Activation of Uroporphyrinogen Decarboxylase by Ferrous Iron in Porphyria Cutanea Tarda

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SUMMARY

Uroporphyrinogen decarboxylase was measured in the presence of ferrous iron, using mitochondria-free rat liver extracts as enzyme source. The activity of the enzyme was found to be increased at concentrations of ferrous iron from 0.01 mM, with maximal activity exhibited from 0.1 mM. Enzyme kinetics indicate that uroporphyrinogen decarboxylase reversibly binds ferrous iron, with a binding constant of approximately $5 \times 10^{-5}$ mol$^{-1}$. It is proposed that the effect of phlebotomy on patients with porphyria cutanea tarda is to mobilize storage iron in the liver to the active ferrous form, which activates hepatic uroporphyrinogen decarboxylase, the enzyme which is defective in this syndrome, with resultant clinical and biochemical remission.


Porphyria cutanea tarda (PCT) or symptomatic porphyria is one of the most common of the hepatic porphyrias encountered in South Africa. Clinically, the disease is manifested by skin lesions indistinguishable from those seen in variegate porphyria. Biochemically, a unique porphin excretory pattern is observed, with characteristic overproduction, accumulation and excretion of uroporphyrin of the I isomer series and, to a lesser extent, of porphyrins with 7, 6 and 5 carboxyl groups. Hepatic accumulation and excretion of the higher carboxylated porphyrins in PCT is consistent with the concept that the primary enzyme defect in the haem biosynthetic pathway in this syndrome is a decrease in the activity of hepatic uroporphyrinogen decarboxylase (EC 4.1.1.37), as has been demonstrated recently.

Iron has been implicated in the pathogenesis of PCT on the basis of clinical observation. Siderosis is almost invariably present in patients with the syndrome, although the degree of iron deposition may not be severe. Indeed, studies by Turnbull et al. indicate that total body iron in PCT is only modestly increased by 1-2 g. Clinical remission of PCT accompanied by a decrease in porphyrin excretion can be effected by repeated venesection, whereas this process can be prevented or reversed by oral iron ingestion.

Kushner et al. have tendered a possible biochemical explanation for the pattern of porphyrin excretion in PCT and the clinical association with disordered iron metabolism. These workers observed firstly that porcine liver uroporphyrinogen cosynthetase is inhibited by ferrous iron and that a greater conversion of porphobilinogen to uroporphyrinogen I occurs. Secondly, it was shown that uroporphyrinogen decarboxylase activity is inhibited in mitochondria-free porcine liver extracts pre-incubated with ferrous iron. It was proposed that, since uroporphyrinogen III and its oxidized form, uroporphyrin III, as well as iron, are inhibitors of uroporphyrinogen cosynthetase, the accumulation of these compounds resulting from impaired activity of uroporphyrinogen decarboxylase could lead to synthesis of uroporphyrin I and its accumulation.

The present report describes the activating effect of ferrous iron on rat liver uroporphyrinogen decarboxylase when ferrous iron is present in the assay system, and an alternative mode of action of iron in the pathogenesis of PCT is discussed.

METHODS

Animals and Treatment

Female Wistar rats weighing 160-200 g were used. One group of rats was fed with a diet of powdered rat cubes containing 0.2% hexachlorobenzene (practical grade, Eastman Kodak Co., Rochester, NY, USA) for 45 days. Animals were killed by decapitation after a 24-hour starvation period, during which time water was allowed ad libitum. Livers were excised, washed with ice-cold isotonic saline, blotted dry and weighed.

