

Sn-Protoporphyrin Rapidly and Markedly Enhances the Heme Saturation of Hepatic Tryptophan Pyrrolase

Evidence that This Synthetic Metalloporphyrin Increases the Functional Content of Heme in the Liver

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Abstract

Sn-protoporphyrin is a potent competitive inhibitor of heme oxygenase, the rate-limiting enzyme in heme degradation to bile pigment, and has been successfully utilized to suppress hyperbilirubinemia in a variety of experimental and naturally occurring forms of jaundice in animals and man. The compound is presumed to act *in vivo* primarily by inhibiting heme oxidation; thus it would be reasonable to expect that preservation of some functional moiety of cellular heme from degradation by heme oxygenase would occur after Sn-protoporphyrin administration. We have examined this question in liver by studying the heme saturation of tryptophan pyrrolase, the heme-dependent enzyme which controls the first and rate-limiting step in the catabolism of L-tryptophan.

Sn-protoporphyrin, in doses (10 $\mu\text{mol/kg}$ body wt) which entirely suppress neonatal hyperbilirubinemia in the experimental animal, leads to a very rapid (~ 30 – 60 min) increase in the heme saturation of tryptophan pyrrolase from normal levels of ~ 50 – 60% to nearly 100% . The effect peaks at 1–2 h and lasts for at least 12 h. Sn-protoporphyrin is also able to block the rapid and marked decline in heme saturation of tryptophan pyrrolase elicited by inorganic cobalt, a potent inducer of heme oxygenase in liver. These findings establish clearly that after the administration of Sn-protoporphyrin in the whole animal, a functionally active heme pool, the one related to tryptophan pyrrolase, is rapidly increased in liver, confirming that the metalloporphyrin inhibits the degradation of endogenous heme by heme oxygenase.

Introduction

The synthetic heme analogue, Sn-protoporphyrin, significantly inhibits heme oxidation rates in various tissues (1–4) and has been successfully used to completely suppress neonatal hyperbilirubinemia in experimental animals (1, 2, 5) and to partially suppress elevated plasma levels of bilirubin in a variety of experimentally induced or naturally occurring form of jaundice in animals and man (6–8). The mode of action of this compound in suppressing jaundice appears to be based primarily on its ability to act as potent and sustained competitive inhibitor substrate (1, 9) for heme oxygenase, the rate-limiting

enzyme in the degradation of heme to bile pigment. Evidence based on metabolic studies of the conversion of endogenous as well as exogenous heme to bilirubin in bile duct-cannulated animals supports this view (8, 10), as do recent findings concerning the ability of Sn-protoporphyrin to diminish carbon monoxide production from endogenous as well as exogenous heme sources in adult animals (11).

There has been, however, up to the present time, no unequivocal proof that some functionally active fraction of cellular heme is in fact preserved from degradation by Sn-protoporphyrin inhibition of heme oxygenase. In this report we have examined this question by exploring the changes which are induced by Sn-protoporphyrin in the activity of the heme-dependent hepatic enzyme, tryptophan pyrrolase. The results of these experiments provide clear evidence that this synthetic metalloporphyrin does inhibit the degradation of heme in the whole animal, as reflected in the striking and prompt changes in the heme saturation of tryptophan pyrrolase which the metalloporphyrin produces in liver.

Methods

Male Sprague-Dawley rats (180–200 g), purchased from Taconic Farms, Inc., Germantown, NY, were used throughout and were injected with the synthetic heme analogues Sn-, Cr-, Zn-, or Mn-protoporphyrin (10 $\mu\text{mol/kg}$ body wt s.c.), or CoCl_2 (250 $\mu\text{mol/kg}$ body wt s.c.) as noted. Control animals received an equivalent volume of 0.9% NaCl. Tissue preparation of liver fractions for enzymatic assays was carried out as previously described (1); δ -aminolevulinic acid (ALA)¹ synthase was determined in mitochondria (12) and tryptophan pyrrolase, both in the absence (holoenzyme) and the presence (total enzyme) of added heme (2 μM) in liver homogenates (13). The latter enzyme activity was calculated from the linear phase of kynurenine formation. The percent heme saturation for tryptophan pyrrolase was expressed as the ratio of holoenzyme to total enzyme ($\times 100$). Protein determinations (14) and statistical analyses were made as described previously (7). Each data point represents the mean value of determinations in three to six animals.

