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TRYPTOPHAN PYRROLASE IN HEME METABOLISM

COMPARATIVE ACTIONS OF INORGANIC TIN AND COBALT AND THEIR PROTOPORPHYRIN CHELATES ON TRYPTOPHAN PYRROLASE IN LIVER

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Abstract—The effects of various metals and metalloporphyrins, which are known to alter markedly heme metabolism *in vivo*, on the heme saturation of tryptophan pyrrolase in liver were examined. At early time points, up to 120 min, administration of $CoCl_2$ to rats caused a rapid and marked decrease in the degree of heme saturation of tryptophan pyrrolase; in contrast, Co-protoporphyrin produced a slight increase in heme saturation of the enzyme. $SnCl_2$ did not alter the heme saturation of tryptophan pyrrolase; however, treatment of rats with Sn-protoporphyrin, a potent competitive inhibitor of heme oxygenase activity both *in vivo* and *in vitro*, produced a rapid and complete heme saturation of tryptophan pyrrolase. In addition, upon simultaneous administration of Sn-protoporphyrin and $CoCl_2$, Sn-protoporphyrin prevented the $CoCl_2$-mediated decrease in heme saturation of tryptophan pyrrolase. These findings provide further evidence that the measurement of the heme saturation of tryptophan pyrrolase is a sensitive indicator of changes in the availability of heme in the "regulatory" heme pool, particularly in the immediate period after administration of compounds which are known to perturb heme metabolism *in vivo*.

The hepatic enzyme tryptophan pyrrolase (L-tryptophan 2,3-dioxygenase, EC 1.13.11.11), which catalyzes the oxidative cleavage of L-tryptophan to *N*-formylkynurenine, is the first and rate-limiting enzyme in the catabolism of L-tryptophan [1]. In rat liver, tryptophan pyrrolase exists in two forms, the active heme-containing holoenzyme and the inactive heme-free apoenzyme; as such, the amount of heme saturation determines which form is present in the liver. It has been postulated that the relative changes in the holo- and apoenzyme activities reflect the availability of "free" heme of the intracellular "regulatory" heme pool [2–5]. Thus, the administration of chemicals which perturb the rates of heme synthesis and degradation, resulting in fluctuations in the availability of "free" heme, will produce subtle changes at the level of heme saturation of tryptophan pyrrolase. For example, the administration of the porphyrinogenic drug 2-allyl-2-isopropylacetamide results in a rapid depletion of heme saturation of tryptophan pyrrolase [6]. In contrast, administration of either the heme precursor δ-aminolevulinate, or heme itself, results in the rapid and complete heme saturation of tryptophan pyrrolase [7–9].

Previous studies from this laboratory have demonstrated that a number of inorganic metals and organometallic compounds can significantly enhance the rate of heme degradation by inducing heme oxygenase [10–13], the rate-limiting enzyme in the oxidative degradation of heme to bile pigment [14, 15]. The induction of heme oxygenase is accompanied by the concomitant characteristic well-documented perturbations in δ-aminolevulinate synthase activity, decreases in microsomal cytochrome P-450 and heme content, and diminished cytochrome P-450-dependent mixed-function oxidase activity which is responsible for the metabolism of a variety of foreign chemicals, drugs and carcinogens [16, 17].

In the present investigation, we have examined the changes that occur in the heme saturation of tryptophan pyrrolase in the immediate period after metal and metalloporphyrin administration. The results of the present investigation indicate that $CoCl_2$ administration results in a prompt decrease (<60 min) in the heme saturation of tryptophan pyrrolase [4, 18]. In contrast Sn(tin)-protoporphyrin, a potent inhibitor of heme oxidation both *in vivo* and *in vitro* [19–22], produced a prompt increase in heme saturation of tryptophan pyrrolase and blocked the decrease in heme saturation of tryptophan pyrrolase produced by $CoCl_2$. As it is not yet possible to measure small changes in the level of intracellular heme directly, measurement of the heme saturation of tryptophan pyrrolase appears indeed to be a very sensitive indicator of changes in the availability of intracellular "free" heme.

