PORPHOBILINOGEN OXYGENASE INDUCTION BY 2-ALLYL-2-ISOPROPYLACETAMIDE TREATMENT

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1. Introduction

It is known that 2-allyl-2-isopropylacetamide (AIA) induces in rodents a chemical porphyria which mimicks human hepatic porphyria [1,2]. This derrangement in porphyrin metabolism is characterized by a strong increase in the urinary excretion of the porphyrin precursors porphobilinogen)PBG) and 5-aminolevulinic acid (ALA), and these increases have been traced to the excessive induction of ALA synthetase in the rats by the porphyria-inducing chemical [3]. ALA synthetase is considered to be the main regulatory enzyme of porphyrin biosynthesis [4], and its induction raises ALA concentration above the normal values. The next enzyme in the metabolic sequence – ALA dehydratase – transforms the excess of ALA into PBG with the net result of an increase in the concentration of both porphyrin precursors, part of which is excreted in urine. We have reported [5] that the administration of pregnenolone to ALA pretreated rats produced a marked decrease in the amount of the PBG excreted in urine, and a simultaneous strong increase in the activity of hepatic porphobilinogen oxygenase activity. The latter is a novel mixed-function type of oxidase which we have isolated from plant and animal sources [6,7], which oxidizes PBG to 5-oxo-2-hydroxy-porphobilinogen, a metabolite which is not transformed into porphyrins anymore. PBG-oxygenase isolated from either rat liver or wheat-germ is a cationic iron–sulfur protein possessing allosteric kinetics and a multimer structure [8]. We want to report now that the administration of AIA not only induces ALA-synthetase in rats, but that it also induces PBG -oxygenase as well. The maximum induction of the latter is coincident in time with the peak of PBG excretion, and has a temporal separation of the maximum of ALA-synthetase activity. The data suggest that the sequential induction of ALA-synthetase and PBG-oxygenase might involve a metabolic response to the problem of excessive PBG formation.

2. Materials and methods

2-Allyl-2-isopropylacetamide (AIA) was a generous gift of Hoffman-La Roche Inc. (Nutley, NJ). Female Wistar rats (170–200 g), were fed freely on rat chow supplemented with wheat grain as an additional carbohydrate source. One week before starting the experiments the wheat grain was removed from the diet, and after the week was completed the rats were fasted during the remaining course of the studies. They were kept in metabolic cages which allowed the collection of the urines, and they were given water freely. Twenty-four hours after the fasting was started, the control rats were injected subcutaneously with a saline solution, while the treated rats were injected with AIA (250 mg/kg) in a NaCl saline solution. Urine was collected 24, 48 and 72 h after the injections and the amount of excreted ALA and PBG was measured by filtering the pooled urines of three rats through a Dowex 1-X4 resin column (2 × 20 cm) in its acid form. The column was washed with 50 ml water to elute ALA, and then with 50 ml 0.8 M acetic acid to elute PBG. The eluates were evaporated to dryness, the residues were dissolved in 0.5 ml water, and the amounts of ALA and PBG present were estimated on aliquots

using standard methods [9]. Both ALA and PBG were absent in the urine of the control rats.

2.1. *Assay of hepatic enzymes*

The livers of the control and treated rats were excised from anesthesized animals, washed with an isotonic NaCl saline solution, and homogenized in a Potter-Elvejhem homogenizer in 8 vol. 0.25 M sucrose (w/v). The suspension was centrifuged at 20 000 × *g* for 15 min, and the supernatant was centrifuged for 1 h at 105 000 × *g*. The microsomal pellet thus obtained was suspended in a saline solution (phosphate buffer 0.5 M, KCl 0.15 M, pH 7.4), again centrifuged at 105 000 × *g* for 30 min, and the pellet was suspended in phosphate buffer 0.05 M (pH 7.4) to protein concentration of 10–15 mg/ml. PBG-oxygenase was assayed by incubating in final vol. 100 μl; phosphate buffer (10 μmol, pH 7.4), PBG (15 nmol), sodium dithionite (100 nmol), and 50 μl microsomal suspension. Incubations were performed at 37°C for 60 min. PBG consumption was estimated with Ehrlich's reagent as described [6]. ALA-synthetase was assayed in liver homogenates as described by Marver et al. [10].

