Urinary excretion of 17-oxosteroids in hereditary coproporphyria

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Summary
1. Urinary 17-oxosteroid conjugates were measured by gas–liquid chromatography in five patients with hereditary coproporphyria.
2. Three patients were in an acute attack and showed significantly increased excretion of sulphate or glucuronide conjugates of aetiocholanolone. There was increased excretion of several other related steroids but no consistent pattern was apparent.
3. In the two patients in remission, excretion of urinary 17-oxosteroids was not increased.
4. The ratio of total urinary aetiocholanolone to androsterone (5β:5α) was found to be significantly elevated for the three patients in an acute attack. Serial measurements were made in two of these patients and showed a highly significant linear correlation between this ratio and the urinary content of δ-aminolaevulic acid and porphobilinogen.
5. These observations suggest the involvement of the 17-oxosteroids, especially aetiocholanolone, in the pathogenesis of hereditary coproporphyria.

Key words: aetiocholanolone, androsterone, coproporphyria, 17-oxosteroids.

Introduction
The hepatic porphyrias are a group of disorders characterized by excessive excretion of porphyrins and porphyrin precursors (Moore & Goldberg, 1974). One of the less common of these diseases in Britain is hereditary coproporphyria, which is distinguishable from the other hepatic porphyrias by the excessive excretion of coproporphyrin in the faeces at all times. In attacks, the urine contains excessive quantities of δ-aminolaevulic acid and porphobilinogen, uroporphyrin and coproporphyrin (Goldberg, Rimington & Lockhead, 1967). It has been established that patients have a marked increase in the activity of the hepatic enzyme δ-aminolaevulic acid synthetase (EC 2.3.1.37), the initial and rate-limiting enzyme in the biosynthesis of haem (Kaufman & Marver, 1970; McIntyre et al., 1971).

In previous work (Goldberg, Moore, Beattie, Hall, McCallum & Grant, 1969; Paxton, Moore, Beattie & Goldberg, 1974) certain urinary and plasma 17-oxosteroids were found to be elevated in acute intermittent porphyria. This prompted the measurement of conjugated 17-oxosteroids in the urine of five patients with hereditary coproporphyria. Of these, three patients were investigated during an acute attack, and two were in remission.

Materials and methods
Solvents and analyses. Solvents were obtained from the Reeve Angel Co. CT range, except ethanol and methanol which were BDH Aristar grade, and hexane, which was BDH AnalaR grade. All solvents were used without further purification, except hexane, which was washed three times with 100 ml of conc. H2SO4 for 1 h, followed by two washes of 100 ml of 2 mol/l NaOH and finally by several washes of 100 ml of distilled water until the solvent was neutral.
**Table 1. Urinary excretion of 17-oxosteroids in patients with hereditary coproporphyria**