EXPERIMENTAL PROCEDURES

Materials. Male Sprague–Dawley rats (180–200 g) supplied by Taconic Farm (Germantown, NY) were used throughout this study. The animals were injected subcutaneously with $CoCl_2$ or $SnCl_2$ (250 μmoles/kg body wt), Co-protoporphyrin (50 μmoles/kg body wt) or Sn-protoporphyrin (10 μmoles/kg body wt); control animals were

injected with an equivalent volume of 0.9% NaCl. Fresh solutions of metalloporphyrins were made immediately prior to injection by dissolving the metalloporphyrin in 0.2 ml of 0.2 N NaOH, adjusting the pH to 7.5 with 0.5 N HCl, and making up to final volume with 0.9% NaCl. The purity of Sn- and Co-protoporphyrin preparations was assessed as described previously [23]. Rats were starved for 24 hr before being killed. $CoCl_2$ and $SnCl_2$ were purchased from the Fisher Scientific Co. (Pittsburg, PA). Co-protoporphyrin and Sn-protoporphyrin were purchased from Porphyrin Products (Logan, UT). All other chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO).

Tissue preparation and enzyme assays. Tissue preparation of liver was as described previously [12]. The animals were killed by decapitation, and livers were removed and immediately placed in ice-cold 0.9% NaCl. After blotting and cleaning, the liver was minced with scissors, and a portion was homogenized in 3 vol. of 0.1 M potassium phosphate buffer pH 7.4, containing 0.25 M sucrose, in a glass Potter–Elvehjem homogenizer with a Teflon pestle. An aliquot of the homogenate (3 ml) was removed, and the activity of δ-aminolevulinate synthase was determined in the mitochondrial fraction as described earlier [24]; the presence of hemoglobin did not interfere with the assay. The rest of the homogenate was processed for the preparation of microsomes which were used for the determination of heme oxygenase activity [12] and cytochrome P-450 content [25]. A portion (1 g) of liver was homogenized in 6.5 vol. of 0.14 M KCl–2.5 mM NaOH, and tryptophan pyrrolase activity was determined in the presence (total enmzyme) and in the absence (holoenzyme) of added hemin (2 μM) [26]. The 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 of total enzyme (×100). Protein concentration was determined by the method of Lowry *et al.* [27] using crystalline bovine serum albumin as standard. Three to six animals were used for each data point, and the statistical analyses of data were performed by Student's *t*-test.

RESULTS

Effects of Sn- and Co-protoporphyrin on hepatic tryptophan pyrrolase activity in vitro. Since heme is required for the expression of the functional activity of tryptophan pyrrolase, the effects of the synthetic heme analogues Co-protoporphyrin and Sn-protoporphyrin were studied on tryptophan pyrrolase activity *in vitro*. The addition of Sn-protoporphyrin to the assay mixture resulted in the inhibition of tryptophan pyrrolase activity in a dose-dependent manner (Table 1). However, when equimolar concentrations of Sn-protoporphyrin and hemin (2 μM) were present, the inhibitory effect of Sn-protoporphyrin on the enzyme activity was minimal (~6.8%), suggesting that heme binds to apo-tryptophan pyrrolase more tightly than does Sn-protoporphyrin. In addition, Co-protoporphyrin produced a similar dose-dependent inhibition of tryptophan pyrrolase activity. However, the inhibitory effect of Co-protoporphyrin on the enzyme activity was more profound than that of Sn-protoporphyrin (Table 1), notably at the highest concentrations (50 μM and 100 μM) examined.

