The Excretion and Formation of Aminoacetone and \( \delta \)-Aminolevulinic Acid in Man*

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The excretion of the aminoketones, aminoacetone (AA) and \( \delta \)-aminolevulinic acid (ALA) has been studied in 7 children. One of the children had ketotic hyperglycinemia and one had nonketotic hyperglycinemia. The formation of these aminoketones has been studied in vivo using \(^{14}\)C-labeled glycine and threonine.

The amounts of AA in the urine were slightly smaller than those of ALA in the subjects studied. Neither was increased by the infusion of large amounts of glycine. The amounts of aminoketone were independent of the concentrations of glycine in the plasma and did not differ from control in patients with disordered glycine metabolism. The amounts of aminoketone excreted varied directly with the weight of the child.

The injection of labeled glycine did not yield labeled urinary AA but small amounts of labeled ALA were found. Significant labeling of urinary AA was found after the administration of labeled threonine.

It is concluded that the metabolic precursor of urinary AA is threonine. Conversion to aminoketones, either AA or ALA, is not a major catabolic pathway for glycine in man. These pathways are not involved in either the etiology or the adaptation to hyperglycinemia.

A number of clinical disorders are present with chemical findings of elevated concentrations of glycine in body fluids. These include ketotic and nonketotic hyperglycinemia,\(^1,2\) methylmalonic acidemia (Morrow, Barness, Auerbach, DiGeroge, Ando & Nyhan: Observations on interrelationships between methylmalonic acidemia and glycinemia, J. Pediat., in press), and isovaleric acidemia (Ando, Nyhan & Klingberg, unpublished observations). In none of these conditions has the mechanism for the abnormality in glycine metabolism been established. In the study of these problems we have begun a systematic study of the metabolism of glycine in man.

Conversion of glycine to \( \delta \)-aminolevulinic acid (ALA) has been thought to represent an important mechanism for the metabolism of glycine, as well as an essential step in the synthesis of heme.\(^3\) Similarly, acetyl-CoA may condense with glycine to form aminoacetone (AA). This reaction has been demonstrated in

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guinea pig liver mitochondria and has been thought to have important meaning in the oxidative metabolism of glycine.\(^4\)

It was the purpose of this investigation to assess the excretion of the amino-ketones, AA and ALA, in control and hyperglycinemic children and to examine their rates of formation from labeled glycine and threonine.

**MATERIALS AND METHODS**

The subjects represented a variety of conditions in which increased concentrations of glycine are found (Table I) including 2 forms of hyperglycinemia, methylmalonic acidemia and isovaleric acidemia. Three control individuals, J.E., D.G. and M.S., were documented to have normal amounts of glycine in the blood and urine. In M.S. the glycine pool was increased to levels found in patients with nonketotic hyperglycinemia by infusion of unlabeled glycine.\(^5\) They were all under very good control so that clinical conditions were stable, and glycine concentrations were not very high. Isotopic amino acids were obtained from the Nuclear Chicago Corp. The specific activities of glycine-\(^2\)\(^{14}\)C and L-threonine-U-\(^{14}\)C were 21.5 and 156 mCi/mole, respectively. Radioactive purity and stability of the labeled amino acids were repeatedly assessed by column chromatography and liquid scintillation counting. Solutions for injection were made isotonic with saline and sterilized by passing through a sterile millipore filter system.

<table>
<thead>
<tr>
<th>Table 1. Urinary excretion of aminoketones</th>
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<tbody>
<tr>
<td>Subject</td>
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<tr>
<td></td>
</tr>
<tr>
<td>K.H.</td>
</tr>
<tr>
<td>R.H.</td>
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<tr>
<td>T.M.</td>
</tr>
<tr>
<td>J.E.</td>
</tr>
<tr>
<td>M.S.*</td>
</tr>
<tr>
<td>D.G.</td>
</tr>
<tr>
<td>L.G.</td>
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</tbody>
</table>

* This subject was infused with nonisotopic glycine for 2 hours in order to maintain elevated pools of glycine.

Following an overnight fast each subject was given 2.0 µCi/kg of labeled amino acid by intravenous injection. Urine was collected in plastic bottles cooled in ice and stored frozen until analyzed.

AA and ALA were assayed by the method of Marver et al.\(^6\) using 4 to 8 ml aliquots of each 25-hour urine sample. The procedure was modified by washing the resin with 0.01 M...
sodium acetate buffer, pH 4.6, after applying the pyroles of the aminoketones to the second Dowex-1-acetate column. This procedure prevented the contamination of the AA-pyrrole with radioactive amino acids. The eluates from the second column were collected in 2.0 ml fractions. A 0.5 ml aliquot of each fraction was mixed with Bray's solution for liquid scintillation counting. Another 0.5 ml aliquot of each fraction was mixed with an equal volume of modified Ehrlich's reagent. The intensity of color was read at 556 nm at 10 minutes after mixing for AA and at 25 minutes for ALA. The concentration of each aminoketone was calculated using the molar extinction coefficients obtained from 1-amino 2-propanone hydrobromide (Eastman) and δ-aminolevulinic acid hydrochloride (Calbiochem), 6.84 × 10⁴ for AA pyrrole and 6.04 × 10⁴ for ALA pyrrole. Confirmation of the identity of the aminoketone-pyrroles isolated from the column was done using the paper chromatographic methods of Mauzerall and Granick. Two different solvent systems were employed with the Rf values of the isolated pyroles corresponded to those reported.

