PORPHOBILINOGEN EXCRETION IN CHEMICAL INDUCED PORPHYRIA: REVERSAL BY INDUCTION OF PORPHOBILINOGEN OXYGENASE

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1. Introduction

Porphobilinogen, the metabolic precursor of all the natural porphyrins, is oxidized by an enzyme called porphobilinogen oxygenase which is present both in animals and plants [1]. In the former it was isolated from rat liver microsomes where its low activity can be strongly induced by previous administration to the rats of pregnenolone or progesterone [2]. In this paper we describe the effect of pregnenolone administration to rats previously treated with 2-allyl-2-isopropylacetamide (AIA). This chemical induces in rodents the so-called chemical porphyria which mimicks the human hepatic porphyria. This abnormality is characterized by a strong increase in the urinary excretion of the porphyrin precursors porphobilinogen and δ-aminolevulinic acid. The increase is due to the 'de novo' sythesis of the hepatic enzyme δ-aminolevulinic acid synthetase (ALA-synthetase), the rate-controlling enzyme in porphyrin and heme biosynthesis [3]. The administration of pregnenolone to AIA-pretreated rats results in;1) a strong decrease in the amount of urinary excreted porphobilinogen, and in excretion of 5-oxo-2-hydroxyporphobilinogen, 2) a strong increase in the levels of porphobilinogen oxygenase activity, and 3) a slight increase in the levels of ALA-synthetase. Those results lend strong support to the idea of porphobilinogen oxygenase participation together with ALA-synthetase in the regulation of the physiological levels of porphobilinogen.

2. Materials and methods

2-Allyl-2-isopropylacetamide (AIA) was a generous gift of Hoffman-La Roche Inc. (Nutley, N. J.). Female Wistar rats of the Instituto de Fisiologia strain (100 to 150 gr) were used. All the experiments were carried out on fasted rats. During each experiment a group of eight rats were treated with AIA (250 mg/Kg) dissolved in a saline solution and administered in one subcutaneous injection. This treatment was followed at the indicated times (see Results) by the administration of pregnenolone dissolved in corn oil to four of the eight rats, while the other four were injected with the vehicle only and were used as controls. The pregnenolone was injected subcutaneously in four doses (2 mg/Kg each) over a period of 48 hr. Fasting conditions were kept throughout the experiment.

2.1. *Assay of hepatic enzymes*

To assay the hepatic enzymes the rats were decapitated, the excised livers were washed with an isotonic saline solution and microsomes were prepared as described elsewhere [2]. The final microsomal pellet was suspended in 0.05 M phosphate buffer (pH 7.4) and disrupted by treatment with deoxycholate (0.1%). An aliquot (0.5 ml) of the resulting suspension was applied to a DEAE-cellulose column (1 × 20 cm) equilibrated with phosphate buffer 0.01 M (pH 7.4) and eluted with the same buffer. Fractions of 1.5 ml were collected and porphobilinogen oxygenase activity was immediately measured as described elsewhere [1]. To assay ALA-synthetase activity liver homogenates were prepared and assayed as described by Marver et al. [4].

2.2. *Assay of urinary metabolites*

The urines of both groups of four rats each (those treated with pregnenolone and those injected with vehicle only), were collected at the indicated times,

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pooled separately and filtered through a Dowex 1-X4 resin column (2 × 20 cm) in its acid form. The column was washed with 50 ml of water to elute the δ-aminolevulinic acid, and then with 50 ml of 0.8 M acetic acid to elute the porphobilinogen [5]. The eluates were evaporated to dryness, the residues were dissolved in 0.5 ml of water, and the amounts of porphobilinogen and δ-aminolevulinic acid present were estimated on aliquots (50 μl) using standard methods [1,5]. The remaining aqueous solution containing the residue of the 0.8 N acetic acid eluate was applied to a Sephadex G-10 column (1 cm × 90 cm), equilibrated with water. The column was eluted with water and fractions of 0.5 ml were collected and examined by thin-layer chromatography on cellulose using the upper layer of butanol: acetic acid: water (4:1:5) mixture as developer [1]. 5-Oxo-2-hydroxyporphobilinogen (R_f 0.25) was detected in several fractions (14-16) together with porphobilinogen (R_f 0.50) after spraying with Ehrlich's reagent [1]. The oxoporphobilinogen containing fractions were evaporated to dryness at 30°C in vacuo, the pooled residues were dissolved in water-saturated *n*-butanol, and the solution was applied to a cellulose powder column (2 × 20 cm) previously equilibrated with the upper layer of the *n*-butanol: acetic acid: water (4:1:5) mixture. The column was eluted with the same solvent, and the fractions containing oxoporphobilinogen were combined and evaporated to dryness in vacuo.

