ENZYMES OF HEME METABOLISM IN THE KIDNEY

Regulation by Trace Metals Which Do Not Form Heme Complexes*

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On the basis of the responses (1–3) of intact liver as well as of cultured hepatocytes to exogenously administered heme (Fe-protoporphyrin-IX, Fe-heme), the regulation of δ-aminolevulinate synthetase (ALAS),[1] the rate-limiting enzyme in the heme biosynthetic pathway (4), by heme itself has been proposed (2–4). According to this proposal, heme regulation of ALAS formation reflects the functioning of this metalloporphyrin complex as a repressor of the formation of the enzyme protein, with cellular heme content and the rate of ALAS formation being reciprocally related.

However, it was recently shown in studies from this laboratory (5–9) and others (10) that transition metals such as $Fe^{2+,3+}$, $Cu^{1+,2+}$, and Co^{2+} have a potent ability to alter cellular heme metabolism in various tissues of intact animals and in cells in culture as well. Of these metals, Co^{2+} has been most extensively studied. The effect of Co^{2+} on hepatic ALAS formation in animals was found to be biphasic, with an initial inhibition of the enzyme being followed by secondary or "rebound" induction of ALAS activity (6, 7). Moreover, both in whole animals and in cultured hepatocytes, Co^{2+} and Cu^{1+} were capable of inhibiting the induction of ALAS by a number of potent chemical inducers of the enzyme (7, 8). These findings raised the possibility that metals directly alter ALAS activity through their binding at a regulatory site for the synthesis of the enzyme or that they regulate heme synthesis after the formation of metalloporphyrin complexes (7, 8) which in turn would repress ALAS by mimicking the action of heme. The latter mechanism was recently shown not to be required for metals to regulate ALAS in liver, because Pt^{4+} and Ni^{2+}, which do not enzymatically form heme complexes, act similarly to heme in controlling ALAS production (11).

The present study was undertaken to study the enzymes of heme metabolism in kidney because this organ responds differently from liver to chemical agents—such as porphyria-inducing drugs and steroids—which potently alter heme metabolism in the latter organ. In addition, metal ion effects on heme pathway enzymes in kidney were examined to test our hypothesis that nonheme complexed metals have the capacity to directly regulate heme synthesis in cells. Pt^{4+} and Ni^{2+} were used in these studies because they do not enzymatically

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[1] *Abbreviations used in this paper:* ALAS, δ-aminolevulinate synthetase; ALAD, δ-aminolevulinate dehydratase; UROS, uroporphyrinogen-I-synthetase.

form protoporphyrin-IX complexes. The results indicate that although these two elements have somewhat differential effects on heme pathway enzymes in kidney as compared with liver (11) they are capable of regulating ALAS and heme oxygenase in the same manner as does heme itself.

Materials and Methods

In Vivo Studies. Male Sprague-Dawley rats were treated (subcutaneously) with $NiCl_2 \cdot 6H_2O$ (250 μmol/kg), and $PtK_2Cl_6 \cdot 6H_2O$ (125 μmol/kg). Tissues were prepared as described elsewhere (7). The whole homogenate was used for the determination of ALAS as described by Marver et al. (12), δ-aminolevulinate dehydratase (ALAD), according to the method of Mauzerall and Granick (13), ferrochelatase by the method of Maines et al. (7), using dicarboxylic mesoporphyrin-IX as substrate and total porphyrin content by the method of Granick et al. (14). The postmitochondrial cell fraction was used for assaying uroporphyrinogen-I-synthetase (UROS) as described by Sassa et al. (15). Microsomal fractions were prepared and used for the determination of heme content (16).

Whole cell homogenates and microsomal fractions obtained from untreated animals were used for in vitro studies. Small volumes of solutions of Ni^{2+} and Pt^{4+} were added to the enzyme preparation used for the various assays to obtain desired final concentrations of compounds.

