Biochimica et Biophysica Acta, 673 (1981) 339—350

BBA 29538

ZINC · PROTOPORPHYRIN IS A SELECTIVE INHIBITOR OF HEME OXYGENASE ACTIVITY IN THE NEONATAL RAT

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(Received August 4th, 1980)

Key words: Hyperbilirubinemia; Heme biosynthesis; Cytochrome P-450; Metalloporphyrin; Heme oxygenase inhibitor; (Neonatal rat)

Summary

The present study was undertaken to examine the liver, spleen and kidney heme oxygenase activity in the rat, and also to investigate the response of the enzyme to a variety of metalloporphyrin complexes. The enzyme activity in the liver and the kidney of 3—4-day-old rats was several-fold greater than the corresponding values in the adult animals; however, the splenic enzyme activity was markedly depressed in comparison to that of adult rats. During the first 2—3 weeks post-parturation period, the activity of heme oxygenase in the spleen progressively increased, and in 4 weeks approached the adult values. The treatment of the newborn animals with the metalloporphyrin complex, Zn · protoporphyrin-IX, inhibited heme oxygenase activity in the spleen, liver and the kidney. Sn · protoporphyrin treatment also inhibited the activity of the enzyme in the liver and the spleen. The mechanism of the inhibition appeared to be competitive in nature. In contrast, the treatment of the the newborn animals with Co · protoporphyrin increased the activity of the enzyme in the tested organs. The treatment of newborn animals with Fe · protoporphyrin (heme) also increased heme oxygenase activity in the spleen and the kidney. In addition, Co · and Fe · protoporphyrin complexes inhibited the activity of δ-aminolevulinate synthetase in the spleen; Sn · protoporphyrin and Zn · protoporphyrin, however, did not alter the activity of this enzyme. The effects of Co · protoporphyrin and Zn · protoporphyrin on the microsomal contents of cytochromes *P*-450, b_5, the total heme, and the microsomal drug metabolism activity in the liver were compared. Zn · protoporphyrin was ineffective in altering the indicated cellular variables. According to these findings Zn · protoporphyrin may be useful as an experimental tool for the selective suppression of heme degradation activity.

Introduction

The rapid degradation of fetal hemoglobin and the oxidation of its heme moiety (Fe · protoporphyrin) is a contributing factor in the development of post-parturition hyperbilirubinemia. Heme oxygenase, which constitutes the rate-limiting enzyme in the degradation of heme [1], cleaves oxidatively the closed tetrapyrrole molecule to form biliverdin, with the concomitant release of iron and the formation of a molecule of CO. Biliverdin is subsequently reduced to form bilirubin, an activity which is catalyzed by the cytosolic enzyme, biliverdin reductase. In contrast to the rather innocuous pigment biliverdin, bilirubin is a lipid-soluble neurotoxic compound. Recent studies in the rat have shown an elevated rate of heme oxygenase activity in various organs such as the liver, kidney and the skin during early neonatal life [2—5]. It also has been demonstrated that in the newborn of various species, including man and rat, the ability to carry out certain oxidative functions which are catalyzed by the microsomal hemoproteins cytochrome *P*-450 and b_5 are very limited [6—9]. Moreover, in the rat during the first 2—3 weeks of life the activity of δ-aminolevulinate synthetase is severely depressed [5,10,11]. The latter enzyme is the initial and the rate-limiting step in the pathway of heme biosynthesis [12], and its developmental pattern in the liver and the kidney is reciprocally related to that of heme oxygenase [5]. The reciprocal relationship between the activity of heme oxygenase and δ-aminolevulinate synthetase has been attributed to post-parturition hemolysis and the regulatory action of heme on its own metabolism. It is known that heme oxygenase is highly inducible by heme and the metalloporphyrin compound Co · protoporphyrin [13,14]. However, the above complexes are potent inhibitors of the synthesis of δ-aminolevulinate synthetase [12,15,16]. The inhibitory effect of heme on δ-aminolevulinate synthetase is biphasic and the inhibition is followed by a rebound increase in the activity [15].