Results

The injection of Sn-protoporphyrin in a single dose (10 $\mu\text{mol/kg}$ body wt s.c.) which is known to completely suppress neonatal hyperbilirubinemia in the experimental animal (1, 2) caused a marked and rapid (within 30') increase in the percent heme saturation of hepatic tryptophan pyrrolase (Fig. 1). This increase reached nearly 100% saturation from a normal value of ~ 50 – 60% ; the increase peaked at 1–2 h and then subsided

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1. Abbreviations used in this paper: ALA, δ -aminolevulinic acid.

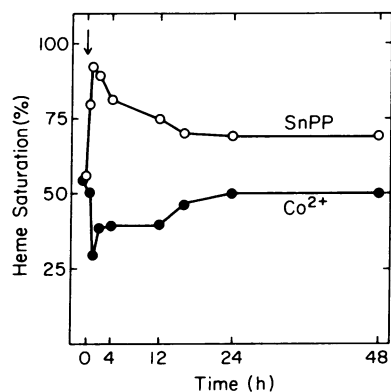


Figure 1. Changes in the percent heme saturation of hepatic tryptophan pyrrolase in response to Sn-protoporphyrin (SnPP) and CoCl₂ administration. Animals were injected with Sn-protoporphyrin (10 μ mol/kg body wt) or CoCl₂ (250 μ mol/kg body wt) subcutaneously at 0 h, 1, and the percent heme saturation of tryptophan pyrrolase was calculated at different time intervals as described in the text. Sn-protoporphyrin treated, —○—; CoCl₂-treated, —●—. Data are expressed as the means for three to six animals for each time point.

to a level of ~80% during the period of 4–12 h after injection of the metalloporphyrin. At 72 h (data not shown), heme saturation of the enzyme had returned to near-normal levels (~60%).

CoCl₂ elicits an approximately 10-fold increase in heme oxygenase activity in the liver 16 h after its administration in rats (15), with the induction response being detectable within 1–2 h; a concurrent transient (2–6 h) suppression followed by a rebound (~16 h) increase of ALA-synthase activity also occurs (15). These enzymic changes lead to substantial declines in the contents of cellular heme and the heme protein, cytochrome P-450 (15). The heme-depleting effects of CoCl₂ were manifested in the liver by a rapid decline in the degree of heme saturation of tryptophan pyrrolase (Fig. 1), confirming previous studies (16, 17); this decline was nearly reciprocal in extent and in time course to the increase in heme saturation of the enzyme produced by Sn-protoporphyrin (Fig. 1). These findings are consistent with the ability of the metalloporphyrin to potentially inhibit heme oxygenase and that of the inorganic metal to potentially induce the enzyme, thereby enhancing or diminishing cellular contents of heme, respectively. The combined administration of both agents produced no significant changes in heme saturation of tryptophan pyrrolase (data not shown), demonstrating the ability of Sn-protoporphyrin to block completely the CoCl₂-induced depression in the degree of heme saturation of this enzyme.