Materials. Reagents were purchased from the Sigma Chemical Co., St. Louis Mo. Porphobilinogen, δ-aminolevulinic acid, and porphyrin standards (copro- and uroporphyrins) were purchased from Porphyrin Products, Salt Lake City, Utah. Dicarboxylic mesoporphyrin-IX was prepared as described elsewhere (17).

Results

Effect of Nickel on Heme Metabolism. The time-courses of the effects of a single dose of Ni^{2+} on renal ALAS and heme oxygenase activities and heme contents are depicted in Fig. 1. In contrast to hepatocytes (11) in which a single injection of Ni^{2+} produces a biphasic response in ALAS, with an initial sharp decline in enzyme activity followed by an eventual increase to levels above normal, ALAS activity of kidney did not exhibit the early inhibition response, but remained unchanged for 12 h, after which the enzyme activity was substantially increased. As shown (Fig. 1), in the kidney Ni^{2+} had a very marked effect on heme oxygenase with the enzyme activity being elevated at 16 h by 12 times over the control. Moreover, the initial inhibition of heme oxygenase which was observed in the liver (11) was not noted in kidney. Microsomal content of heme in kidney which did not change during the first 3 h decreased substantially thereafter despite the elevation of ALAS.

A differential effect of Ni^{2+} on ALAD from hepatic and renal sources was observed. As shown in Table I, hepatic ALAD activity was not inhibited by Ni^{2+}, whereas ALAD of kidney was significantly inhibited 12 h after treatment and the inhibition was sustained for more than 24 h. Ferrochelatase and UROS activities were decreased slightly in both liver and kidney (10–15%) during the first 24 h and the total porphyrin contents of liver and kidney showed decreases 3 h after Ni^{2+} treatment but had returned to normal at 16 h (data not shown).

The time-courses of the Pt^{4+} effects on enzymes of heme metabolism in kidney are shown in Fig. 2. ALAS was significantly depressed in the initial hours after treatment with the metal. The early decline in ALAS lasted for 12 h and was followed by a sharply increased enzyme activity thereafter. A decrease in cellular content of heme was noted by 3 h after Pt^{4+} treatment and was sustained for at least 24 h despite the highly elevated ALAS activity at