3. Results

3.1. *Influence of fasting on the induction of porphobilinogen oxygenase*

Since the effect of pregnenolone on porphobilinogen oxygenase activity had been previously determined in rats fed ad libitum [2], and since the effect of AIA administration on the activity of the oxygenase was unknown, a preliminary study on its enzymatic activity under those conditions was carried out. Rats fasted for 24 hr, were treated with either AIA or pregnenolone as described in Methods. Non-fasted rats were fed laboratory chow freely and were given the same treatment. Porphobilinogen oxygenase was assayed after 72 hr of starting the experiment (table 1). Fasting had a slight enhancing effect on porphobilinogen oxygenase activity. The effect was increased by AIA administration, while pregnenolone had the strongest inducing effect either in fasted or non-fasted rats. Fasted rats were then chosen to carry out the further studies.

3.2. *Effect of pregnenolone on the urinary porphobilinogen excretion in AIA-pretreated rats*

The administration of AIA to a group of eight fasted rats resulted in a strong increase in urinary porphobilinogen excretion which reached a maximum after approximately 33 to 39 hr and then decreased rapidly (fig.1). Fifteen hours after the AIA treatment,

Table I

Induction of porphobilinogen oxygenase by pregnenolone and 2-allyl-isopropylacetamide (AIA) in fasted and non-fasted rats.

Rats	Treatment	Porphobilinogen oxygenase activity (nmol of porphobilinogen consumed/gr liver/hr)
Non-fasted	—	54 ± 15
Non-fasted	Pregnenolone	200 ± 25
Fasted	—	85 ± 11
Fasted	AIA	135 ± 20
Fasted	Pregnenolone	455 ± 50

Pregnenolone (2 mg/Kg) was dissolved in corn oil and injected subcutaneously. Four injections were made in 48 hr. Controls received the vehicle only. The AIA was dissolved in saline solution (250 mg/Kg) and injected subcutaneously. Porphobilinogen oxygenase was prepared by processing each liver separately. The preparations were purified through DEAE-cellulose [1]. Each value represents an average of twelve animals.

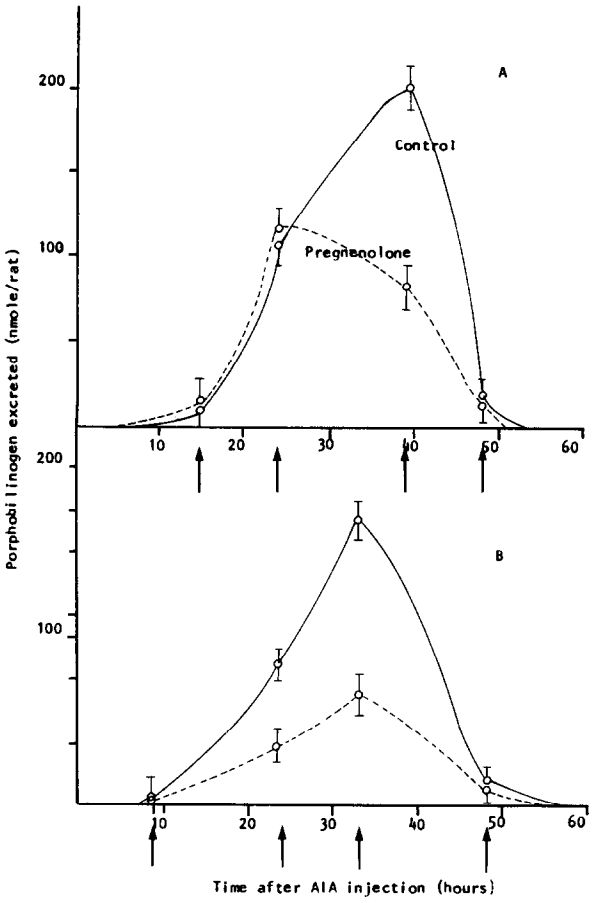

Fig.1. Effect of pregnenolone on urinary porphobilinogen excretion in AIA-pretreated rats. Fasted rats were injected subcutaneously with AIA (250 mg/Kg) dissolved in corn oil, was injected subcutaneously at the indicated times (↑) after AIA injection. Controls were simultaneously injected with vehicle only. Porphobilinogen was measured as described in Materials and methods. Each value represents an average of 40 pregnenolone treated rats (o---o)and 40 control rats (o——o).