In studies with heme oxygenase isolated from rat liver [17] and pig spleen [18] it has been established that the enzyme is not a hemoprotein; however, upon binding heme substrate, it assumes a hemoprotein nature [19—22] capable of binding molecular oxygen with the consequent degradation of the heme substrate. In addition, it has been shown that heme oxygenase does not exhibit specificity towards the chelated metal ion of the heme molecule. It was observed that the additions of non-physiological metalloporphyrins, such as Mn · and Ni · protoporphyrins, to the heme oxygenase assay system competitively inhibited the binding and the oxidation of Fe · protoporphyrin by the enzyme [19]. The present investigation sought to determine whether the in vivo treatment of rats with several non-physiological metalloporphyrins can also inhibit the activity of the enzyme towards Fe · protoporphyrin, when assessed in vitro. In this report it is demonstrated that the post-parturation increase in cellular heme oxygenase activity can be markedly suppressed in the newborn rats by treatment with the metalloporphyrin complex, Zn · protoporphyrin, which is not a substrate for the heme oxygenase system. Moreover, this inhibition occurs without inhibiting the activities of the enzymes of the heme biosynthetic pathway and the hemoprotein-dependent oxidative activities of the liver.

Experimental procedures

Materials, treatments and tissue preparations

Pregnant Sprague-Dawley rats were purchased from Harlan Resources Laboratories, Madison, WI. The metalloporphyrins, Co · protoporphyrin-IX, Sn · protoporphyrin-IX and Zn · protoporphyrin-IX (dimethylester and free acid), were purchased from Porphyrin Products, Logan, UT. The compounds were analyzed for purity by thin-layer chromatography on pre-coated silica gel G plates (Merck), using the following systems, water, *n*-propanol and pyridine (5.5 : 0.1 : 0.4, by volume); and 2,6-lutidine and water (5 : 3.5 by volume) with NH_3 vapor [23]. In most preparations only a slight contamination with protoporphyrin was detected. These compounds were used without further purification. Fe · protoporphyrin-IX (heme) and co-factors were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

For all experiments metalloporphyrin solutions were prepared immediately before use by dissolving in minimum volumes of NaOH (0.1 N), and adjusting the pH to 7.4 with 0.1 N HCl. All injections were made subcutaneously, the volume of injections were 0.1 ml/10 g body weight, and the doses were 0.4 μmol/10 g body weight. The regimens of treatments are indicated in the legends of appropriate figures and tables. In all instances one-half of the newborn rats of each litter were treated, and the others served as the controls. The newborn rats were kept with their mothers at all times. The controls received saline treatment. The organs of 2—10 neonatal rats were pooled for each experiment. All tissue preparations were made in 0.1 M potassium phosphate buffer (pH 7.4). The liver microsomal fractions were prepared as described elsewhere [5].

Enzyme assays

The activity of δ-aminolevulinate synthetase was measured in the whole cell homogenate, as recently detailed [24]. The procedure used was an adaptation of the method described by Marver et al. [25]. The activity of uropophyrinogen I synthase in the red blood cells and the liver was measured as described by Granick et al. [26]. For the measurement of enzyme activity in the erythrocytes, pooled blood was obtained by cardiac puncture immediately before killing the animals. The blood cells were washed with saline, centrifuged (1000 rev./min, 5 min), and to the final pellet a 1.0 ml aliquot of 0.1 M potassium phosphate buffer (pH 7.4) was added. The cells were lysed by rapid freeze-thawing of the samples, the stroma was removed by centrifugation, and the clear supernatant fraction was used for the assay. The activity of ferrochelatase was measured as described by Jones and Jones [27], and that of δ-aminolevulinate dehydratase was measured by the method of Mauzerall and Granick [28].

