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Research Article

The ability of Sn(tin)-protoporphyrin to inhibit the induction of hepatic delta-aminolevulinate (ALA) synthase by allylisopropyl acetamide (AIA) was examined in the adult rat. Doses of Sn-protoporphyrin of 1, 10, and 50 mumol/kg body wt resulted in decreases in AIA-induced hepatic ALA-synthase activity of 32, 52, and 60%, respectively, compared with rats treated with AIA alone; inhibition of ALA-synthase was not a direct effect of Sn-protoporphyrin. This inhibition of the enzyme activity in liver was reflected in concurrent decreases in urinary excretion of ALA and porphobilinogen (PBG). The increased urinary excretion of ALA and PBG observed following AIA treatment was reduced by the lowest dose of Sn-protoporphyrin (1 mumol/kg body wt) and abolished completely by the higher doses of the metalloporphyrin (10 and 50 mumol/kg body wt). These findings in a rat model of hepatic porphyria suggest that Sn-protoporphyrin may be useful in the treatment of acute exacerbations of "inducible" hepatic porphyrias in man, especially since Sn-protoporphyrin, unlike hematin which is presently used for this purpose, is neither degraded by nor induces the activity of heme oxygenase.

Sn-Protoporphyrin Suppresses Chemically Induced Experimental Hepatic Porphyria

Potential Clinical Implications

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Abstract

The ability of Sn(tin)-protoporphyrin to inhibit the induction of hepatic δ-aminolevulinate (ALA) synthase by allylisopropyl acetamide (AIA) was examined in the adult rat. Doses of Sn-protoporphyrin of 1, 10, and 50 μmol/kg body wt resulted in decreases in AIA-induced hepatic ALA-synthase activity of 32, 52, and 60%, respectively, compared with rats treated with AIA alone; inhibition of ALA-synthase was not a direct effect of Sn-protoporphyrin. This inhibition of the enzyme activity in liver was reflected in concurrent decreases in urinary excretion of ALA and porphobilinogen (PBG). The increased urinary excretion of ALA and PBG observed following AIA treatment was reduced by the lowest dose of Sn-protoporphyrin (1 μmol/kg body wt) and abolished completely by the higher doses of the metalloporphyrin (10 and 50 μmol/kg body wt). These findings in a rat model of hepatic porphyria suggest that Sn-protoporphyrin may be useful in the treatment of acute exacerbations of "inducible" hepatic porphyrias in man, especially since Sn-protoporphyrin, unlike hematin which is presently used for this purpose, is neither degraded by nor induces the activity of heme oxygenase.

Introduction

The porphyrias comprise a group of diseases each of which is characterized by a specific genetically determined defect of one of the enzymes of the heme biosynthetic pathway (1). While the defect is usually inherited, the internal milieu, especially the hormonal and nutritional status of the individual, is a vital factor in the clinical expression of the genetic defect in forms of the disease such as acute intermittent porphyria. Clinical expression of the disease is almost invariably accompanied by the accumulation of those products of heme biosynthesis generated proximal to the defective enzyme (1, 2). The first and rate-limiting enzyme in heme synthesis is mitochondrial δ-aminolevulinic acid (ALA)[1] synthase which catalyzes the condensation of glycine with succinyl-CoA to form ALA (3). In the "inducible" forms of hepatic porphyria (AIP, hereditary coproporphyria, and variegate porphyria), treatment of an acute attack is directed toward suppression of ALA-synthase and the effectiveness of therapy is monitored by measuring porphyrin precursor excretion and by clinical status (1, 2). Many therapeutic approaches have been applied to these diseases but only two clinical strategies are widely accepted: high carbohydrate loading (4–6), and intravenous hematin administration (7–9). Recently, we have described a third therapeutic approach, the use of synthetic luteinizing hormone-releasing hormone agonist analogues in those porphyric women who have cyclical attacks of the disease in relation to their menses (10).

We have previously reported the ability of the synthetic heme analogue Sn(tin)-protoporphyrin to suppress the activity of heme oxygenase and decrease bilirubin production and hyperbilirubinemia in animals and humans (11–15). Sn-protoporphyrin administration has also been shown to result in a rapid (within 60 min), nearly complete, heme saturation of rat hepatic tryptophan pyrrolase (16), which strongly suggests that some fraction of functional hepatic heme content is transiently increased following administration of the compound. We hypothesized that Sn-protoporphyrin might inhibit ALA-synthase and porphyrin precursor accumulation. This report describes our studies to test this hypothesis in a rat model of porphyria in which ALA-synthase is induced after the destruction of endogenous heme by allylisopropyl acetamide (AIA) (17–19). The results of this study indicate that Sn-protoporphyrin is highly effective in suppressing AIA-induced hepatic porphyria in the rat.