3. Results

3.1. *Excretion of ALA and PBG in the urines of AIA treated rats*

The maximum excretion of both ALA and PBG was observed in the urines collected between 24 h and 48 h after the AIA injection, though some ALA was already present in the 24 h urine (table 1). We have reported [5] that PBG excretion reaches a peak about 33 h after the administration of AIA. The

Table 1
Urinary ALA and PBG excretion in AIA treated rats

Time after AIA injection (h)	ALA excreted (nmol/rat)	PBG excreted (nmol/rat)
0–24	21.4 ± 1.6	0
24–48	120 ± 14	146 ± 11
48–72	0	0

ALA and PBG were determined at the indicated times as described in the text. Each value represents a mean ± standard error of the mean (SEM) of 20 rats

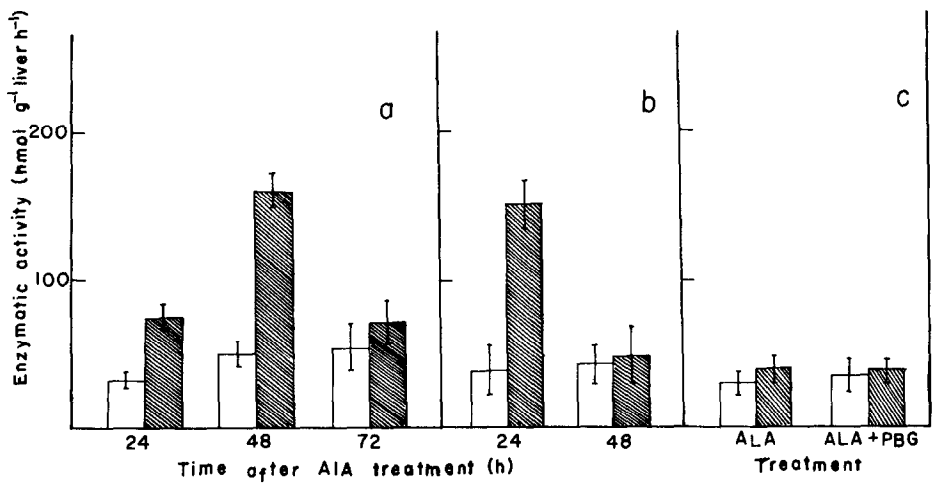

Fig.1. Effect of AIA on: (a) induction of hepatic microsomal PBG-oxygenase; (b) induction of hepatic ALA-synthetase; (c) effect of ALA (8 mg/kg) and PBG (4 mg/kg) on the induction of PBG-oxygenase. Livers from control (clear bars) and treated (shaded bars) rats were obtained and used as described in the text. Results are expressed as mean ± standard error of the mean (SEM) from at least 6 experiments, each utilizing livers from 3 control rats and 3 treated rats.

urines collected in the 48–72 h period were devoid of both metabolites. When larger rats were used (230–250 g), the excretion peak of PBG was reached during the first 24 h, and then decreased rapidly.

3.2. *Hepatic PBG-oxygenase and ALA-synthetase activity in AIA treated rats*

Microsomal PBG-oxygenase activity and ALA-synthetase activity in the excised livers of the control and AIA treated rats was investigated (fig.1). Oxygenase activity in the AIA treated rats increased simultaneously with ALA and PBG excretion and reached a maximum 48 h after the AIA treatment. No significant difference in PBG-oxygenase activity between the control and the treated rats was found 72 h after the AIA treatment (fig.1a). The higher values of ALA-synthetase activity were found in the livers excised 24 h after the AIA treatment; while in those excised 48 h after the treatment the values were almost normal (fig.1b). These last results are in agreement with [3], which reported a peak in ALA-synthetase induction in the 8–24 h period after AIA treatment.

When larger rats were used, and the peak of PBG excretion was reached during the first 24 h period after the AIA treatment (see above), the peak of oxygenase activity was also found in the livers excised 24 h after the treatment and was absent in the livers excised after 48 h.

To examine if ALA or PBG, or both together, were involved in the observed PBG oxygenase induction, they were administered to the rats, and oxygenase activity was examined. ALA (4 mg/kg, 8 mg/kg and 12 mg/kg) was subcutaneously injected into the rats and the excretion of these compounds in the urines was assayed 6, 15 and 24 h later. The 6 h urines contained 90% of all the excreted ALA and PBG formed at expense of the injected ALA, in a 7:10 ratio of ALA:PBG. No significant increase of PBG-oxygenase activity over the control runs was detected in the ALA-treated animals (fig.1c). When ALA (8 mg/kg) and PBG (4 mg/kg) were administered together, similar results were obtained. Approx. 30% the injected porphyrin precursors were recovered in the urines. It was evident thet PBG-oxygenase activity was induced by the AIA treatment, and not by the porphyrin precursors produced as consequence of the treatment.

4. Discussion

The above-mentioned results show that a chemical which increases the physiological levels of the porphyrin precursors ALA and PBG, induces simultaneously the formation of a microsomal mixed-function oxidase (PBG-oxygenase) which can partially dispose of the excessive PBG. The same is true for a hormonal imbalance such as a prolonged fasting, which is known [11,12] to induce the urinary excretion of ALA and PBG in humans. The transfer of the rats from the carbohydrate-rich diet to the carbohydrate-poor diet followed by a relatively prolonged fasting, is by itself a factor in the induction of the hepatic microsomal PBG-oxygenase (fig.1, clear bars). The activity of the microsomal enzyme decreases with the disappearance of excess PBG, which again lends support to the idea of a participation of the oxygenase in the balance of the in vivo concentration of the first porphyrin precursor.

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