The rate-limiting enzyme of heme synthesis, ALA-synthase, is also responsive to the content of cellular heme, with repression of the enzyme synthesis occurring with doses of heme markedly smaller than those which induce heme oxygenase (18, 19); in the experiment depicted in Fig. 2, ALA-synthase was not significantly reduced in activity by the same dose of Sn-protoporphyrin which resulted in a rapid and complete heme saturation of tryptophan pyrrolase (Fig. 2) as well as in a marked reduction of heme oxygenase activity (data not shown). Thus, these data confirm the responsiveness of the heme saturation index of tryptophan pyrrolase to subtle changes in cellular heme content and indicate that the percent heme

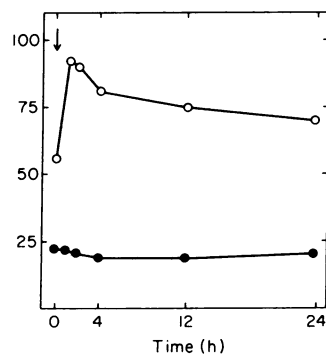


Figure 2. Effects of Sn-protoporphyrin on the percent heme saturation (—○—) of tryptophan pyrrolase and on ALA-synthase activity (—●—) in rat liver. Sn-protoporphyrin (10 μ mol/kg body wt.) was administered subcutaneously at 0 h, 1. Animals were sacrificed at the times indicated and the percent heme saturation of tryptophan pyrrolase and ALA-synthase activities were determined as described in the

text. ALA-synthase activities is expressed as (nanomoles of ALA per milligram per hour) $\times 10^{-2}$. Data are expressed as the mean values for three to six animals at each time point.

saturation of this enzyme is a more sensitive index of changes in cellular heme concentration than is ALA-synthase activity, as has been suggested previously (13).

The response of tryptophan pyrrolase to the administration of other synthetic heme analogues was variable. Cr-protoporphyrin, the only other heme analogue which has been shown to be capable of completely suppressing neonatal hyperbilirubinemia in the rat (3) in a dose comparable with that of Sn-protoporphyrin, produced an increase in heme saturation of tryptophan pyrrolase reaching a peak at 2 h of ~80% (control, ~60%) with the effect remaining at this level through 24 h. Zn- and Mn-protoporphyrin did not significantly affect the heme saturation of tryptophan pyrrolase (Table I).

Discussion

Tryptophan pyrrolase catalyzes the oxidative cleavage of L-tryptophan to N-formylkynurenine and is the first and rate-limiting enzyme in the catabolism of L-tryptophan (20). The active holoenzyme is normally ~50–60% saturated with heme, but fluctuations in the availability of cellular free heme produce rapid changes in the enzyme activity by converting the inactive heme-free apoenzyme to the active heme-containing holoenzyme (21, 22). Many chemicals which perturb cellular heme metabolism by altering the synthesis or degradation of heme

Table I. Effects of Sn-, Cr-, Mn-, and Zn-Protoporphyrin on Heme Saturation of Hepatic Tryptophan Pyrrolase

Metalloporphyrin	Tryptophan pyrrolase (percent heme saturation)
Control	60.4
Sn-protoporphyrin	95.0*
Cr-protoporphyrin	64.9‡
Zn-protoporphyrin	60.5
Mn-protoporphyrin	57.2

* $P < 0.05$.

‡ Peak saturation of 80% reached at 2 h.

Tryptophan pyrrolase activity measured 1 h after metalloporphyrin administration (10 μ mol/kg body wt s.c.). Values shown are the means of determinations in three to six animals for each compound.

produce changes in cellular heme content, and thereby, in total tryptophan pyrrolase activity (23, 24); because of this responsiveness to subtle changes in cellular heme, it has been proposed that the degree of heme saturation of tryptophan pyrrolase may serve as a useful index of the "regulatory" heme fraction in liver cells (24). The responsiveness of this heme-dependent enzyme activity thus served, in these experiments, as a useful index of the increase in cellular heme content which would be expected to occur if Sn-protoporphyrin significantly inhibited heme oxygenase activity in the liver in the whole animal.