Methods

Male Sprague-Dawley rats (150–200 g), purchased from Taconic Farms Inc., Germantown, NY, were maintained in metabolic cages at 22°C on a 12:12-h light/dark cycle with free access to powdered rat chow and water. After a minimum of 5 d acclimatization to the cages, animals were injected subcutaneously with saline, Sn-protoporphyrin, and/or AIA at the doses indicated in the legends to figures. During experiments, urine was collected in light-shielded containers and frozen at −20°C until assay. Tissue preparation and measurement of mitochondrial ALA-synthase activity and protein were carried out as previously described (20, 21). Urinary ALA and porphobilinogen (PBG) were measured as described (22) using modified Ehrlich's reagent (23), and the results were expressed as micrograms per 24 h. Sn-protoporphyrin concentrations in urine were measured fluorometrically by the method of Simionatto et al. (24). Differences between means were examined by the *t* test.

Results

Fig. 1 depicts the time course of the effect of Sn-protoporphyrin (10 μmol/kg body wt., subcutaneously) on hepatic ALA-synthase

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1. *Abbreviations used in this paper:* AIA, allylisopropyl acetamide; ALA, δ-aminolevulinic acid; PBG, porphobilinogen.

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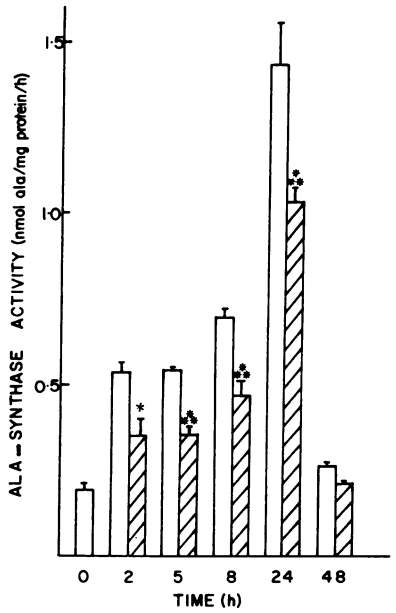

Figure 1. Time course of hepatic mitochondrial ALA-synthase activity after AIA (□; 400 mg/kg body wt; subcutaneously) or AIA with Sn-protoporphyrin (■; 10 μmol/kg body wt; subcutaneously). Animals were treated at 0 h and killed at the indicated times. Means± SEM are presented; $n = 3$, $*P < 0.05$, $***P < 0.01$ compared with respective AIA-treated control.

activity in AIA-treated rats. Both induction of the enzyme by AIA and concomitant inhibition of the induction response by Sn-protoporphyrin were detectable at 2 h, maximal at 24 h, and had returned to base-line levels by 48 h. Accordingly we used 24-h time-points in subsequent experiments. The dose response of Sn-protoporphyrin inhibition of AIA-stimulated ALA-synthase activity is shown in Fig. 2. Doses of Sn-protoporphyrin of 1, 10, and 50 μmol/kg body wt led to 32, 52, and 60% decreases, respectively, in ALA-synthase activity compared with AIA treatment alone. Direct addition of Sn-protoporphyrin (250 μM) to mitochondria from control or AIA-treated rats was without effect on ALA-synthase activity (data not shown).

Fig. 3 displays the concomitant effects of Sn-protoporphyrin on urinary excretion of ALA and PBG during three successive 24-h periods following the treatments indicated. The AIA-induced increases in urinary ALA and PBG were maximal during the period of 24–48-h after treatment and had returned to base

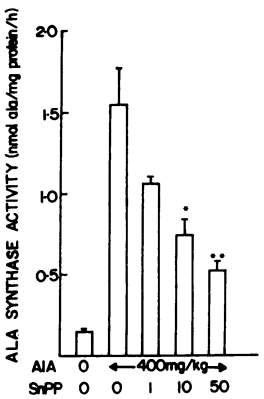

Figure 2. The dose response of Sn-protoporphyrin (*SnPP*) inhibition of ALA-synthase after AIA. All rats except controls were treated with AIA (400 mg/kg body wt; subcutaneously)±Sn-protoporphyrin subcutaneously in the doses indicated. Animals were killed at 24 h. Means±SEM are presented; $n = 3$. $*P < 0.05$, $**P < 0.02$, $***P < 0.01$ compared with previous point.