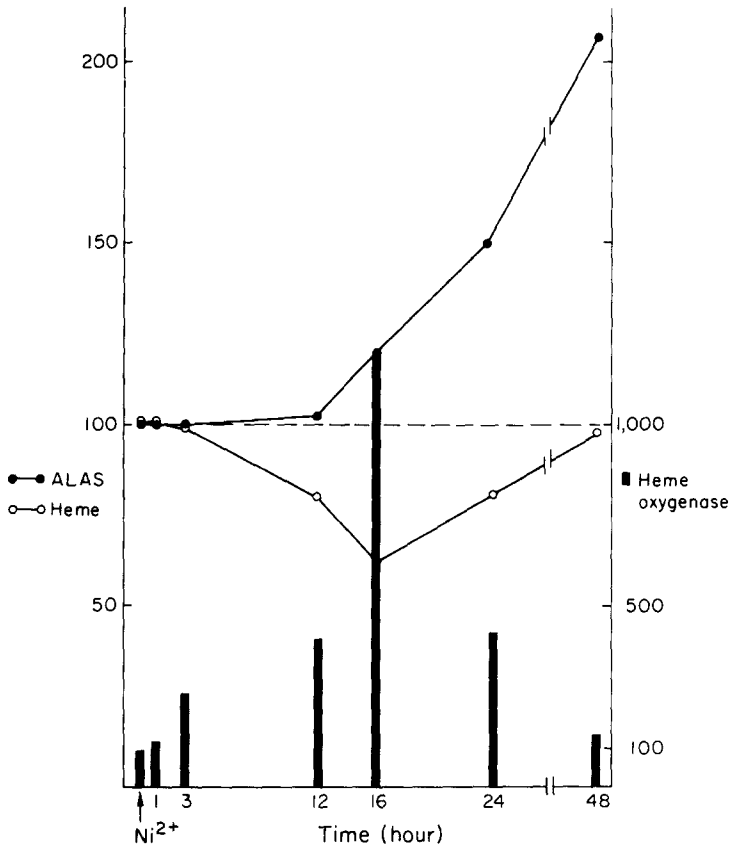

FIG. 1. The time-course of response to Ni^{2+} of heme pathway enzymes in rat kidney. Rats were treated (subcutaneously) with nickel chloride and then killed at indicated intervals. Various assays were performed using procedures detailed in the text. The values shown are the means of three determinations and are expressed as the percent of the control. The control value for heme oxygenase was 0.84 ± 0.21 nmol bilirubin/mg per h; control value for ALAS activity was 80 ± 18 pmol/mg per h; and that of microsomal heme was 0.30 ± 0.05 nmol/mg.

TABLE I

Effect of In Vivo Ni^{2+} Treatment on ALAD Activity in Kidney and Liver

Tissue	Time	ALAD
	h	*nmol/mg/h*
Kidney	0	2.87
	3	2.75
	12	2.01
	24	1.40
Liver	0	4.50
	3	4.95
	12	4.72
	24	3.97

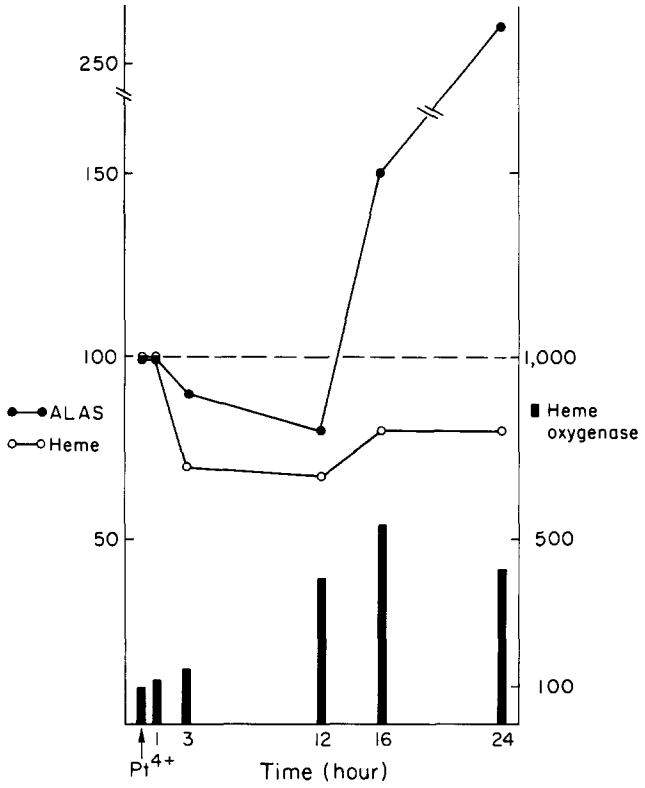

FIG. 2. The time-course of response to Pt^{4+} of heme pathway enzymes in rat kidney. Rats were treated with platinum (ic) chloride. Various assays were carried out as described in the text. The control values for the measured parameters were within ±10–20% of the values given in the legend of Fig. 1.

this time. The effects of Pt^{4+} on heme oxygenase activity are also shown in this figure. Pt^{4+} was a potent inducer of heme oxygenase in kidney as it was in the liver (11).

Table II shows the effects of Pt^{4+} on porphyrin content and on other enzymes of heme metabolism in kidney as compared with liver. In liver, ALAD, UROS, and ferrochelatase were decreased by 10–20% within 3–12 h after Pt^{4+} treatment but the porphyrin content of this organ was not altered by the metal. In kidney, Pt^{4+} was a potent inhibitor of −SH-dependent enzymes of heme biosynthesis so that ALAD activity was depressed by 50–70% in 12 h with the decrease in enzyme activity being noted within 3 h after treatment; UROS activity at this time was depressed almost equivalently, and ferrochelatase activity was reduced by 10–15%. The total porphyrin content of kidney at 24 h was decreased markedly (60%) in contrast to the effect of the metal on liver porphyrin.