Table 1. Effect *in vitro* of Sn- and Co-protoporphyrin on rat liver tryptophan pyrrolase activity

Inhibitor	Conc (μM)	Tryptophan pyrrolase (total enzyme activity)	% Inhibition
Sn-protoporphyrin	0	3.77 ± 0.13	0
	1	3.73 ± 0.14	0.97 ± 0.58
	2	3.51 ± 0.10	6.81 ± 0.62
	5	2.97 ± 0.05*	21.05 ± 2.06
	10	2.61 ± 0.09*	30.83 ± 1.10
	20	2.18 ± 0.12†	42.07 ± 2.14
	50	1.73 ± 0.09†	54.17 ± 2.27
	100	1.22 ± 0.08†	67.86 ± 1.08
Co-protoporphyrin	0	5.26 ± 0.13	0
	1	4.91 ± 0.17	6.74 ± 0.94
	2	4.62 ± 0.07	12.23 ± 0.93
	5	4.17 ± 0.10*	20.92 ± 2.37
	10	3.38 ± 0.09†	35.60 ± 2.74
	20	2.34 ± 0.11†	55.52 ± 2.51
	50	1.37 ± 0.14†	73.88 ± 2.68
	100	0.82 ± 0.06†	84.38 ± 1.11

Rats were starved for 24 hr before being killed. The livers were removed, and tryptophan pyrrolase (total enzyme activity expressed as μmoles kynurenine formed/hr/g liver wt) was determined in the absence and presence of various concentrations of Sn- and Co-protoporphyrin as described in Experimental Procedures. Data are expressed as the means ± S.E. of three different experiments.

* $P < 0.01$ as compared with control.

† $P < 0.001$ as compared with control.

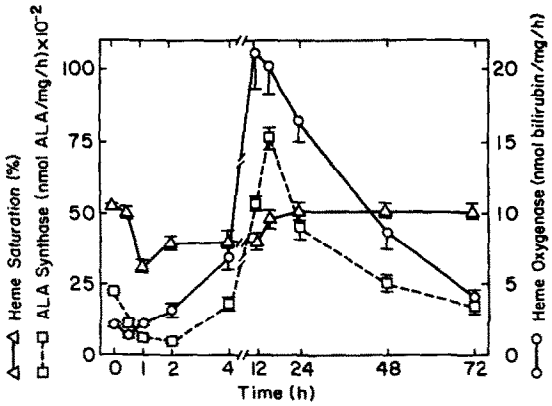

Fig. 1. Time course of the effects of $CoCl_2$ on heme oxygenase and δ-aminolevulinate (ALA) synthase activities and on the heme saturation ratio of rat liver tryptophan pyrrolase. Male rats were injected subcutaneously with a single dose of $CoCl_2$ (250 μmoles/kg body wt) at 0 hr and killed at the time intervals indicated on the abscissa. Three rats were used at each time point and the assays were performed as described in Experimental Procedures. Key: (○) heme oxygenase activity (nmoles bilirubin/mg protein/hr); (□) 10^{-2} × ALA synthase activity (nmoles ALA/mg protein/hr); and (△) percent heme saturation ratio of tryptophan pyrrolase.