The activity of heme oxygenase was measured in the microsomal fraction of the liver. The microsomal fraction of the spleen was used for kinetics studies. Due to the limitation of tissue samples, the 9000 × *g* supernatant fractions of the kidney and the spleen were used as the enzyme source in certain indicated experiments. The source of biliverdin reductase was perfused liver cytosol (105 000 × *g* supernatant) of untreated adult rats. The assay of heme oxygenase

was a modification of that described earlier [29], with the procedural modifications described below. The protein concentration of the microsomal or the 9000 × *g* fractions were adjusted to 4—6 mg/ml. The test system with a final volume of 1.5 ml had a pH of 7.4, and was 0.09 M in phosphate buffer. It contained 0.5 ml of enzyme source; 0.1 ml of 105 000 × *g* supernatant fraction (5.0 mg of protein/ml); NADPH-generating system consisting of 0.8 mM NADP/0.8 mM glucose 6-phosphate/1.0 unit glucose-6-phosphate dehydrogenase; and 1.0 mM $MgCl_2$. 20 μl 2.5 mM heme solution was added to start the reaction. The blank incubation medium did not contain the NADPH generation system. The mixtures were prepared at 4°C and then aerobically incubated in the dark in a shaking incubator at 37°C (10 min for the liver and the spleen, 15 min for the kidney). At the end of incubation time, the amount of bilirubin formed was measured spectrophotometrically in the split wavelength mode of an Aminco Dual Wavelength spectrophotometer. An extinction coefficient of 40 $cm^{-1} \cdot mM^{-1}$ for absorption between 464 nm and 530 nm was used [29].

The microsomal content of heme was determined from the pyridine hemochromogen spectrum [30]. The microsomal contents of cytochromes *P*-450 and b_5 were measured by the methods described by Omura and Sato [31]. The cytochrome b_5 measurements were made using NADH as the reducing agent, and an extinction coefficient of 183 $cm^{-1} \cdot mM^{-1}$ for absorption between 408 nm and 423 nm was used [31]. The hemoprotein-dependent oxidation activity was measured using a prototype drug, ethylmorphine, as the substrate. The *N*-demethylation of ethylmorphine was assessed by measuring the formation of formaldehyde [32]. Protein was measured by the method of Lowry et al. [33]. Each experiment was repeated at least five times, and the data were analyzed using Student's *t*-test, and the value $P \leqslant 0.05$ was regarded as denoting significance.

Results

The developmental pattern of splenic heme oxygenase activity in the neonatal rat

Fig. 1 shows the pattern of heme oxygenase activity in the developing rat spleen during the first 4 weeks of life. As shown, in the unborn rats the heme oxygenase activity of the spleen measured only a fraction of the adult value. However, after birth the enzyme activity progressively increased with age, and in 4 weeks approached that of the adult rats. The observed developmental change in heme oxygenase activity of the spleen differs from those of the liver and the kidney; in the latter organs the enzyme activity is highly elevated during the first 2 weeks of post-parturition life, and thereafter decreases to approximate the adult values in the subsequent 2 weeks (Table I and Ref. 6).

The kinetics of the inhibition of heme oxygenase by Zn · protoporphyrin and Sn · protoporphyrin

The kinetics of the oxidation of Fe · protoporphyrin (heme) by the neonatal spleen microsomal heme oxygenase and the effects of metalloporphyrins Zn · protoporphyrin and Sn · protoporphyrins are shown in Fig. 2.

As shown, the apparent K_m = 20 μM of the neonatal splenic enzyme for Fe ·

TABLE I

EFFECTS OF IN VIVO TREATMENT WITH Zn · , Sn · , Co · AND Fe · PROTOPORPHYRIN COMPLEXES ON THE HEME OXYGENASE ACTIVITY OF THE LIVER, SPLEEN AND THE KIDNEY OF THE NEONATAL RAT

Newborn rats were injected within 82—96 h after birth with the indicated metalloporphyrin complexes (0.4 μmol/10 g, twice per day for 2 days). The control animals received saline treatment. In all experiments one-half of the newborn rats of each litter were treated with a metalloporphyrin and the others served as the controls. 12 h after the last injection the animals were killed. For the determination of heme oxygenase activity the pooled organs of 8—10 newborn rats were used for each experiment. The heme oxygenase activity of the organs were measured using the microsomal fraction of the liver and the 9000 × *g* supernatant fractions of the spleen and the kidney. The adult values ($n = 5$) represent those of young male rats (150—200 g). The data presented are the mean ± S.D. of five experiments with each metalloporphyrin, and 20 with the controls.