Table III shows the in vitro effects of Pt^{4+} and Ni^{2+} on activities of ALAS, ALAD, and heme oxygenase in kidney and liver tissue preparations. In kidney, ALAD and heme oxygenase, the latter of which is also a −SH-dependent enzyme (18), were markedly inhibited by Pt^{4+}; high concentrations of Ni^{2+} were

TABLE II
Effect of In Vivo Pt^{4+} Treatment on Enzymes of Heme Biosynthesis and the Total Porphyrin Content in Kidney and Liver

Tissue	Time	ALAD	UROS	Ferrochelatase	Total porphyrin
	h	*nmol/mg/h*	*pmol/mg/h*	*nmol/mg/h*	*pmol/mg*
Kidney	0	2.55	220	2.33	15.80
	3	1.91	147	2.07	12.64
	12	1.02	125	2.05	9.95
	24	0.87	105	2.09	6.32
Liver	0	4.45	460	4.47	4.12
	3	4.00	460	4.02	4.18
	12	4.00	373	3.66	4.12
	24	3.96	336	3.93	4.00

TABLE III
In Vitro Effect of Ni^{2+} and Pt^{4+} on Hepatic and Renal Enzymes of Heme Metabolism

Organ	Concentration	ALAS		ALAD		Heme oxygenase	
		Ni^{2+}	Pt^{4+}	Ni^{2+}	Pt^{4+}	Ni^{2+}	Pt^{4+}
	μM			*% of control*			
Kidney	125	100	101	93	70	100	69
	250	105	118	91	56	80	14
Liver	125	100	105	111	51	105	96
	250	100	116	106	35	75	54

ALAS and ALAD activities were assayed in whole cell homogenates after the addition of the indicated concentrations of Ni^{2+} and Pt^{4+}. Microsomal fractions were used for heme oxygenase assays.

also slightly inhibitory to these enzymes. In liver, Ni^{2+} did not inhibit ALAD but Pt^{4+} markedly reduced its activity. Ni^{2+} at high concentrations was also inhibitory to heme oxygenase but Pt^{4+} was considerably more effective in this regard. Pt^{4+} did not inhibit the activity of either hepatic or renal ALAS; in fact, it increased this enzyme activity slightly at high concentrations.

Discussion

The results of the present study demonstrate that metal ions can regulate the activities of the two enzymes which are rate limiting in cellular heme synthesis and heme degradation—ALAS and heme oxygenase, respectively—in the kidney as they do in the liver (11). These findings, considered together with previously published data from this laboratory (5–9), thus support the idea that these enzymes are under metal control in physiological circumstances. The general unresponsiveness of kidney heme pathway enzymes to those foreign chemicals that potently alter their activities in liver emphasizes the physiological significance of the renal enzyme response to metals, as shown in this study.

In the present report, Ni^{2+} and Pt^{4+} were shown to produce long-lasting and substantial reductions in renal content of heme after single injections of the

metals. Microsomal heme in this study paralleled total cellular heme contents, as reported previously (7). However, in the kidney, as was shown earlier with liver (11), the activity of ALAS was not consistently increased as a reciprocal response to the substantial reductions observed in heme content. ALAS is a mitochondrial enzyme with a very short half-life (2, 3), and prompt and reciprocal increases in ALAS would have been expected if formation of this enzyme were solely regulated by a feedback mechanism involving heme as is generally believed. Alternatively, if the metal-induced reductions of cellular heme had not decreased below the threshold levels of heme which would result in feedback derepression of ALAS synthesis (3), this enzyme activity would have been expected to remain unaltered. Rather, Pt^{4+} produced concurrent reductions in both ALAS and total cellular heme content, and Ni^{2+} produced a substantial reduction of cellular heme at a time (12 h, Fig. 1) when ALAS activity was unaltered.