pregnenolone (2 mg/Kg) was injected into four rats and this injection was repeated at intervals of 9, 15 and 9 hr. The remaining four rats were injected with vehicle at the same time intervals. After the second pregnenolone injection (fig.1A), a strong decrease in the concentration of urinary porphobilinogen was observed in the four pregnenolone treated rats, as compared to the four non-treated controls. The difference reached a maximum after 24 hr of starting the pregnenolone treatments (fig.1A). When the pregnenolone treatment was started after 9 hr of the initial AIA administration, the difference in the amount of excreted porphobilinogen between the treated and non-treated rats is noticeable after the first pregnenolone injection and the maximum difference is also reached after 24 hr of starting the pregnenolone treatment (fig.1B). Although fluctuations in the absolute values of excreted porphobilinogen were obtained after the AIA treatment, the effect of pregnenolone in decreasing the amount of excreted porphobilinogen was permanently observed after a total of twenty experiments with eight animals each. Pregnenolone administration had no systematic effect on the urinary concentration of δ-aminolevulinic acid that could be correlated with the observed decreased in porphobilinogen urinary levels.

5-Oxo-2-hydroxyporphobilinogen was isolated from the urines of the pregnenolone treated rats. The urines of four groups of four pregnenolone treated rats were collected after 33 hr of the AIA administration (fig.1B) and were purified through a Dowex 1-X4 column and 5-oxo-2-hydroxyporphobilinogen was isolated following the procedure described in Materials and methods. It was absent (or present in trace amounts) in the urines of the control groups.

3.3. *Effect of pregnenolone on hepatic porphobilinogen oxygenase and ALA-synthetase activities in AIA pretreated rats*

An increase in hepatic porphobilinogen oxygenase activity was observed in the AIA pretreated rats which were injected with pregnenolone, as compared with the non injected controls. Oxygenase activity increased constantly with the increasing amounts of pregnenolone injected (fig.2). When porphobilinogen oxygenase and ALA-synthetase were simultaneously determined in the livers of control and of pregnenolone treated animals, a slight increase in the ALA-synthetase activity was found in the latter (fig.2C). Hence the decrease in porphobilinogen formation cannot be attributed to an hypothetical inhibitory effect of the pregnenolone on ALA-synthetase.

