbutyryl-CoA inhibit the enzyme complex strongly (Kølvraa, 1979).

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