Uroporphyrinogen Decarboxylase (EC 4.1.1.37) Assay

Liver samples were homogenized under ice (1:10 w/v in 0.1M tris:HCl, pH 6.8) and homogenates centrifuged at 32,000 g in a refrigerated centrifuge at 4°C for 45 minutes. Supernatants thus obtained were used as the enzyme source. Uroporphyrinogen decarboxylase activity was measured by a radiochemical method as described previously. Briefly, the reaction mixture contained the following, in a total volume of 0.5 ml: \textsuperscript{14}C-porphobilinogen, 1.33 \mu mol; uroporphyrinogen synthetase (sufficient to produce 25 - 30 nmol uroporphyrinogen I); tris:HCl 50 \mu mol, pH 7.65. In some experiments, 0.01 - 2 \mu mol ammonium ferrous sulphate was included in the reaction mixture. After an initial incubation period of 30 minutes at 37°C in the dark, KH$_2$PO$_4$ (40 \mu mol), postmitochondrial liver supernatant containing 1.5 - 2.5 mg protein and 0.1M
tris: HCl, pH 6.8, were added in a volume of 1 ml. Reactions were terminated by the addition of 1 ml 3N HCl after a further incubation period of 30 minutes at 37°C. After centrifugation, the pH of the supernatant was adjusted to 3.1 with saturated sodium acetate, and porphyrins were adsorbed on talc and esterified overnight in 5% H₂SO₄ in methanol. The porphyrin methyl esters were transferred into chloroform and chromatographed on aluminium-backed silica-gel thin-layer chromatography plates; the silica gel at those areas which showed red fluorescence when viewed under ultraviolet light was scraped off and counted with 10 ml Instagel (Packard) in a Packard Tricarb scintillation counter.

The amount of uroporphyrinogen I formed in each assay was determined in a separate assay. The incubation conditions were identical to those described for the uroporphyrinogen decarboxylase assay, except that the enzyme source for uroporphyrinogen decarboxylase was replaced by the appropriate volume of 0.1M tris: HCl, pH 6.8. The quantity of uroporphyrinogen I formed was determined spectrophotometrically immediately after the second incubation period by conversion of uroporphyrinogen I to uroporphyrin I with 0.2M iodine in 0.3M KI, excess iodine immediately being reduced with sodium thiosulphate.

Separate control assays were performed, incubation conditions again being identical to those for the uroporphyrinogen decarboxylase assay, but the enzyme source for uroporphyrinogen decarboxylase was replaced by 0.1 tris: HCl, pH 6.8. Apparent uroporphyrinogen decarboxylase activity observed in these samples (due to the presence of uroporphyrinogen decarboxylase as a contaminant in the uroporphyrinogen synthetase preparation) was applied as a correction factor in the calculation of uroporphyrinogen decarboxylase activity.

**Other Methods**

Protein was determined by the method of Lowry et al. with bovine serum albumin as standard.

**RESULTS**

The in vitro effect of increasing concentrations of ferrous iron (as ammonium ferrous sulphate) up to 0.25 mM on rat liver uroporphyrinogen decarboxylase is demonstrated in Fig. 1. Similar results, with the same degree of activation by ferrous iron at the various concentrations, were obtained on the three other occasions when the experiment was performed (results not shown). Maximal activity was manifested at concentrations of ferrous iron from 0.10 mM, and further, the activity remained elevated at this level up to the highest concentration used, namely 2 mM (Table 1). The curve shown in Fig. 1 is a typical example of activator binding reversibly with enzyme. Using Michaelis-Menten kinetics, an approximate value of 5 × 10⁶ mol⁻¹ was obtained for the apparent binding constant of ferrous iron to uroporphyrinogen decarboxylase.

![Fig. 1. The in vitro effect of added ferrous iron as ammonium ferrous sulphate on the activity of rat liver uroporphyrinogen decarboxylase. The supernatant (32 000 g) of a liver homogenate from an untreated rat was used as enzyme source. Each point is the mean of two observations.](image)

**TABLE I. IN VITRO ACTIVATING EFFECT OF ADDED FERROUS IRON (AMMONIUM FERROUS SULPHATE) ON RAT LIVER UROPORPHYRINGEN DECARBOXYLASE**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Fe²⁺ (mM)</th>
<th>Uroporphyrinogen I decarboxylated per mg protein per h (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>—</td>
<td>1.9</td>
</tr>
<tr>
<td>0.4</td>
<td>—</td>
<td>9.9</td>
</tr>
<tr>
<td>0.8</td>
<td>—</td>
<td>10.6</td>
</tr>
<tr>
<td>1.2</td>
<td>—</td>
<td>10.3</td>
</tr>
<tr>
<td>1.6</td>
<td>—</td>
<td>9.4</td>
</tr>
<tr>
<td>2.0</td>
<td>—</td>
<td>10.6</td>
</tr>
<tr>
<td>45 days with 0.2% hexachlorobenzene</td>
<td>—</td>
<td>0.8 (0.4 - 1.4)</td>
</tr>
<tr>
<td>1.0</td>
<td>2.1</td>
<td>(1.4 - 2.7)</td>
</tr>
</tbody>
</table>

The mean value for two determinations of uroporphyrinogen decarboxylase activity at each concentration of Fe²⁺ is given, except in the case of rats treated with hexachlorobenzene, where 3 animals were used and values are expressed as a mean with the range for individual data in parenthesis.

