Family studies on the activity of uroporphyrinogen I synthase in diagnosis of acute intermittent porphyria

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Summary

1. Ten subjects with acute intermittent porphyria from three different families, and 92 relatives, were investigated for their erythrocyte uroporphyrinogen I synthase (EC 4.3.1.8) activities by the spectrofluorimetric method described and for their urinary concentrations of δ-aminolaevulinic acid and porphobilinogen.

2. The mean uroporphyrinogen I synthase activity in 41 healthy women and 41 healthy men showed a significant ($P < 0.001$) sex difference.

3. A reduction of about 32% of the enzyme activity was observed in the porphyric subjects as compared with values in healthy normal subjects and the values from the porphyric subjects overlapped those of the reference subjects.

4. With the values from the normal subjects in each family used as reference, however, the enzyme activity in normal subjects was twice that in affected subjects. Thus by using an internal family reference uroporphyrinogen I synthase values became more reliable in disclosing latent cases of the disorder. Furthermore, these measurements were shown to have a stronger discriminative power than urinary δ-aminolaevulinic acid and porphobilinogen determinations.

Key words: erythrocytes, porphyria, porphyrin biosynthesis, uroporphyrinogen I synthase.

Introduction

An attack of acute porphyria may be precipitated by certain drugs, steroids, starvation or infections (Eales, 1974; Tschudy, Valsamis & Magnussen, 1975). The prognosis of the latent acute intermittent porphyria subject (i.e. symptomless carrier of the genetic defect) could be improved if the subjects at risk could be informed about the precipitating agents. So far, urinary determinations of the porphyrin precursors δ-aminolaevulinic acid and porphobilinogen have been used to try to diagnose the latent condition, but these analyses may be inadequate (With, 1963; Meyer, Strand, Doss, Rees & Marver, 1972). A partial block has been demonstrated in the pathway for haem biosynthesis at the level of uroporphyrinogen I synthase (EC 4.3.1.8) in liver cells (Heilmeyer & Clotten, 1969; Strand, Felsher, Redeker & Marver, 1970; Miyagi, Cardinal, Bossermaier & Watson, 1971) and in erythrocytes (Meyer et al., 1972; Strand, Meyer, Felsher, Redeker & Marver, 1972) of these patients. Since deficiency of this enzyme is thought to reflect the primary genetic defect in the porphyria (Meyer et al., 1972; Meyer, 1973; Kappas, Sassa, Granick & Bradlow, 1974), this deficiency should also be present in the latent condition. The purpose of this study was to quantify the erythrocyte uroporphyrinogen I synthase activity in normal subjects and in those with clinically manifest porphyria. The enzyme activities were subsequently compared with urinary concentrations of δ-aminolaevulinic acid and porphobilinogen to assess these methods in the diagnosis of latent acute intermittent porphyria.
Subjects

Patients

102 non-hospitalized subjects from three different families, A, B and C, were examined. Ten spouses were included as control subjects. Ten subjects had clinically manifest acute intermittent porphyria: subject nos. 4, 8, 9, 10 and 11 (Fig. 3) belonged to family A, nos. 3, 6 and 8 (Fig. 4) to family B, and nos. 6 and 17 (Fig. 5) belonged to family C. Clinically manifest acute intermittent porphyria was diagnosed on the basis of one or more acute attacks, from clinical history, regardless of δ-aminolaevulinic acid, porphobilinogen and enzyme activity values.

Control subjects

The urinary excretion of δ-aminolaevulinic acid and porphobilinogen was measured in 25 healthy women and 25 healthy men, aged 16–67 years (mean 33.6 years). The erythrocyte uroporphyrinogen I synthase activity was studied in 41 healthy women and 41 healthy men, aged 17–67 years (mean 31.4 years). Fifty-six of the subjects were blood donors. None of these subjects was related to the families A, B and C.

Methods

Urine analysis

The first urine voided in the morning was collected, the pH adjusted to 7.0 and the sample stored at −20°C. δ-Aminolaevulinic acid and porphobilinogen were separated by ion-exchange chromatography (Fluka, Switzerland) (Mauzerall & Granick, 1956) as modified by With (1963). Porphobilinogen was eluted in one step with 10 ml of acetic acid (1 mol/l) (With, 1968). Correction for loss of δ-aminolaevulinic acid and porphobilinogen was done by subjecting aqueous standard solutions of the pure crystalline substances (Sigma Chemical Co., St Louis, Mo., U.S.A.) to the chromatographic procedure.

Enzyme assay

Capillary blood (finger prick) was collected into heparinized Caraway tubes and mixed immediately. Packed cell volume was determined (Cellocriit 2, A.B. Ljungberg and Co., Stockholm, Sweden). Pilot experiments showed that varying the amounts of sodium heparin in the samples had no influence on the enzyme activity, and that whole-blood samples could be stored for 1 day at room temperature and at least 1 week at +4°C or −20°C without loss of activity.