Treatment	Heme oxygenase (nmol bilirubin/h per mg protein)		
	Liver	Spleen	Kidney
Control	11.20 ± 1.6	2.71 ± 0.29	2.41 ± 0.31
Zn · protoporphyrin	5.06 * ± 1.6	1.62 * ± 0.30	1.10 * ± 0.30
Sn · protoporphyrin	7.22 * ± 2.2	2.01 * ± 0.38	1.97 ± 0.28
Co · protoporphyrin	15.90 * ± 2.3	4.00 * ± 0.48	3.31 * ± 0.29
Fe · protoporphyrin	13.74 ± 2.0	3.41 * ± 0.35	4.22 * ± 0.34
Control adult	1.34 * ± 0.11	6.26 * ± 0.75	0.83 * ± 0.30

* $P \leq 0.05$ when compared with the control values.

protoporphyrin closely approximates that previously reported [19] for the adult spleen (17 μM). Moreover, as with the adult spleen perparations [19] the substrate binding site of heme oxygenase did not exhibit specificity towards the metal chelate moiety of the metalloporphyrins. Zn · protoporphyrin, when present in the assay system in a final concentration of 5 μM, competitively inhibited the oxidation of Fe · protoporphyrin. The lack of specificity of the enzyme binding site for metalloporphyrins was further established in experiments in which the effect of Sn · protoporphyrin on the oxidation of Fe · pro-

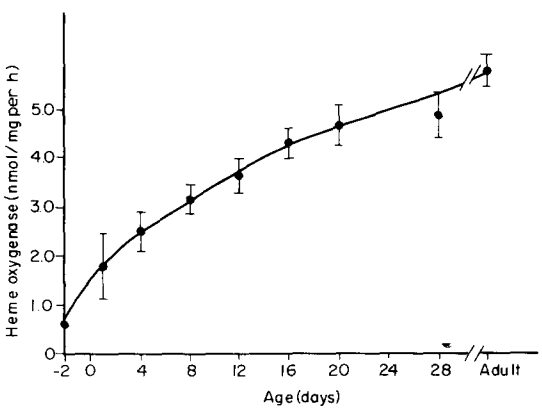

Fig. 1. The development pattern of splenic heme oxygenase activity in the rat. Rat spleen 9000 × *g* supernatant fractions were prepared and assayed for heme oxygenase activity. Depending on the age of the animals, 2—10 spleens were pooled for each experiment, and each point represents the mean value of five experiments. The adult value represents that of young male rats (150—200 g).

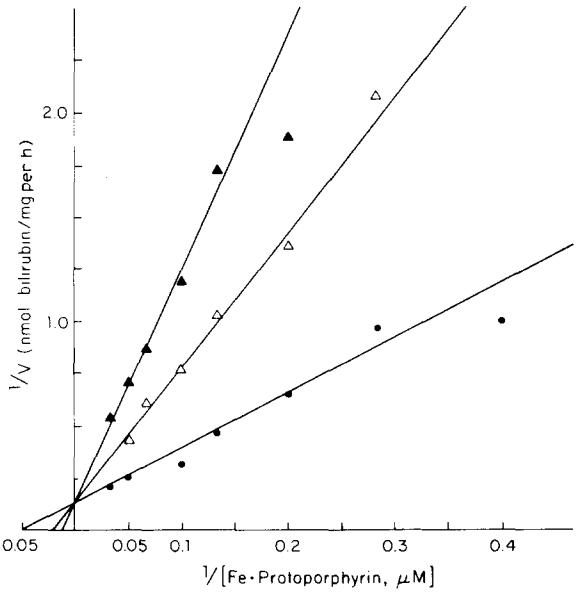

Fig. 2. The kinetics of the inhibition of Fe · protoporphyrin oxidation by Zn · protoporphyrin and Sn · protoporphyrin. The assay was carried out utilizing the splenic microsomal fraction as described in the Experimental procedures section. ●———●, control; △———△, plus Sn · protoporphyrin, 5 μM; ▲———▲, plus Zn · protoporphyrin, 5 μM.