Effects in vivo *of* $CoCl_2$ *and* $SnCl_2$ *administration on the heme saturation of tryptophan pyrrolase, δ-aminolevulinate synthase and heme oxygenase activities in liver.* The effects of $CoCl_2$ administration on the percent heme saturation of tryptophan pyrrolase, δ-aminolevulinate synthase activity, and the induction of heme oxygenase in rat liver were followed with time (Fig. 1). The percent heme saturation of tryptophan pyrrolase dropped significantly (~30%) within 1 hr of $CoCl_2$ administration and remained low for 12 hr, suggesting a rapid decrease in the availability of intracellular heme as a result of $CoCl_2$ administration. The levels of heme saturation of tryptophan pyrrolase had returned to control levels by 16 hr and remained at these levels for up to 72 hr. $CoCl_2$ caused a characteristic initial decline in δ-aminolevulinate synthase activity followed by the well-documented "rebound" increase at 12–16 hr after metal administration [16, 28]; therafter, the enzyme activity gradually returned to control levels. $CoCl_2$ administration produced a 10-fold induction of heme oxygenase which reached a maximum 16 hr after metal treatment. The level of heme oxygenase activity slowly decreased but was still double the initial level even 72 hr after $CoCl_2$ treatment. The effect of $SnCl_2$ on these aspects of heme metabolism was also studied in liver. The animals were treated with $SnCl_2$ (250 μmoles/kg body wt) at 0 hr and the effects on the percent heme saturation of tryptophan pyrrolase, δ-aminolevulinate synthase activity, and the induction of heme oxygenase in rat liver were followed up to 72 hr. $SnCl_2$ induces heme oxygenase in kidney but not in liver [29]. Administration of $SnCl_2$ to rats produced no change in the heme saturation of tryptophan pyrrolase during the 72-hr period of study. In addition, the activities of δ-aminolevulinate synthease and heme oxygenase remained unaltered throughout this time period (results not shown).

Effect in vivo *of Sn-protoporphyrin on heme metabolism in liver.* Sn-protoporphyrin has been shown to be a competitive inhibitor of heme oxygenase activity both *in vivo* and *in vitro* [19–22]. It was thererfore of interest to determine if a specific decrease in heme oxidation *in vivo* as a result of Sn-protoporphyrin administration would produce changes in the heme saturation of tryptophan pyrrolase. Figure 2 shows that a single dose of Sn-protoporphyrin (10 μmoles/kg body wt) caused a marked and rapid increase (within 1 hr) in the percent heme saturation of tryptophan pyrrolase, indicating a significant increase in the availability of heme for saturation of tryptophan pyrrolase. The maximum heme saturation effect on tryptophan pyrrolase elicited by Sn-protoporphyrin lasted for approximately 4 hr before gradually decreasing, but remained higher than the initial values at all the subsequent time points studied up to 72 hr. In addition, there was a significant decrease in heme oxygenase activity within 30 min of metalloporphyrin administration, and this diminished enzyme activity persisted for a minimum of 72 hr. There were no significant changes in δ-aminolevulinate synthase activity or cytochrome P-450 content during this period.

Effect in vivo *of Co-protoporphyrin on heme metabolism in liver.* Co-protoporphyrin is another competitive inhibitor of both microsomal and homogenously purified heme oxygenase activity *in vitro* [19, 20]. Paradoxically, the administration of Co-protoporphyrin to rats results in a potent induction of hepatic heme oxygenase activity *in vivo*; the enzyme activity remains elevated for several weeks [30] after a single dose of the metalloporphyrin. In compari-

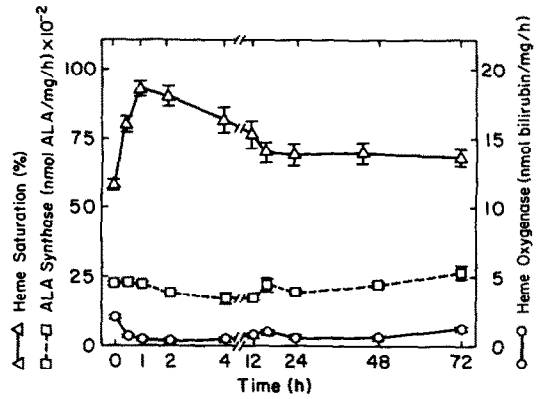

Fig. 2. Time course of the effects of Sn-protoporphyrin on heme oxygenase and δ-aminolevulinate (ALA) synthase activities and on the heme saturation ratio of rat liver tryptophan pyrrolase. Male rats were injected subcutaneously with a single dose of Sn-protoporphyrin (10 μmoles/kg body wt) at 0 hr and killed at the times indicated. Three rats were used at each time point, and the enzyme assays were performed as described in the text. Key: (○) heme oxygenase activity (nmoles bilirubin/mg protein/hr); (□) 10^{-2} × ALA synthase activity (nmoles ALA/mg protein/hr); and (△) percent heme saturation ratio of tryptophan pyrrolase.