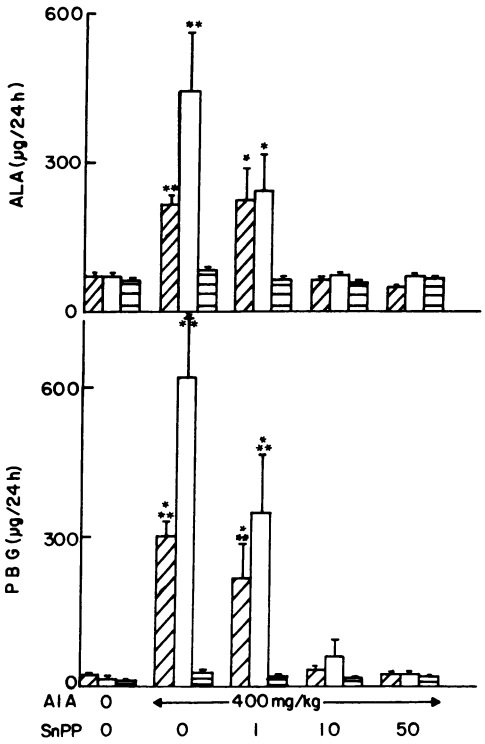

Figure 3. The effect of Sn-protoporphyrin (*SnPP*) on the urinary excretion of ALA and PBG in AIA-treated rats. *Top:* ALA; *bottom:* PBG. All rats except controls were treated at 0 h with AIA (400 mg/kg body wt; subcutaneously)±Sn-protoporphyrin at the indicated concentrations. All urines were collected for three successive 24-h periods (■, 0–24 h; □, 24–48 h; ▤, 48–72 h) and analyzed for ALA and PBG content. Means±SEM are presented; $n = 3$–9. $*P < 0.05$, $**P < 0.02$, $***P < 0.01$ compared with controls. Additionally, for ALA, at 24–48 h, Sn-protoporphyrin, 10 μmol/kg body wt, was significantly different from 1 μmol/kg body wt ($P < 0.05$). For PBG, both 0–24 h, and 24–48 h, Sn-protoporphyrin, 10 μmol/kg body wt, was significantly different from 1 μmol/kg body wt ($P < 0.05$).

line in the 48–72-h period. Sn-protoporphyrin administration resulted in a dose-dependent decrease in the content of ALA and PBG in urine of AIA-treated rats; 1 μmol/kg body wt resulted in an ~50% reduction, while 10 and 50 μmol/kg body wt. completely eliminated the AIA-induced increases in both ALA and PBG. To ensure that Sn-protoporphyrin was not interfering with the urinary ALA and PBG determinations, the concentration of Sn-protoporphyrin was measured (24) in the urine of rats treated with high (50 μmol/kg body wt) and low (1 μmol/kg body wt) doses of Sn-protoporphyrin. These concentrations, 140 and 3 μmol/liter, respectively, were added directly to urine samples from rats treated with AIA only, and then, ALA and PBG were measured. No significant changes were noted (e.g., comparing AIA-treated rat urine with and without 140 μmol/liter Sn-protoporphyrin, respective values [μg/3 ml] were: ALA, 12.72±0.84 vs. 11.75±0.70; PBG, 17.38±1.07 vs. 19.74±2.49; $n = 3$, x±SEM).

Discussion

This study demonstrates that Sn-protoporphyrin administration to AIA-treated rats results in a dose-dependent decrease in the

induction of hepatic ALA-synthase. The synthetic metalloporphyrin also produced a dose-dependent decrease in urinary excretion of ALA, the product of ALA-synthase, and of PBG, the product of ALA-dehydratase, which is the next enzyme in the heme pathway.

We chose to test the effect of Sn-protoporphyrin on AIA induction of hepatic ALA-synthase in fed rats because most patients with porphyria consume a relatively high carbohydrate diet which is usually increased either orally or intravenously during an acute crisis. However, the compound is equally effective in starved rats although basal and AIA-stimulated ALA-synthase activities are twofold higher proportionately than in fed rats (data not shown).

There are several potential mechanisms for the observed effect of Sn-protoporphyrin on AIA-induced ALA-synthase activity. The increased heme saturation of tryptophan pyrrolase after Sn-protoporphyrin administration (16) reflects a transiently increased functional hepatic heme pool resulting from Sn-protoporphyrin inhibition of hepatic heme catabolism (11), and heme is well known to inhibit ALA-synthase messenger RNA transcription (25) and its translation (26, 27), and the translocation of the protein into mitochondria (28, 29), as well as to directly inhibit ALA-synthase (30). We have recently reported that following Sn-protoporphyrin administration to bile duct-cannulated rats, there is a large increase in excretion of heme into bile (31) but this probably represents a compensatory mechanism to eliminate heme following heme oxygenase inhibition by Sn-protoporphyrin. It is also possible that Sn-protoporphyrin bears sufficient structural similarity to heme that it can itself directly mimic the regulatory action of heme in repressing ALA-synthase formation.

Whatever the proximate mechanism of the inhibitory effect of Sn-protoporphyrin on ALA-synthase formation, this compound might be useful in the treatment of acute exacerbations of "inducible" hepatic porphyrias. Unlike hematin, Sn-protoporphyrin is not degraded by heme oxygenase nor does it induce this enzyme, an action which would enhance exogenous and endogenous heme catabolism, and thus attenuate the therapeutic response to hematin. In animal studies, we have observed no overt toxicity with doses of Sn-protoporphyrin greatly exceeding those used in this study and administered at weekly intervals for a period as long as 32 wk (32). Whether low doses (i.e., ~1.0 μmol/kg body wt) of Sn-protoporphyrin would be as effective in decreasing the enhanced activity of hepatic ALA-synthase in the porphyria patient in crisis as they are in suppressing heme oxygenase activity and hyperbilirubinemia in animals and man (15) is not yet known, but this question is presently under investigation in this laboratory. Finally, other potential advantages of Sn-protoporphyrin are that there is no known in vivo mechanism for the enzymatic degradation of the metalloporphyrin and that the compound persists in tissues for periods of up to 7 d after a single dose (33). These facts suggest that low doses of Sn-protoporphyrin may prove useful in the treatment of acute attacks of the "inducible" hepatic porphyrias.

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