toporphyrin was investigated. As shwon, Sn · protoporphyrin also competitively inhibited the oxidation of Fe · protoporphyrin. The possibility of the oxidation of Zn · protoporphyrin and Sn · protoporphyrin by the liver and the spleen heme oxygenase was studied. No activity was detected when these complexes in the final concentrations of 5—50 μM were added to heme oxygenase assay system. The lack of specificity of heme oxygenase for the substrate was not restricted to the above conditions, in which the metalloporphyrins were added to the heme oxygenase assay system. When the kinetics of Fe · protoporphyrin oxidation was measured using the splenic microsomal preparations obtained from Zn · protoporphyrin-treated newborn rats, the apparent K_m value was also markedly increased (60 μM). In addition, the V of the enzyme, using the microsomal fractions obtained from newborn rats treated in vivo with Zn · protoporphyrin or Sn · protoporphyrin did not differ from that of the enzyme preparations obtained from control animals. The absence of change in V indicates that catalytically active (Fe · protoporphyrin) and nonactive (Zn · protoporphyrin and Sn · protoporphyrin) metallporphyrins bind to heme oxygenase at a common catalytic site. The inhibitory activity of Zn · protoporphyrin (free acid) on heme oxygenase with that of the dimethylester derivative was compared, the former complex was a somewhat more effective inhibitor of the enzyme than the latter. The kinetic studies were also carried out using the liver microsomal fraction, and similar kinetic patterns were observed. However, with higher concentrations of heme, in the presence of metalloporphyrins spectral distortions were noted.

The effects of Zn · , Sn · , Co · and Fe · protoporphyrin complexes on heme oxygenase activity in the neonatal rat

Table I shows the effects of in vivo treatment of the neonatal rat with Zn · , Sn · , Co · and Fe · protoporphyrin complexes on heme oxygenase activity in the liver, spleen and kidney. This table also shows the comparative activities of the enzyme in the organs of the neonatal rats to those of the adult animals. As shown, the enzyme activity in the liver and the kidney of the newborn animals was highly elevated when compared to the adult levels. The administration of Zn · protoporphyrin for two consecutive days to the newborn rats significantly inhibited the heme oxygenase activity in all organs studied. Also as shown, the activity of the enzyme in the liver and the spleen, but not in the kidney, were inhibited by Sn · protoporphyrin; although the extent of inhibitory effects were less pronounced than those of the Zn · protoporphyrin complex. This table also demonstrates the differential effects of in vivo treatments with Zn · protoporphyrin and Sn · protoporphyrin on heme oxygenase activity in comparison to treatment with Fe · protoporphyrin and Co · protoporphyrin. As shown, the activity of the enzyme in the spleen and the kidney were increased in response to treatment with Fe · protoporphyrin and Co · protoporphyrin. This activity was also increased in response to Co · protoporphyrin treatment in the liver. However, Fe · protoporphyrin treatment did not exert a significant effect on the liver heme oxygenase activity. The inhibitory activity of Sn · protoporphyrin and Zn · protoporphyrin on the neonatal liver heme oxygenase most likely did not represent the hematopoietic nature of the organ; as noted, the inhibitory effect was also exerted on the splenic and the kidney enzymes, the organs which do not exhibit a developmental change in cell populations.

The comparative effects of metalloporphyrins on the activity of δ-aminolevulinate synthetase in the spleen; and the drug metabolism activity and microsomal contents of heme and cytochromes in the liver

As noted earlier, it is known that in the neonatal rat increased heme oxygenase and depressed δ-aminolevulinate synthetase activities are accompanied by decreases in the cellular levels of heme and hemoproteins, and depressed rates of heme-dependent oxidative activities. Accordingly, the effects on δ-aminolevulinate synthetase of zinc and tin metalloporphyrins, which inhibited heme oxygenase activity, and those of Fe · protoporphyrin and Co · protoporphyrin which augmented the enzyme activity were investigated. In addition, the effects of Zn · protoporphyrin and Co · protoporphyrin on oxidative drug metabolism activity of the liver and the microsomal contents of heme and hemoproteins were compared.

As shown in Table II, in the newborn rats, the activity of δ-aminolevulinate synthetase in the spleen was not responsive to treatment with Zn · protoporphyrin and Sn · protoporphyrin. However, the administration of Co · protoporphyrin and Fe · protoporphyrin to the animals significantly inhibited the activity of the enzyme in the spleen. The effects of the additions of the above metalloporphyrin complexes to the δ-aminolevulinate synthetase assay system were also investigated. When added in the final concentrations of 25 μM and 50 μM to the assay medium containing splenic enzyme preparation, none of the above metalloporphyrin complexes significantly altered the enzyme activity.

