Concentrations in Serum and Urinary Excretion of Guanidine, 1-Methylguanidine, and 1,1-Dimethylguanidine in Chronic Renal Failure

Israel M. Stein and Michael J. Micklus

Guanidine (G), 1-methylguanidine (MG), and 1,1-dimethylguanidine (DMG) have long been implicated as uremic "toxins." A method has been developed for determining G, MG, and DMG in serum and urine. Specimens were chromatographed on carboxylate resin, with use of 1 molar NaOH, and quantitated colorimetrically with a modification of the Voges-Proskauer reaction. The mean values for G and MG in the serum of uremic patients were 0.3 and 0.4 mg per liter, respectively. DMG was not detected. Although the urinary excretion of MG is significantly increased in renal failure, the concentrations of G, MG, and DMG in serum are not markedly increased, and it is therefore unlikely that G, MG, or DMG contribute to the toxic manifestations of the uremic syndrome.

Additional Keyphrases: "toxins" in uremia • ion-exchange column-chromatography • Voges-Proskauer reaction • uremic syndrome

Since the early work of Pfiffner and Myers (1), and Major and Weber (2), it has been suspected that guanidine compounds contribute to the toxic manifestations of chronic renal disease. The lack of suitable analytical techniques has long hindered attempts to identify individual derivatives of guanidine in the body fluids of patients with renal insufficiency.

We have developed a method for the determination of guanidine (G), 1-methylguanidine (MG), and 1,1-dimethylguanidine (DMG) by ion-exchange chromatography and a modified Voges-Proskauer colorimetric reaction.

This method has been applied to the study of these compounds in the serum and urine of patients with chronic renal failure.

Materials and Methods

Analytical Methods

Isolation of G, MG, and DMG: G, MG, and DMG were isolated by use of a weakly acidic ion-exchange resin (carboxylic acid resin, "Bio-Rex 70," 100-200 mesh, sodium form; BioRad Laboratories, Richmond, Calif. 94804). Fifty grams of this resin was prepared for use by washing with 600 ml of 1 molar NaOH, followed by 500 ml of distilled water, and was finally equilibrated with 400 ml of 0.2 molar NaOH. The resin was next packed into 30 × 1 cm columns to a height of 25 cm, and 50 ml of 0.2 molar NaOH was passed through the columns.

Four-milliliter specimens of serum or 10-ml aliquots of 24-h urine collections were applied directly to the column, without prior treatment. After the specimens had entered the resin, elution was begun with 40 ml of 0.2 molar NaOH, followed by 80 ml of 1 molar NaOH. The effluent from 0.2 molar NaOH was discarded; the 1 molar NaOH effluent, which contained G, MG, and MDG, was collected in 4-ml fractions on an automatic fraction collector. The fractions were then analyzed by a modification of the Voges-Proskauer reaction as detailed below. Concentrations of G, MG, and DMG were determined from calibration curves of 5, 10, and 20 μg/ml of each of these compounds (Sigma Chemical Co., St. Louis, Mo. 63178). Recoveries were determined by adding G, MG, and DMG (1-100 μg) to split samples of urine and serum.

Chromatographically pure arginine, guanidinoacetic acid, guanidinosuccinic acid, creatine, creatinine, and urea (Sigma) were chromatographed to determine their pattern of elution from carboxylate resin.

Colorimetric reaction: The eluate was analyzed by a modification of the Voges-Proskauer reaction (7). This method is equally sensitive for both mono- and disubstituted guanidines. The lower limit of detection for the Voges-Proskauer reaction is less than 1 μg/ml for G, MG, or DMG.

From the Departments of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, Mass. 02215.

Requests for reprints should be addressed to: Israel M. Stein, M.D., Beth Israel Hospital, 330 Brookline Ave., Boston, Mass. 02215.

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To 2 ml of eluate, 1 ml of 2 molar NaOH and 1 ml of an α-naphthol-diacyetyl reagent were added. The α-naphthol-diacyetyl reagent was prepared freshly every four days by adding 2.5 ml of a 10 g/liter solution of diacyetyl (2,3-butanedione) to 97.5 ml of n-propanol that contained 5 g of α-naphthol (Sigma). The color was allowed to develop at room temperature for 35 min and was measured at 535 nm in a Gilford spectrophotometer.

Subjects

Subjects included both ambulatory and hospitalized patients with clinical evidence of chronic renal failure, whose serum creatinine concentrations ranged from 5.5 to 21 mg/100 ml. Serum specimens were obtained from eight patients whose mean blood urea nitrogen was 140 mg/100 ml (range, 66 to 195 mg/100 ml). Twenty-four-hour urine specimens were obtained from seven patients whose blood urea nitrogen averaged 113 mg/100 ml (range, 66 to 156 mg/100 ml).