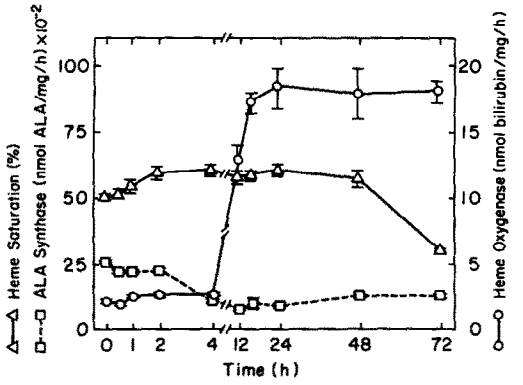

Fig. 3. Time course of the effects of Co-protoporphyrin on heme oxygenase and δ-aminolevulinate (ALA) synthase activities and on the heme saturation ratio of rat liver tryptophan pyrrolase. Male rats were injected subcutaneously with a single dose of Co-protoporphyrin (50 μmoles/kg body wt) at 0 hr and killed at the time intervals indicated. At least three rats were used at each time point, and the enzyme assays were performed as described in Experimental Procedures. Key: (○) heme oxygenase activity (nmoles bilirubin/mg protein/hr); (□) 10^{-2} × ALA synthase activity (nmoles ALA/mg protein/hr); and (△) percent heme saturation ratio of tryptophan pyrrolase.

son, the perturbations of heme metabolism produced by $CoCl_2$ administration are almost complete within 72 hr. The percent heme saturation of tryptophan pyrrolase increased slightly (~15–20%) within 2 hr and remained elevated at this level when compared to the initial value for as long as 48 hr after Co-protoporphyrin administration (Fig. 3). The heme saturation of tryptophan pyrrolase, however, was markedly lower than the initial value (40%) at 72 hr (Fig. 3). As expected, a single injection of Co-protoporphyrin caused a profound induction of heme oxygenase activity (10-fold) at 12–16 hr after administration of the metalloporphyrin, and the enzyme activity remained markedly elevated through 72 hr. The activity of δ-aminolevulinate synthase was inhibited (~60%) at 4 hr and remained low through 72 hr (Fig. 3).

Effect of Sn- and Co-protoporphyrin on the $CoCl_2$*-mediated decrease in heme saturation of tryptophan pyrrolase in liver.* Table 2 shows the effect of simultaneous administration of $CoCl_2$ and Sn-protoporphyrin on the percent heme saturation of tryptophan pyrrolase. The simultaneous treatment of rats with Sn-protoporphyrin and $CoCl_2$ resulted in the blockade of the $CoCl_2$-mediated decrease in heme saturation of tryptophan pyrrolase at 1 and 2 hr (Table 2). In addition, Sn-protoporphyrin administration produced a marked inhibition of the $CoCl_2$-mediated induction of heme oxygenase activity (Table 2). However, the $CoCl_2$-mediated decline in δ-aminolevulinate synthase activity remained unchanged. Since Co-protoporphyrin also increased the heme saturation of tryptophan pyrrolase in the period immediately after metalloporphyrin administration, the effect of the simultaneous administration of $CoCl_2$ and Co-protoporphyrin on percent heme saturation of tryptophan pyrrolase was also studied (Table 2). Co-

Table 2. Blockade of $CoCl_2$-mediated decrease in percent heme saturation of tryptophan pyrrolase by Sn- and Co-protoporphyrin