The uroporphyrinogen I synthase activity was determined by a modification of the micromethod of Granick, Sassa, Granick, Levere & Kappas (1972). Whole blood was frozen and thawed and 2 μl of haemolysate was mixed with 25 μl of Tris/HCl buffer (0.05 mol/l) (Trizma, Sigma Chemical Co.), pH 7.66, containing porphobilinogen (10⁻⁴ mol/l). The mixture was incubated at 37°C for 90 min in the dark. The reaction was stopped by adding 300 μl of ice-cold ethyl acetate/acetic acid (2:1, v/v). The porphyrins were extracted (Sassa, Granick, Granick, Kappas & Levere, 1973b) and the two phases separated by centrifuging. Fluorimetric determinations were carried out in a microcuvette, with a Hitachi–Perkin Elmer MPF-2A spectrofluorimeter, equipped with a R-106 photomultiplier. Fluorescence was determined (Strand et al., 1972) at excitation and emission wavelengths of 404 nm and 595 nm respectively and compared with standard solutions prepared from preweighed vials containing 5.0 μg of coproporphyrin I (Sigma Chemical Co.), ε₄₀₀ nm = 7.47 x 10³ in HCl (0.1 mol/l), dissolved in aqueous HCl solution (0.5 mol/l) which had been shaken with a solution of Tris/HCl and ethyl acetate/acetic acid in the same proportions as the samples. The standard curve was linear between concentrations of 25 and 250 pmol of coproporphyrin I/ml. The amount of porphyrin formed was determined as uroporphyrin (Granick et al., 1972; Strand et al., 1972). The enzymes activity was calculated as nmol of uroporphyrin formed h⁻¹ ml⁻¹ of erythrocytes at 37°C.

\[
\text{Activity} = \frac{\hat{X} \times 0.75 \times \frac{0.4}{0.002 \times \text{PCV} \times 10^3}}{10 \times \text{PCV}}
\]

Where \( \hat{X} = \text{pmol of coproporphyrin (mean of duplicate analysis),} \) 0.4 is the volume (ml) of the final aqueous phase, 0.002 is the volume (ml) of the haemolysate used, \( \frac{1}{3} \) is the incubation-time factor, 0.75 is the fluorescence intensity correction factor and PCV is packed cell volume.

The reaction velocity was linear for at least 2 h. Duplicate assays from 82 reference subjects and
Uroporphyrinogen I synthase activity

Intra-individual variation. In 25 persons, including normal subjects \((n = 8)\), the patients \((n = 4)\) and the group of relatives \((n = 13)\), individual variation of the uroporphyrinogen I synthase activities over a 2 years period was ±1.6 units. The day-to-day variation (30 day period) was ±1.0 unit for a normal woman and ±2.0 units for a normal man studied. The enzyme activities were therefore investigated in blood samples taken at random.

Control subjects. A highly significant \((P < 0.001)\) difference was observed between the mean enzyme activity in women \((32.6 ± 5.2\) units\) and men \((27.9 ± 4.3\) units\) (Fig. 2). Mean activity in blood donors was not significantly different from non-donors.

Subjects with acute intermittent porphyria

Among the ten subjects with the clinically manifest disorder, the activity of uroporphyrinogen I synthase ranged from 19.2 to 32.1 units (mean 22.8) in six women, and from 15.2 to 22.3 units (mean 18.4) in four men. Thus a marked reduction of 30% in men and 34% in women in the enzyme activity was observed in subjects with the clinically manifest condition as compared with normal subjects (Fig. 2). Furthermore, enzyme activities in the former subjects overlapped those observed in the control subjects (Fig. 2).

Analysis of results of enzyme assays from relatives of manifest cases (detailed results have

Fig. 1. Concentration of δ-aminolaevulinic acid and porphobilinogen in the morning urine, collected from 50 healthy adults.
been deposited as Clinical Science and Molecular Medicine Table 7 with the Librarian of The Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE, from whom copies can be obtained on request), however, suggested that the prediction of which enzyme activities should be considered reduced might be facilitated when activities from each subject's siblings and/or parents were used as an internal reference. Thus the enzyme activity of a suspected porphyric subject was of greater predictive value when activity values were available from both healthy and identified cases of the disease, in the family studied. In most cases the enzyme activity in unaffected subjects was then twice that in the affected subjects in that family.