TABLE II

THE COMPARATIVE EFFECTS OF Zn · , Sn · , Co · AND Fe · PROTOPORPHYRIN COMPLEXES ON THE δ-AMINOLEVULINATE SYNTHETASE ACTIVITY OF THE SPLEEN OF THE NEONATAL RAT

Newborn rats were treated within 82—96 h after birth subcutaneously with solutions of Zn · , Sn · , Co · or Fe · protoporphyrin complexes. The control animals received saline. In all instances one-half of the neonates of each litter were treated with a metalloporphyrin and the others served as the controls. The metalloprophyrin solutions were prepared immediately before use. The dose of all metalloporphyrins was 0.4 μmol/10 g body weight (twice per day for 2 consecutive days) and the animals were killed 12 h after the last injection. The activity of δ-aminolevulinate synthetase was measured in the cellular homogenates. Organs from 8—10 animals were pooled for each experiment. The data presented are the mean ± S.D. of five experiments with each metalloporphyrin, and 20 with the controls.

Treatment	δ-Aminolevulinate synthetase (pmol δ-aminolevulinate/h per mg protein)
Control	546 ± 43
Zn · protoporphyrin	554 ± 48
Sn · protoporphyrin	509 ± 37
Co · protoporphyrin	315 * ± 52
Fe · protoporphyrin	436 * ± 54

* $P \leq 0.05$ when compared with the control value.

The comparative effects of Zn · protoporphyrin and Co · protoporphyrin treatments on the microsomal contents of heme, hemoproteins and the oxidative metabolism of ethylmorphine are shown in Table III. In the neonatal rat Zn · protoporphyrin treatment did not decrease the microsomal contents of cytochromes *P*-450, b_5 and heme. Also, the cytochrome *P*-450 dependent *N*-demethylation of ethylmorphine was not decreased in response to Zn · protoporphyrin treatment. These findings contrast with the cellular response

TABLE III

THE COMPARATIVE EFFECTS OF Zn · PROTOPORPHYRIN AND Co · PROTOPORPHYRIN ON THE MICROSOMAL DRUG METABOLIZING ACTIVITY AND THE MICROSOMAL CONTENTS OF HEME AND HEMOPROTEINS IN THE NEONATAL RAT LIVER

Neonatal rats were treated 82—96 h after birth with Zn · protoporphyrin or Co · protoporphyrin (0.4 μmol/10 g, twice per day) for 2 days. The control animals received saline. In all instances one-half of the neonates of each litter were treated with a metalloporphyrin and the others served as controls. 12 h after the last injection the animals were killed, the livers from 8—10 animals were pooled, and the microsomal fractions were prepared [5]. This preparation was used for the measurement of the indicated cellular variables. Ethylmorphine *N*-demethylase activity was measured by Nash's method [32]. The cytochrome b_5 content was determined from the NADH-reduced absorption spectrum [31], the total microsomal heme content was measured from the pyridine hemochromogen spectrum [30], and the cytochrome *P*-450 values were obtained from the reduced — CO difference spectrum [31]. The data presented are the mean ± S.D. of five experiments with each metalloporphyrin, and then experiments with the controls.

Treatment	Ethylmorphine *N*-demethylase (nmol HCHO/h per mg protein)	Microsomal heme (nmol/mg)	Cytochrome *P*-450 (nmol/mg)	Cytochrome b_5 (nmol/mg)
Control	109.0 ± 29	0.89 ± 0.09	0.29 ± 0.04	0.22 ± 0.04
Zn · protoporphyrin	92.6 ± 25	0.80 ± 0.08	0.28 ± 0.05	0.21 ± 0.05
Co · protoporphyrin	72.2 ± 23	0.67* ± 0.10	0.21* ± 0.04	0.15 ± 0.04

* $P \leq 0.05$ when compared with the control values.

elicited by Co · protoporphyrin. As shown, significant decreases in the cytochrome *P*-450 and heme contents of the liver were produced as the result of this treatment. Zn · protoporphyrin treatment did not cause any alterations in the activities of ferrochelatase, δ-aminolevulinate dehydratase, and uroporphyrinogen I synthase in the liver.