Treatment	Percent heme saturation of tryptophan pyrrolase	ALA synthase activity	Heme oxygenase activity
Saline	53.70 ± 2.76	0.218 ± 0.017	1.95 ± 0.12
$CoCl_2$ (1 hr)	30.26 ± 1.34*	0.071 ± 0.008†	2.33 ± 0.23
$CoCl_2$ + Sn-protoporphyrin (1 hr)	70.39 ± 3.07‡	0.067 ± 0.012†	0.55 ± 0.11*
$CoCl_2$ + Co-protoporphyrin (1 hr)	67.25 ± 5.52§	0.041 ± 0.010†	2.16 ± 0.13
$CoCl_2$ (2 hr)	42.28 ± 1.89‖	0.038 ± 0.009†	3.13 ± 0.74
$CoCl_2$ + Sn-protoporphyrin (2 hr)	62.41 ± 4.09§	0.042 ± 0.016†	0.50 ± 0.10*
$CoCl_2$ + Co-protoporphyrin (2 hr)	63.34 ± 2.64‡	0.030 ± 0.003†	2.27 ± 0.27

Rats were starved for 24 hr and received a subcutaneous injection of $CoCl_2$ (250 μmoles/kg body wt), $CoCl_2$ plus Sn-protoporphyrin (10 μmoles/kg body wt) or $CoCl_2$ plus Co-protoporphyrin (50 μmoles/kg body wt) at 1 and 2 hr before killing. Tryptophan pyrrolase-heme saturation, ALA-synthase (expressed as nmoles ALA formed/mg homogenate protein/hr) and heme oxygenase (expressed as nmoles bilirubin formed/mg microsomal protein/hr) activities were determined as described in Experimental Procedures. Results are the means ± S.E.M. for three rats.

* $P < 0.01$ as compared with saline-treated control group.
† $P < 0.001$ as compared to saline-treated control.
‡ $P < 0.01$ as compared with $CoCl_2$ treatment (1 hr).
§ $P < 0.05$ as compared with $CoCl_2$ treatment (2 hr).
‖ $P < 0.05$ as compared with saline-treated control group.

protoporphyrin blocked the $CoCl_2$-mediated decline in percent heme saturation of tryptophan pyrrolase at 1 and 2 hr after metalloporphyrin treatment (Table 2) and, as such, behaved in a manner identical to Sn-protoporphyrin.

DISCUSSION

The results of the present investigation demonstrate that the heme saturation of cytosolic tryptophan pyrrolase is a sensitive index of the rapid changes that occur in the availability of intracellular heme as a result of metal-mediated perturbations in heme metabolism. $CoCl_2$ administration, which produces a potent induction of heme oxygenase with a concomitant early depression followed by the late "rebound" of δ-aminolevulinate synthase activity, resulted in a rapid decrease in the heme saturation of tryptophan pyrrolase. As such, this decrease in the heme saturation of tryptophan pyrrolase which occurs when heme oxygenase activity is unchanged, is presumably due to the initial rapid decrease in δ-aminolevulinate synthase activity, resulting in a marked decrease in hepatic heme levels. The late recovery of heme saturation of tryptophan pyrrolase 12–16 hr after $CoCl_2$ administration coincided with the "rebound" increase in δ-aminolevulinate synthase activity and maximal heme oxygenase activity. It thus appears that a balance in heme supply for the "free" heme pool of tryptophan pyrrolase was achieved and maintained after ~12 hr by increased δ-aminolevulinate synthase activity which resulted in increased heme synthesis and that this compensated for the increased heme degradation that occurred as a consequence of the induction of heme oxygenase.

It has been suggested that agents which induce heme oxygenase might act by labilizing the heme moiety of cytochrome P-450, with the released heme then acting as the proximate inducer of the enzyme [31]. The induction of heme oxygenase by endotoxin has been postulated to be due to increased dissociation of heme from cytochrome P-450; the released heme produced an enhanced saturation of tryptophan pyrrolase prior to the induction of heme oxygenase [32]. In contrast, the present findings with $CoCl_2$ clearly show a significant decrease in tryptophan pyrrolase heme saturation within 1 hr of metal treatment (Fig. 1), thereby suggesting that the $CoCl_2$ induction of heme oxygenase does not involve any release of intracellular heme from other hemoproteins. These findings are in agreement with previous studies in which concurrent administration of SKF-525A [33] with an inducing metal did not affect the extent and time course of heme oxygenase induction; the decrease in cytochrome P-450 content normally associated with metal administration was, however, prevented. These findings provide further evidence that heme oxygenase induction by metals can proceed without the labilization of the heme moiety of cytochrome P-450.