The results of this study demonstrated that Sn-protoporphyrin administration rapidly leads to a prompt and near-total heme saturation of tryptophan pyrrolase lasting for a number of hours, thus clearly establishing that the synthetic metalloporphyrin transiently increased the functional content of heme in the liver cells of the treated animals. Direct quantitation of the actual amount of heme in liver protected from degradation by the metalloporphyrin was not feasible. It should be noted that the heme which transiently accumulated in the liver and which produced the near-complete saturation of tryptophan pyrrolase could be derived not only from the liver but from other tissue sources as well. Moreover the time-course of the effect of Sn-protoporphyrin on the heme saturation of tryptophan pyrrolase may reflect, in a general way, the time-frame in which the synthetic metalloporphyrin may be biologically effective in the whole animal. This time-frame of biological activity is much shorter in duration than the period during which the metalloporphyrin can be extracted from liver tissue (4); the latter may, as we have suggested earlier (8), reflect nonspecific tissue binding of Sn-protoporphyrin rather than the heme oxygenase bound compound.

The ability of inorganic cobalt to promptly and markedly deplete tryptophan pyrrolase holoenzyme of its heme component is consistent with the known capacity of this metal to rapidly and potentially induce heme oxygenase and to transiently inhibit ALA-synthase in the liver thus leading to a depletion in hepatic cells of both total heme and cytochrome P-450 contents. However, since Sn-protoporphyrin does not alter the CoCl_2 -mediated inhibition of ALA-synthase (data not shown), the ability of Sn-protoporphyrin to block the CoCl_2 effect on tryptophan pyrrolase is most reasonably explained by the ability of the metalloporphyrin to inhibit the activity of CoCl_2 -induced heme oxygenase, thus diminishing the rate of the metal-induced depletion of cellular heme available for binding to tryptophan pyrrolase apoenzyme. We have previously shown that Sn-protoporphyrin can in fact block completely the very high levels of hepatic heme oxygenase which can be induced by CoCl_2 or Co-protoporphyrin administration in the whole animal (25).

In vitro studies carried out in connection with these experiments indicated that Sn-protoporphyrin added to the assay mixture for tryptophan pyrrolase can, if provided in substantial excess, compete with heme for binding to the enzyme apoprotein; however, at equimolar concentrations of heme and Sn-protoporphyrin, the enzyme activity is not significantly inhibited (~6.3%). The fact that such inhibition does not occur in vivo is reflected in the early and near-complete saturation of tryptophan pyrrolase with heme which was produced by a single dose of Sn-protoporphyrin (Fig. 1); at later time periods, the regression of the heme saturation index of the enzyme toward normal may reflect a balance

involving the excretion of Sn-protoporphyrin from the liver, coupled with the concurrent, substantially enhanced biliary excretion of heme, which we have recently identified (7), as a consequence of Sn-protoporphyrin administration.

The ability of other metalloporphyrins to increase the heme saturation of tryptophan pyrrolase was variable; Cr-protoporphyrin induced such a response, though to a lesser degree than Sn-protoporphyrin (Table I). Interestingly Cr-protoporphyrin, like Sn-protoporphyrin, also suppresses neonatal hyperbilirubinemia in the experimental animal (3) and its inhibitory potency (i.e., K_i) for both human and rat spleen heme oxygenase approaches that of Sn-protoporphyrin (3). The other metalloporphyrins studied did not enhance the heme saturation of tryptophan pyrrolase when administered at the same dose in which Sn-protoporphyrin displayed such activity nor have they been shown capable, in our studies, of suppressing neonatal hyperbilirubinemia in the rat, even when administered in doses 50-fold greater than the effective dose of Sn-protoporphyrin (2, 7).

The ability of Sn-protoporphyrin administration to lead to the rapid and complete heme saturation of tryptophan pyrrolase is a newly defined biological property of this heme analogue and confirms that this synthetic metalloporphyrin can significantly inhibit the degradation of endogenous heme to bile pigment. The importance of hepatic tryptophan pyrrolase activity in relation to overall tryptophan metabolism in the whole animal is potentially substantial and could involve consequences for metabolic systems in other tissues in which tryptophan may be involved. Thus, control of tryptophan pyrrolase activity by synthetic heme analogues may have pharmacological implications in the whole animal.

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