Andro. = androsterone; Aetio. = aetiocholanolone; DHA = dehydroepiandrosterone (3β-hydroxyandrost-5-en-17-one); EPI = epandrosterone (3β-hydroxy-5α-androstan-17-one); 11-oxoandro. = 11-oxoandrosterone; 11-oxaoetio. = 11-oxoaetiocholanolone; 11-hydroxyandro. = 11-hydroxyandrosterone; 11-hydroxyaetio. = 11-hydroxyaetiocholanolone. Values greater than those from normal subjects of the appropriate age group are italicized. Patient no. 3 and patient no. 5 were in remission and had normal values.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Andro. (μmol/24 h)</th>
<th>Aetio. (μmol/24 h)</th>
<th>DHA (μmol/24 h)</th>
<th>EPI (μmol/24 h)</th>
<th>Normal (control)</th>
<th>Patient no. 1</th>
<th>Patient no. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (control)</td>
<td>20-29</td>
<td>0.230</td>
<td>0.071-1.42</td>
<td>0.202</td>
<td>0.082</td>
<td>5.19-15.25</td>
<td>0.168</td>
<td>0.01</td>
</tr>
<tr>
<td>Patient no. 1</td>
<td>0.64</td>
<td>1.68</td>
<td>0.08</td>
<td>2.51</td>
<td>2.17</td>
<td>4.13</td>
<td>0.08</td>
<td>0.23</td>
</tr>
<tr>
<td>Patient no. 2</td>
<td>0.01</td>
<td>0.23</td>
<td>0.25</td>
<td>1.08</td>
<td>6.85</td>
<td>14.1</td>
<td>0.79</td>
<td>0.33</td>
</tr>
<tr>
<td>Normal (control)</td>
<td>30-39</td>
<td>0.052-0.48</td>
<td>0.15-0.98</td>
<td>0.02-2.07</td>
<td>0.10-0.85</td>
<td>0.55-6.54</td>
<td>1.82</td>
<td>0.15</td>
</tr>
<tr>
<td>Patient no. 4</td>
<td>0.10</td>
<td>1.19</td>
<td>0.15</td>
<td>0.05</td>
<td>0.79</td>
<td>0.33</td>
<td>0.93</td>
<td>40.66</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Andro. (μmol/24 h)</th>
<th>Aetio. (μmol/24 h)</th>
<th>DHA + EPI (μmol/24 h)</th>
<th>11-Oxoandro. (μmol/24 h)</th>
<th>11-Oxoaetio. (μmol/24 h)</th>
<th>11-Hydroxyandro. (μmol/24 h)</th>
<th>11-Hydroxyaetio. (μmol/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (control)</td>
<td>0.162</td>
<td>0.23-0.88</td>
<td>0.49-5.04</td>
<td>0.95-4.5</td>
<td>0-3.26</td>
<td></td>
</tr>
<tr>
<td>Patient no. 1</td>
<td>0.65</td>
<td>2.23</td>
<td>1.41</td>
<td>0.36</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>Patient no. 2</td>
<td>0.79</td>
<td>0.33</td>
<td>2.39</td>
<td>1.37</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Normal (control)</td>
<td>0.110</td>
<td>0.0-0.88</td>
<td>0.65-3.40</td>
<td>0.064-4.50</td>
<td>0-2.44</td>
<td></td>
</tr>
<tr>
<td>Patient no. 4</td>
<td>0.79</td>
<td>0</td>
<td>5.93</td>
<td>40.66</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Steroids and coproporphyria

Table 2. Urinary excretion of porphyrin precursors and porphyrins in five patients with hereditary coproporphyria

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Clinical state</th>
<th>No. of 24 h collections</th>
<th>δ-Aminolaevulic acid (µmol/24 h)</th>
<th>Porphobilinogen (µmol/24 h)</th>
<th>Uroporphyrin (µmol/24 h)</th>
<th>Coproporphyrin (µmol/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>20</td>
<td>Attack</td>
<td>5</td>
<td>232 ± 163</td>
<td>168 ± 147</td>
<td>1.36 ± 0.18</td>
<td>6.57 ± 3.37</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>25</td>
<td>Attack</td>
<td>7</td>
<td>162 ± 54</td>
<td>345 ± 57</td>
<td>3.04 ± 1.63</td>
<td>1.62 ± 0.29</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>28</td>
<td>Remission</td>
<td>1</td>
<td>21</td>
<td>3</td>
<td>0.07</td>
<td>0.64</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>30</td>
<td>Attack</td>
<td>1</td>
<td>141</td>
<td>81</td>
<td>1.22</td>
<td>12.14</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>47</td>
<td>Remission</td>
<td>1</td>
<td>23</td>
<td>4</td>
<td>0.04</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Normal range
0-40 0-16 0-0.05 0-0.43

The solvent was then dried with CaCl₂ and redistilled.

Analysis of 17-oxosteroids in urine was carried out by the method of Paxton et al. (1974). Urinary δ-aminolaevulic acid was measured by the method of Mauzerall & Granick (1956) and urinary porphobilinogen and porphyrins by the method of Rimington (1971).

Normal control subjects. The 24 h urinary secretion of 17-oxosteroid conjugates was estimated in thirty-eight males, age 17-63 (mean 32) years. The individual steroids in urine were found to vary considerably with age, reaching a peak during the third decade and then declining with advancing years. For the purpose of comparing these normal values with those found in patients with hereditary coproporphyria, the male and female control groups were divided into smaller groups according to age.