4. Discussion

The obtained results indicate that an increased

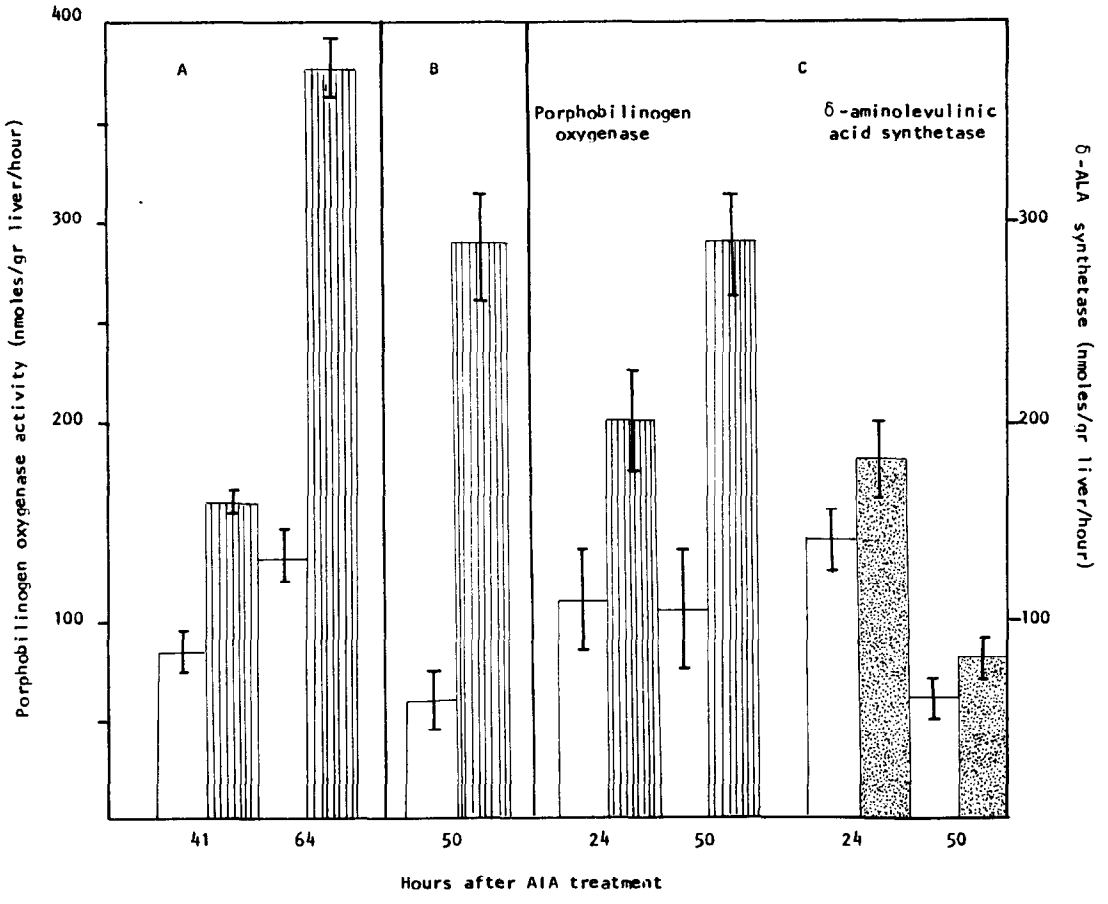

Fig.2. Effect of pregnenolone on hepatic porphobilinogen oxygenase and ALA-sythetase in AIA-pretreated rats. Female rats were treated with AIA followed by pregnenolone or with AIA followed by vehicle as described in the text. At the indicated times after the AIA treatment the rats were killed and hepatic porphobilinogen oxygenase activity was determined [2] in the controls ( ) and in the pregnenolone treated animals ( ). A) Pregnenolone was injected 15 hr after the AIA-treatment, B) Pregnenolone was injected 9 hr after AIA treatment. C) The experiment was carried out as in B); the livers were then halved, ALA-synthetase activity [4] was determined on one half and porphobilinogen oxygenase activity on the other half. ALA-synthetase activity was determined in the pregnenolone treated ( ) and the control animals ( ). The values are an average of 20 pregnenolone treated animals and of 20 control animals.

metabolic formation of porphobilinogen can be counteracted by its enzymatic oxidation to 5-oxo-2-hydroxy-porphobilinogen if porphobilinogen oxygenase activity is increased to high levels. Both enzymes might be simultaneously induced under similar conditions (table 1 and fig.2C), and thus check a sudden increase in the physiological concentration of porphobilinogen produced by a rise in ALA-synthetase activity. The properties of the reducing system [2] necessary for oxygenase activity 'in vivo' must further be studied in order to obtain a complete picture of the physiological role of the enzyme.

Acknowledgements

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References

[1] Frydman, R. B., Tomaro, M. L., Wanschelbaum, A., Andersen, E. M., Awruch, J. and Frydman, B. (1973) Biochemistry 12, 5253–5262; Frydman, B., Frydman, R. B. and Tomaro, M. L. (1973) Molec. Cell. Biochem. 2, (2), 121–136.

[2] Tomaro, M. L., Frydman, R. B. and Frydman, B. (1973) Biochemistry 12, 5263–5268.

[3] a) Granick, S. (1966) J. Biol. Chem. 241, 1359–1375; b) Tschudy, D. P., Perlroth, M. G., Marver, H. S., Collins, A., Hunter, Jr. G. and Rechcigl, Jr. M. (1965) Proc. Natl. Acad. Sci. U.S. 53, 841–846.

[4] Marver, H. S., Tschudy, D. P., Perlroth, M. G., Collins, A. (1966) J. Biol. Chem. 241, 2803–2809.

[5] Falk, J. E. (1964) Porphyrins and Metalloporphyrins, 155–157, Elsevier, Amsterdam.