Sn-protoporphyrin is a potent competitive inhibitor of heme oxygenase activity *in vitro* [19, 21, 22] and has also been shown to prevent naturally occurring hyperbilirubinemia in newborn animals as well as to decrease hyperbilirubinemia in strains of mice with congenital hemolytic anemia [19, 34]. In the present study, Sn-protoporphyrin administration resulted in the rapid (~1 hr) and almost complete heme saturation of tryptophan pyrrolase. High levels of heme saturation of tryptophan pyrrolase were maintained for the entire 72-hr period of study. In addition, simultaneous administration of Sn-protoporphyrin and $CoCl_2$ prevented the rapid decrease in heme saturation of tryptophan pyrrolase associated with $CoCl_2$ administration without, however, altering the early $CoCl_2$-mediated inhibition of δ-aminolevulinate synthase activity. The administration of Sn-protoporphyrin resulted in a marked inhibition of heme oxygenase activity *in vivo*, which in turn produced a rapid increase in intracellular heme that was utilized in the immediate heme saturation of tryptophan pyrrolase [35].

We have shown that Co-protoporphyrin has a "dual" effect on heme oxygenase activity in that this metalloporphyrin is a potent competitive inhibitor of heme oxygenase activity *in vitro* [20] and is a profound inducer of this enzyme activity *in vivo* [30]. The administration of this metalloporphyrin resulted in an immediate (within 1 hr) increase in heme saturation of tryptophan pyrrolase, indicating that Co-protoporphyrin, within this time period at least, can also inhibit heme oxygenase activity *in vivo*. In addition, Co-protoporphyrin had the ability to prevent the $CoCl_2$-mediated decrease in heme saturation of tryptophan pyrrolase; this indicates an increased availability of intracellular heme and thus provides additional confirmation that Co-protoporphyrin can act as an inhibitor of heme oxidation *in vivo*. Heme saturation of tryptophan pyrrolase eventually decreased 72 hr after Co-protoporphyrin administration. This is presumably due in part to the prolonged induction of heme oxygenase and in part to the continued inhibition of δ-aminolevulinate synthease. Further studies are needed to clarify the mechanism of action of the metalloporphyrin.

There is a considerable body of evidence which suggests that heme regulates its own synthesis by feedback repression of δ-aminolevulinate synthase, the rate-limiting enzyme in the heme biosynthetic pathway. Indeed, the ability of the porphyrinogenic drugs 2-allyl-2-isopropylacetamide and 3,5-diethoxycarbonyl-1,4-dihydrocollidine to deplete the "regulatory" heme has been suggested to lead to the "derepression" of δ-aminolevulinate synthase and the accumulation of porphyrins [36, 37]. The evidence for the existence of such a "regulatory" heme pool is indirect and is based on the determination of enzyme activity, concentration of heme, and its utilization by several hepatic hemoproteins such as catalase, cytochrome P-450 and tryptophan pyrrolase. The findings in the present investigation strongly suggest that the availability of intracellular heme can be altered by certain metals and metalloporphyrins and that these changes are rapidly reflected in the heme saturation of tryptophan pyrrolase.