**RESULTS**

The excretion of AA and ALA in 7 children is shown in Table I. They ranged in age from 3 months to 7 years and weighed 5 to 17 kg. The mean excretion of AA was 1.57 μmoles/24 hrs or 0.92 μmole/100 mg creatinine. The excretion of AA appeared to correlate well with the weight of the subject. The mean excretion per kg of body weight was 0.125 μmole with a range of 0.095 to 0.204.

The excretion of AA was not greater or less in patients with metabolic disorders in whom glycine concentrations were high than in controls. The infusion of large amounts of glycine, resulting in four-fold elevation of the plasma concentration, did not significantly increase the excretion of AA.

The excretion of ALA was independent on the concentration of glycine in the blood. It did not increase with the infusion of exogenous glycine. Again there appeared to be a correlation with body weight. Mean levels of excretion were 2.31 μmoles/day and 0.180 μmole/kg of body weight.

Following the injection of glycine-2-¹⁴C no isotope could be detected in AA. Isotope was found in ALA after glycine-2-¹⁴C but the level was not high enough to permit accurate calculation. Injection of labeled threonine resulted in the labeling of urinary AA in both subjects studied. There was no difference between the patient with methylmalonic aciduria and the control subject. In the patient with methylmalonic aciduria, the excretion of labeled AA in the first 24 hours after the injection of the isotope amounts to 0.049% of the injected ¹⁴C and in the control it was 0.050%. Isotope of threonine was not found in ALA.

**Table 2. Conversion of glycine and threonine to aminoketones**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Isotope</th>
<th>Aminoacetic acid μCi/day</th>
<th>δ-Aminolevulinic acid μCi/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>K.H.</td>
<td>Glycine-²¹⁴C</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>H.R.</td>
<td>Glycine-²¹⁴C</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>T.M.</td>
<td>Glycine-²¹⁴C</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>J.E.</td>
<td>Glycine-²¹⁴C</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>M.S.</td>
<td>Glycine-²¹⁴C</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>D.G.</td>
<td>Threonine-¹⁴C</td>
<td>13.0</td>
<td>0</td>
</tr>
<tr>
<td>L.G.</td>
<td>Threonine-¹⁴C</td>
<td>8.4</td>
<td>0</td>
</tr>
</tbody>
</table>
The aminoketones, ALA and AA, have been felt for some time to be important in the metabolism of glycine. In fact, Nemeth, Russel and Shemin found that labeled AA was oxidized to CO₂ more efficiently than the α carbon of glycine from which it was presumably derived. The synthesis of ALA is essential to the biosynthesis of porphyrins. The enzyme ALA synthetase is an inducible hepatic enzyme which is thought to catalyze the rate controlling step in porphyrin synthesis. Both aminoketones are found in urine. The measurement of increased amounts of ALA is important in human porphyria and lead intoxication.

ALA is formed from succinyl-coenzyme A and glycine as follows:

\[
\text{HOOCCH}_2\text{CH}_2\text{CO-S-CoA + CH}_3\text{NH}_2\text{COOH} \rightarrow [\text{HOOCCH}_2\text{CH}_4\text{COCHNH}_2\text{COOH}] + \text{CO}_2
\]

The carbon bearing the amino group is a precursor of methionine, purines and the β carbon of serine. Presumably ALA is converted to α-ketoglutaraldehyde, which yields succinate and a Cr unit from the aldehyde group which was originally derived from the α-carbon of glycine. AA may be formed in a similar condensation reaction from acetyl-coenzyme A and glycine:

\[
\text{CH}_3\text{CO-S-CoA + CH}_3\text{NH}_2\text{COOH} \rightarrow [\text{CH}_3\text{COCH}_2\text{NH}_2\text{COOH}] + \text{CO}_2
\]

The intermediate, α-amino-β-ketobutyric acid is known to decarboxylate readily nonenzymatically. The AA synthesized may be converted by transamination or monoamine oxidase to methylglyoxal which can be converted via a glyoxalase reaction to lactate and thereafter to pyruvate. Thus the synthesis of either aminoketone could lead to the complete metabolism of glycine. Either pathway could operate in a cyclic fashion and both have been postulated as major routes for the catabolism of glycine.