The possibility that the metals directly complexed with ALAS to depress its activity can be excluded because high concentrations of Ni^{2+} and Pt^{4+} when directly added to the enzyme in vitro did not diminish its activity. It can be concluded, therefore, that in the experiments described here the exogenous metal ions—rather than the cellular concentrations of Fe-heme—directly exerted an inhibitory effect on the synthesis of ALAS. Direct regulatory actions of Ni^{2+} and of Pt^{4+} on ALAS are consistent with our previous findings with Co^{2+} in whole animal liver and avian embryo hepatocytes grown in tissue culture in which Co^{2+} blocked the *de novo* synthesis of ALAS in the same fashion as does Fe-heme (7, 8).

These findings make it clear that metal ions, independent of their ability to form intracellular complexes with protoporphyrin-IX (19), can mimic the regulatory action of Fe-heme on the rate-limiting enzyme of heme synthesis, ALAS. Fe-heme and metal ion control of ALAS results from inhibition of enzyme protein synthesis (3, 8); this inhibition by metals is followed by a rebound induction phenomenon (6). The exact cause of the induction of ALAS after the initial repression of its synthesis is not known, but it has been suggested (1, 2) that the increase reflects derepression of enzyme synthesis subsequent to the initial inhibition.

The decrease in heme content, which was observed when the heme biosynthetic pathway was functioning at an apparently unaltered rate, as judged by the total porphyrin content, and ALAS and ferrochelatase activities (e.g. 12–24 h after Ni^{2+} treatment) is most likely due to the concomitant increase in the rate of heme degradation mediated by the metal. Ni^{2+} and Pt^{4+} both substantially induced heme oxygenase activity in the kidney and this was associated with depletions of heme contents in this organ. In previous studies in liver, with isotopically labeled heme, we have shown that such decreases of cellular heme content result from induced heme oxygenase activity (20, 21). It can be reasonably assumed therefore that enhanced heme oxygenase activity similarly accounts for the reduction of renal heme contents observed in the present study.

On the basis of the findings described in this report and evidence from previous studies (5–9, 11), it is proposed that metal ions act directly as physiological regulators of ALAS production and that the well-known Fe-heme repression of synthesis of this enzyme essentially reflects the ability of this

metalloporphyrin chelate to facilitate the intracellular transport of metal to the regulatory site of ALAS production. According to this formulation, the metal moiety of Fe-heme, rather than the tetrapyrrole nucleus, is the proximately active chemical species for control of ALAS, and synthesis of this enzyme is regulated either by free metal ion released from the metalloporphyrin chelate through heme oxygenase action, or by coordination of the chelated metal at the enzyme regulatory site. Control of heme oxygenase synthesis directly by metal ions alone is also postulated to occur in similar fashion.

Summary

The in vivo regulation by metal ions of the enzymes of heme metabolism in kidney—particularly of ALAS, the rate-limiting enzyme in heme formation—was investigated. Ni^{2+} and Pt^{4+}, metals which do not enzymatically form metalloporphyrins, were found to regulate ALAS in kidney as they do in liver. The pattern of this regulation was generally similar to that observed with heme and metal ions in liver, i.e., a late increase in enzyme activity after an early period in which ALAS activity was unaltered or inhibited. The metals did not interact with the enzyme in vitro to alter its activity. In this study no direct reciprocal relationship between ALAS activity and total cellular heme content was demonstrated. The metal ions, particularly Pt^{4+}, also altered the activity of other enzymes of heme biosynthesis in kidney. Pt^{4+} severely inhibited the activity of ALAD and UROS. Ni^{2+} and Pt^{4+} were potent inducers of heme oxygenase, the initial and rate-limiting enzyme in heme degradation.

It is proposed that the physiological regulation of ALAS is mediated through the action of metal ions, rather than by the cellular content of heme, and that the regulation of ALAS by heme reflects the action of the central metal ion of heme rather than that of the entire metalloporphyrin complex. In this proposed mechanism for metal ion regulation of ALAS, the tetrapyrrole moiety of heme is considered to function principally as an efficient carrier of metal to the regulatory site for ALAS production, inasmuch as the tetrapyrrole ring itself has been shown in earlier studies not to have any effect on ALAS activity. The production of heme oxygenase is believed to be similarly regulated.

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