Family A (Fig. 3). This family contained two pairs of identical twins with enzyme activities of 17.0 and 18.2 (nos. 17 and 18) and 44.0 and 44.8 (nos. 28 and 29), strongly suggesting that uroporphyrinogen I synthase activities are genetically determined. Furthermore, family A comprised nine healthy subjects, in whom enzyme activity reached values similar to those observed in relatives with the clinically manifest porphyria. By using an internal reference these nine subjects (nos. 14, 15, 17, 18, 19, 20, 30, 33 and 36) demonstrated an enzyme deficiency of about 50%. In contrast they all had normal urinary concentrations of δ-aminolaevulinic acid and porphobilinogen.

Family B (Fig. 4). Five subjects (nos. 5, 7, 15, 16 and 20) with low enzyme activities were thought to be latent carriers of acute intermittent porphyria. In contrast all of them except nos. 7 and 15 had normal urinary concentrations of the porphyrin precursors. Subject no. 14 had a porphobilinogen value (28.6 μmol/l) slightly above what was considered normal (28.2 μmol/l) but no deficiency of enzyme activity. Nineteen of 20 existing children (1–11 years old) in generation III all showed normal enzyme activities.

Family C (Fig. 5). The search for latent acute intermittent porphyria was complicated as each measured uroporphyrinogen I synthase activity overlapped the values in healthy normal subjects, and very few spouses were available. An enzyme activity of about 41 units (nos. 10, 13 and 16), although high, might represent the normal enzyme activity in the corresponding families. Activities of

![Fig. 2. Erythrocyte uroporphyrinogen I synthase activities (nmol of uroporphyrin h⁻¹ ml⁻¹ of erythrocytes at 37°C) from 41 normal women, 41 normal men and 10 subjects with clinically manifest acute intermittent porphyria (AIP). The 10 porphyric subjects were members of three different families: family A (♦), family B (■) and family C (▲).](image)

![Fig. 3. Family A. ©, Females; □, males. ©, □, Subjects with reduced or 50% reduced uroporphyrinogen I synthase activity as compared with healthy relatives (cf. the Results section). ©, □, Subjects with clinically manifest acute intermittent porphyria (see the Subjects section). ©, □, Subjects with clinically manifest acute intermittent porphyria having reduced or 50% reduced uroporphyrinogen I synthase activity and urinary concentrations of porphobilinogen and/or δ-aminolaevulinic acid exceeding that in control subjects. X, Subject not tested; †, subject dead, not tested.](image)
Uroporphyrinogen I synthase in porphyria

A reduction of about 32% in the enzyme activity was found in the ten subjects with clinically manifest acute intermittent porphyria, as compared with values in healthy normal subjects. As also reported by Sassa, Granick, Bickers, Levere & Kappas (1973a), Sassa, Granick, Bickers, Bradlow & Kappas (1974), Koehl & Abecassis (1975), Whitfield, Steward & Hensley (1975) and Mustajoki (1976), the enzyme activities from the porphyric subjects overlapped the values observed in healthy normal subjects. Thus measurement of the enzyme activity is less than completely reliable in the search for latent acute intermittent porphyria. However, when the enzyme activity of a suspected porphyric subject was compared with the values from his parents and/or siblings, a 2:1 ratio between the enzyme activity in a normal and affected subject was usual. By this use of an internal standard for each family, the enzyme-activity test was reliable in families A and B, and to a certain degree this was also true in family C. Both the subjects with clinically manifest porphyria in family C had enzyme activities near to the mean of the values in healthy normal subjects. Similar results were reported from Finland (Mustajoki, 1976), where five patients with acute intermittent porphyria, all relatives of each other, had normal enzyme activities. Furthermore, Peterson, Hamer-nyik, Bird & Labbé (1976) reported a considerable variation in enzyme activities even between manifestly porphyric subjects and possible latent cases.

The upper normal concentration of urinary porphobilinogen is about twice the concentration reported by With (1976). Although the present method elutes other Ehrlich-positive reactants from normal urine than porphobilinogen alone (With, 1968), this method gives correct quantitative determinations in urine samples having abnormally high concentrations of porphobilinogen (With, 1963; With, 1968).

Among the 82 relatives of the ten subjects with acute intermittent porphyria the enzyme test revealed 21 subjects with uroporphyrinogen I synthase deficiency who thus may be possible latent carriers of the condition. Two of these 21 subjects are children, who would not therefore be expected to show increased urinary excretion of the
porphyrin precursors. Among the other 19, only six had increased concentrations of porphobilinogen and/or δ-aminolaevulinate synthase.

Thus the enzyme test revealed 13 adult genetic carriers of acute intermittent porphyria that were not detected by use of urinary δ-aminolaevulinc acid and porphobilinogen measurements alone. Six carriers were detected by both methods. Only one subject had a significantly increased concentration of δ-aminolaevulic acid determinations.

If an internal reference of each family is available, I recommend that the erythrocyte uroporphyrinogen I synthase activities be measured in a search for latent acute intermittent porphyria in both adult and prepubertal subjects. However, equivocal answers should be expected.

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References