Control subjects included hospitalized patients and healthy volunteers who had normal blood urea nitrogen concentrations and who had no history of renal disease.

Results

Figure 1 illustrates a typical chromatographic pattern for a synthetic mixture of guanidine and its derivatives. G, MG, and DMG are distinctly separated from each other and from arginine, guanidinosuccinic acid, guanidinoacetic acid, creatine, creatinine, and urea.

Recoveries determined from aqueous solution, serum, and urine are shown in Table 1.

Serum. A typical chromatographic pattern obtained with uremic serum is shown in Figure 2. The mean concentrations (±SD) of G, MG, DMG, and an unidentified guanidine, labeled “x”—as determined for seven control subjects and eight patients with chronic renal failure—are shown in Table 2. The values for MG and G in uremic serum should not be considered absolute, because of the problems of integrating indistinct peaks. The maximum values, however, for G and MG in the patients studied did not exceed 60 and 100 μg/100 ml, respectively.

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. recoveries</th>
<th>Amount added, µg</th>
<th>Recovery, %</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanidine</td>
<td>30</td>
<td>1–50</td>
<td>98.6 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>1-Methylguanidine</td>
<td>36</td>
<td>1–50</td>
<td>98.9 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>1,1-Dimethylguanidine</td>
<td>16</td>
<td>5–25</td>
<td>85.6 ± 1.9</td>
<td></td>
</tr>
</tbody>
</table>

* Guanidine derivative added to 2 to 10 ml of sample before chromatography.

**Urine.** The 24-h urinary excretion of G, MG, and DMG as determined in eight controls and in seven patients with chronic renal failure is shown in Table 3. Amounts of as much as 24 mg of MG per 24-h urine were noted in one patient with renal failure.

Although MG increased significantly in chronic renal failure, the excretion of G and “x” were not significantly increased. Concentrations of G and MG in serum are higher than in controls, in whom no evidence of G or MG could be found. DMG was not detected in either serum or urine.

Discussion

Toxic metabolites have long been implicated in the pathogenesis of the uremic syndrome. Previously reported concentrations of MG and G in uremic serum have ranged from 0.03 to 10 mg/100 ml (1–5). The methods used by these investigators, however, lacked specificity, were overly complex, or—as in the reports of Giovanetti et al. (6)—actually generated MG in the process of its estimation.
Table 2. Concentration of Guanidine, 1-Methylguanidine, 1,1-Dimethylguanidine, and "x" in Serum

<table>
<thead>
<tr>
<th>BUN, mg/100 ml</th>
<th>Guanidine</th>
<th>Methylguanidine</th>
<th>1,1-Dimethylguanidine</th>
<th>&quot;x&quot;&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7)</td>
<td>11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.025</td>
<td>&lt;0.025</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Renal failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(8)</td>
<td>140</td>
<td>0.03 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.04 ± 0.04</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as arginine equivalent.
<sup>b</sup> Mean.
<sup>c</sup> Mean ± SD.

Table 3. Urinary Excretion of Guanidine, 1-Methylguanidine, 1,1-Dimethylguanidine, and "x"

<table>
<thead>
<tr>
<th>BUN, mg/100 ml</th>
<th>Guanidine</th>
<th>1-Methylguanidine</th>
<th>1,1-Dimethylguanidine</th>
<th>&quot;x&quot;&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(8)</td>
<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.5 ± 0.5</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Renal failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7)</td>
<td>113</td>
<td>1.2 ± 8.6</td>
<td>8.8 ± 7.2</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Expressed as arginine equivalent.
<sup>b</sup> Mean.
<sup>c</sup> Mean ± SD.

Our previous success with the use of ion-exchange chromatography for the isolation of guanidinosuccinic acid and methylguanidine (8, 9), suggested that the ion-exchange technique might be applied to the determination of G, MG, and DMG in body fluids. The method described above confirms that impression. The technique is simple, sensitive, accurate, rapid, and reproducible.

Compound "x," which elutes before DMG, is as yet unidentified. The fact that compound "x" elutes in close proximity to G, MG, and DMG, and gives a positive Sakaguchi reaction—a test that is highly specific for monosubstituted guanidino derivatives—suggests that it is a strongly basic monosubstituted guanidine.

The concentrations of G and MG in the sera of patients with renal failure are not markedly increased, and it is therefore unlikely that G, MG, or DMG contribute to uremic toxicity. The concentration in serum and the urinary excretion of "x" compound are not significantly different from that for controls, suggesting that it also is not a uremic "toxin."

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References