In studies of mammalian synthesis of aminoketones, Urata and Granick found that mitochondrial preparations from guinea pig liver effectively formed AA from acetyl-CoA and glycine. An α-aminoketone was also formed from glycine and propionyl-CoA. These preparations formed only traces of ALA. Similarly, Marver and colleagues found that AA was the major aminoketone in rat urine, occurring in 4–5 times the amounts of ALA. Urata and Granick extrapolated from their data on mitochondria and the turnover rates for glycine in the rat to calculate that conversion to AA could account for as much as one-fourth of the glycine synthesized daily. This would constitute a major pathway for the catabolism of glycine.

AA may also be formed from threonine. The compound was identified by Elliott as a metabolite of Staphylococcus aureus growing in the presence of...
threonine. Neuberger and Tait\textsuperscript{12} demonstrated an enzyme in \textit{Rhodopseudomonas spheroides} which converted threonine to AA. There was a specific requirement for DPN. This reaction would proceed as follows:

\[
\text{CH}_3\text{CHOHCHNH}_2\text{COOH} + \text{DPN} \rightarrow \text{CH}_3\text{COCHNH}_2\text{COOH}
\]  

The product \(\alpha\)-amino-\(\beta\)-ketobutyric acid would then decarboxylate spontaneously to form AA:

\[
\text{CH}_3\text{COCHNH}_2\text{COOH} \rightarrow \text{CO}_2 + \text{CH}_3\text{COCH}_2\text{NH}_2
\]

Marver and colleagues\textsuperscript{6} found that the administration of 2 mmoles of threonine per 100 g rat increased the urinary excretion of AA. With smaller doses this was not seen, and there was no change in the excretion of ALA.

Our studies indicate the conversion of glycine to aminoketones is not a major pathway for the catabolism of glycine in man. No evidence was obtained of the incorporation of isotope of glycine into AA and very little was found in ALA. Furthermore the infusion of very large amounts of nonisotopic glycine did not increase the excretion of either aminoketones. These observations are consistent with the conclusion that the conversation of glycine to ALA is predominantly a synthetic pathway, not a degradative one.

The role of AA in glycine metabolism or \textit{vice versa} is not clear; but the data would indicate that either would be small. On the other hand, significant conversion of the isotope of threonine to urinary AA was demonstrated. It is concluded that the source of urinary AA is threonine, not glycine.

These data obtained in children indicate that the amounts of AA excreted in the urine are somewhat smaller than those of ALA. However, the data reported for adults\textsuperscript{6} showed about equal amounts of excretion of AA and ALA; average 10.5 \(\mu\)moles/24 hrs of AA and 9.7 \(\mu\)moles/24 hrs of ALA in 8 adults. In the rat the amounts of AA far exceed the amounts of ALA excreted; 1.3 \(\mu\)moles/24 hrs/kg of AA and 0.41 \(\mu\)mole/24 hrs/kg of ALA were detected.\textsuperscript{6} In our studies the excretion of both aminoketones varied directly with the body weight of the subject. The excretion of AA particularly per kg of body weight was constant. Comparative biological studies on the relation between growth or body size and the amount of aminoketone excreted over a wide range of human subject size and in different species of mammals will be of great interest. A relevant observation could be the inhibitory role in cell division of methylglyoxal which is the direct metabolic product of AA.\textsuperscript{13}

These studies have bearing on the inborn errors of glycine metabolism. The excretion of the aminoketones was independent of the concentration of glycine in the plasma. Patients with hyperglycinemia excreted aminoketones in the same way as other subjects. Obviously, these are not pathways that are employed to handle extra-quantities of glycine. It is also apparent that in none of the conditions studied are there alterations in these pathways that would account for the hyperglycinemias observed.
In nonketotic hyperglycinemia we have reported\(^5\) evidence for a defect in the conversion of the first carbon of glycine to CO\(_2\) and the second carbon of glycine to the β-carbon of serine. These studies and subsequent observations on the metabolism of labeled glyoxylate in this condition exclude a defect in glycine oxidase. (Gerritsen, Nyhan, Rehberg, & Ando. Metabolism of glyoxylate in nonketotic hyperglycinemia, Pediat. Res., in press). We have postulated a defect in a glycine decarboxylase reaction which converts glycine to CO\(_2\) and a single carbon tetrahydrofolate derivative. This enzymatic reaction has been described in rat liver mitochondria\(^14\) as well as in avian and microbial systems.\(^15-17\) Theoretically a defect in the succinate-glycine cycle, which yields CO\(_2\) and a single carbon derivative from glycine could also explain the findings observed. However, none of the patients with hyperglycinemia have had problems with hemoglobin synthesis. Further in these experiments urinary excretion of ALA and its labeling after glycine did not differ from controls. If patients with hyperglycinemia had a defect in the first step of the succinyl-CoA-glycine condensation, the excretion of ALA or its labeling should have been diminished. A defect in the next step should lead to the accumulation of isotope in α-ketoglutaraldehyde or a related product. Neither was observed. Therefore, the data in nonketotic hyperglycinemia are consistent with a defect in a glycine decarboxylase.

References