An increase in enzyme activity of similar magnitude was observed when hepatic uroporphyrinogen decarboxylase was assayed in the presence of 1 mM ferrous iron, using the postmitochondrial liver supernatants from 3 rats treated with hexachlorobenzene for 45 days as enzyme source (Table 1).

**DISCUSSION**

The results of the present study demonstrate that the activity of hepatic uroporphyrinogen decarboxylase is increased in the presence of ferrous iron. Further, an increase in enzyme activity of similar magnitude in the presence of 1 mM ammonium ferrous sulphate was observed in the livers of rats treated for 45 days with hexachlorobenzene, i.e. rats with decreased uroporphyrinogen decarboxylase activity (Table 1). The observed increase in enzyme activity was real, since corrections
were made in each determination for the effects of the same concentrations of ferrous iron on the production of "C-uroporphyrinogen I substrate and on decarboxylation of "C-uroporphyrinogen I by contaminant enzymes.

Our finding that ferrous iron added in vitro directly activates uroporphyrinogen decarboxylase is in sharp contrast to that of Kushner et al., who have reported inhibition of the enzyme in porcine liver extracts pre-incubated with ferrous iron. This disparity may be attributed to a different methodological approach. When liver extracts are pre-incubated with ferrous iron, the divergent iron may be responsible for oxidative damage to the sulphhydryl-dependent uroporphyrinogen decarboxylase. However, when the activity of the enzyme is measured in the presence of divalent iron, oxidative damage may not have occurred to any marked extent, so that the activity of the enzyme is increased.

The activity of uroporphyrinogen decarboxylase has been shown to be decreased in the livers of both patients with PCT and rats treated with hexachlorobenzene, a compound which, when administered to rats, results in the development of a porphyria which biochemically closely resembles human PCT. Moreover, it has been shown that, in rats rendered siderotic before hexachlorobenzene feeding and in rats treated simultaneously with iron and hexachlorobenzene, iron appears to potentiate the porphyrin process, including a greater decrease in the activity of uroporphyrinogen decarboxylase. However, in vivo studies in rats have shown that hepatic siderosis induced by oral or intraperitoneal administration of iron does not increase the total amounts of excreted or accumulated porphyrins, nor is the activity of hepatic uroporphyrinogen decarboxylase affected. Further, PCT in humans is an unusual accompaniment of haemochromatosis or secondary iron overload. If the findings in rats are extrapolated to man, it is possible that the effect of iron overload is to potentiate the porphyrin process in patients who show a propensity to develop PCT as a result of liver insult (idiosyncratic or sporadic PCT), with resultant decreased hepatic uroporphyrinogen decarboxylase activity, or in patients who have a pre-existing generalized defect of the enzyme (familial PCT). The mechanism of the potentiating effect of iron on PCT is presently being investigated in detail in our laboratories.

Our findings of activation of uroporphyrinogen decarboxylase by ferrous iron may provide an explanation for the remission of clinical and biochemical symptoms of PCT in patients treated by repeated venesection. The effect of this treatment may be to mobilize iron stored as ferritin and haemosiderin in the liver to the active ferrous form. This activates hepatic uroporphyrinogen decarboxylase, the activity of which is decreased in PCT. A second effect of venesection may be to remove the inhibitory effect of iron on uroporphyrinogen cosynthetase and its apparent activating effect on uroporphyrinogen synthetase. This concept is still in keeping with the report that the activity of hepatic uroporphyrinogen decarboxylase was unchanged in a patient with PCT who had undergone successful treatment by repeated venesection. Firstly, the in vitro measurement of the activity of the enzyme is not performed in a situation where the ferrous iron concentration is the same as in the liver, and secondly the enzyme is not rate limiting in the haem biosynthetic pathway, as evidenced by asymptomatic carriers of familial PCT, who have decreased uroporphyrinogen decarboxylase activity but normal urinary and faecal porphyrin excretion.

We thank the South African Medical Research Council for financial support.

REFERENCES