Results

Five patients, four females and one male, with hereditary coproporphyria, were investigated. The two patients examined during remission had normal amounts of the sulphate and glucuronide conjugates of all the 17-oxosteroids in the urine. This was associated with normal amounts of the porphyrin precursors, porphobilinogen and δ-aminolaevulic acid. The three patients in attack, who were all female, had elevated amounts of sulphate or glucuronide conjugates of aetiocholanolone (3α,11β-dihydroxy-5α-androstan-17-one) (Table 1). Elevations of the 11-hydroxy- or 11-oxo-17-oxosteroid glucuronide conjugates were observed in two of the patients in attack, especially in patient no. 4, who had a massive amount of 11β-hydroxyandrosterone (3α,11β-dihydroxy-5α-androstan-17-one) in the urine. These elevated urinary steroids were accompanied by elevated coproporphyrin, uroporphyrin and the porphyrin precursors, δ-aminolaevulic acid and porphobilinogen (Table 2).

The ratio of 5β steroids:5α steroids in the urine, i.e. aetiocholanolone sulphate and glucuronide: androsterone sulphate and glucuronide, was found to be significantly greater for the patients (nos. 1, 2 and 4) in attack (2.14 ± 0.81; 2.03 ± 0.31; and 2.22) compared with normal subjects (0.94 ± 0.5). The ratios for the two patients in remission (1.11 and 1.19) were found to be within the range for normal subjects.

In the two patients in attack in whom serial 24 h collections of urine were obtained, this aetiocholanolone:androsterone ratio gave a highly significant straight-line regression with urinary excretion of δ-aminolaevulic acid and porphobilinogen with equations:

Patient no. 1

δ-Aminolaevulic acid excretion (µmol/24 h) = 140 (aetiocholanolone/androsterone) − 15

(r = 0.82)

Porphobilinogen excretion (µmol/24 h) = 122 (aetiocholanolone/androsterone) − 23

(r = 0.81)

Patient no. 2

δ-Aminolaevulic acid excretion (µmol/24 h) = 210 (aetiocholanolone/androsterone) − 265

(r = 0.78)
These results imply that there is a close association between hereditary coproporphyria and elevated amounts of 17-oxosteroids. There are also many clinical observations which suggest the involvement of endocrine secretions, specifically steroids, in the provocation and aggravation of the acute attacks of hereditary coproporphyria. Thus the acute attacks occur predominantly in the third and fourth decades when steroid production and excretion are at a maximum; the acute attacks have a marked female preponderance and are associated with the luteal phase of the menstrual cycle or with pregnancy; they rarely become manifest before puberty and they are infrequent after the menopause; chemical as well as symptomatic relapse results as a consequence of sex hormone administration in some patients.

In this investigation significant elevations of some 17-oxosteroids were detected in the urine of all patients in attack. The ratio in urine of aetiocholanolone (5β): androsterone (5α) was found to be significantly greater for the patients suffering an acute attack than for normal subjects. There was also a highly significant linear correlation with the urinary amounts of δ-aminolaevulic acid and porphobilinogen in two of these patients, the maximum occurring during the attack then gradually decreasing with corresponding decreases in the amounts of δ-aminolaevulic acid and porphobilinogen. These results suggest that patients with hereditary coproporphyria have an imbalance in their steroid metabolism resulting in an overall increase in the 5β type of steroid metabolite. Gillette, Bradlow, Gallagher & Kappas (1970) have reported that in patients with acute intermittent porphyria the 5α pathway for steroid metabolism was greatly diminished, indicating a gross deficiency of Δ4-5α-steroid reductase activity, which resulted in preferential formation of the 5β metabolites. A similar situation appears to exist in the livers of patients in attack of hereditary coproporphyria.

The mechanism of action of these steroids is unknown. Previous research, especially that of Granick & Kappas (1967), suggests that these 5 β-steroid metabolites act directly as depressors of the operon allowing synthesis de novo of δ-aminolaevulic acid synthetase in the liver. Possibly many of the varied factors which precipitate and aggravate acute episodes of the hepatic porphyrias, may interact with an endogenous regulatory system of steroid metabolism in the liver.

**Acknowledgments**

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**References**