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REFERENCES

1. W. E. Knox, *Br. J. exp. Path.* **32**, 462 (1951).
2. A. A.-B. Badawy, *Biochem. J.* **172**, 487 (1978).
3. A. A.-B. Badawy, *Biochem. Soc. Trans.* **7**, 575 (1979).
4. G. Kikuchi and Y. Yoshida, *Molec. cell. Biochem.* **53**, 163 (1983).
5. D. A. Litman and M. A. Correia, *J. Pharmac. exp. Ther.* **232**, 337 (1985).
6. A. A.-B. Badawy and C. J. Morgan, *Biochem. J.* **206**, 451 (1982).
7. L. Wetterberg, A. Yuwiler and E. Geller, *Life Sci.* **8**, 1047 (1969).
8. R. Druyan and A. Kelly, *Biochem. J.* **129**, 1095 (1972).
9. A. A.-B. Badawy and M. Evans, *Biochem. J.* **150**, 511 (1975).
10. M. D. Maines and A. Kappas, *Proc. natn. Acad. Sci. U.S.A.* **71**, 4293 (1974).
11. M. D. Maines and A. Kappas, *Biochem. J.* **154**, 125 (1976).
12. G. S. Drummond and A. Kappas, *Biochem. J.* **192**, 637 (1980).
13. D. W. Rosenberg, G. S. Drummond and A. Kappas, *Molec. Pharmac.* **21**, 150 (1981).
14. R. Tenhunen, H. S. Marver and R. Schmid, *Proc. natn. Acad. Sci. U.S.A.* **61**, 748 (1968).
15. R. Tenhunen, H. S. Marver and R. Schmid, *J. biol. Chem.* **244**, 6388 (1969).
16. M. D. Maines and A. Kappas, *J. biol. Chem.* **250**, 4171 (1975).
17. A. J. Paine, in *Essays in Biochemistry* (Eds. N. Campbell and R. D. Marshall) Vol. 17, p. 85. The Biochemical Society, London (1981).
18. A. A.-B. Badawy, *Biochem. J.* **164**, 431 (1977).
19. G. S. Drummond and A. Kappas, *Proc. natn. Acad. Sci. U.S.A.* **78**, 6466 (1981).
20. T. Yoshinaga, S. Sassa and A. Kappas, *J. biol. Chem.* **257**, 7778 (1982).
21. G. S. Drummond and A. Kappas, *Science* **217**, 1250 (1982).
22. A. Kappas and G. S. Drummond, in *Microsomes, Drug Oxidations and Drug Toxicity* (Eds. R. Sato and R. Kato), p. 629. Wiley-Interscience, New York (1982).
23. C. S. Simionato, K. E. Anderson, S. Sassa, G. S. Drummond and A. Kappas, *Analyt. Biochem.* **141**, 213 (1984).
24. S. Sassa, A. Kappas, S. E. Bernstein and A. P. Alvares, *J. biol. Chem.* **254**, 729 (1979).
25. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2379 (1966).
26. A. A.-B. Badawy, *J. pharmac. Meth.* **6**, 77 (1981).
27. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
28. F. DeMatteis and A. H. Gibbs, *Ann. clin. Res.* **8**, (Suppl. 17), 193 (1976).
29. A. Kappas and M. D. Maines, *Science* **192**, 60 (1976).
30. G. S. Drummond and A. Kappas, *Proc. natn. Acad. Sci. U.S.A.* **79**, 2384 (1982).
31. D. M. Bissell and L. E. Hammaker, *Archs Biochem. Biophys.* **176**, 103 (1976).
32. D. M. Bissell and L. E. Hammaker, *Biochem. J.* **166**, 301 (1977).
33. G. S. Drummond, D. W. Rosenberg and A. Kappas, *Biochem. J.* **202**, 59 (1982).
34. S. Sassa, G. S. Drummond, S. E. Bernstein and A. Kappas, *Blood* **61**, 1011 (1983).
35. A. Kappas, G. S. Drummond and M. K. Sardana, *J. clin. Invest.* **75**, 302 (1985).
36. S. Granick, *J. biol. Chem.* **244**, 1359 (1965).
37. F. DeMatteis, *Pharmac. Rev.* **19**, 523 (1967).