Table III also shows that in the newborn animals, regardless of the treatment, the microsomal contents of hemoproteins *P*-450 and b_5, when added together, were less than the total microsomal heme content. This observation might be attributed to one or a combination of the following developmental phenomena: a decreased cytochrome b_5 reductase activity of the liver of the neonatal rat, a decreased apocytochrome content of the microsomal fractions, and/or the increased nonspecific binding of heme to the microsomal membranes.

Effect of Zn · protoporphyrin treatment on uroporphyrin I synthase in the erythrocytes

To investigate whether Zn · protoporphyrin treatment caused any alterations in the rate of post-parturation maturation of erythrocytes, the activity of uroporphyrin I synthase in blood was measured. The activity of this enzyme in immature erythrocytes is much higher than that of the mature cells [29,34]. Therefore, alterations in the activity of this enzyme might reflect alterations in the natural maturation and degradation processes of the erythrocytes.

Newborn rats, 82—96-h-old, were treated for 2 days with Zn · protoporphyrin (twice per day), and the activity of uroporphyrinogen I synthase in the erythrocytes was measured. No significant difference in the enzyme activity in the erythrocytes of the treated and the control animals was detected. The enzyme activity in the treated neonates was 33 ± 6 pmol porphyrin/h per mg protein (n = 5), and that of the control animals was 35 ± 8 pmol porphyrin/h per mg protein (n = 5).

Discussion

The occurrence of physiological jaundice in the newborn human presents a clinical condition which occasionally proves to be of serious neurological consequence. Physiological jaundice also occurs in various mammalian species including the rat [35]. The microsomal enzyme system, heme oxygenase, which converts heme to biliverdin is the rate-limiting enzyme in the pathway of heme degradation. In the newborn of the species which experience post-parturition hemolysis of the fetal erythrocytes, the increased rate of heme degradation coupled with other physiological deficiences such as the reduced hepatic bilirubin conjugating activity, and the diminished plasma bilirubin binding capacity [36], could contribute significantly to the development of jaundice.

The present study suggests that increases in the rate of heme degradation activity in various organs may be an important factor in the development of hyperbilirubinemia in the neonatal rat. It may be postulated that when this process is inhibited in the tissues with high heme oxygenase activity, such as the spleen, liver and kidney, significant decreases in the plasma level of bilirubin could be attained. Heme oxygenase utilizes as the substrate not only

hemoglobin heme, but the heme moiety of cellular hemoproteins, such as that of the microsomal cytochrome *P*-450 [37]. Certainly the utilization of cytochrome *P*-450 heme as a substrate by heme oxygenase does not necessarily imply a regulatory role for the enzyme in the cellular levels of cytochrome *P*-450. Moreover, the increased heme oxygenase activity is not the sole determinant of the microsomal concentration of cytochrome *P*-450 [38]. Nonetheless, in the newborn rat the highly elevated heme oxygenase activity not only could promote the increased degradation of heme and the occurrence of hyperbilirubinemia, but in the liver, in conjunction with depressed rates of heme and hemoprotein production [5,10,11], could contribute to decreased drug metabolism activities [5—11]. In turn, the increased activity of heme oxygenase and the decreased heme biosynthesis activity, which are reflected in a depressed activity of δ-aminolevulinate synthetase in liver and kidney [5,10,11], are apparently direct manifestations of the post-parturition hemolysis [5]. The finding that the suppression of heme oxygenase activity by Zn · protoporphyrin did not result in a concomitant increase in the microsomal cytochrome *P*-450 content (Table III) may reflect the suggested [39,40] role of apocytochrome *P*-450 as the limiting factor in the production of the cytochrome.

Previous studies have shown that heme oxygenase does not have an absolute specificity towards Fe · protoporphyrin; rather, other metal complexes of protoporphyrin will also bind [19,41] and compete [19] for the same catalytic site. The kinetics studies conducted in the present study suggest that the lack of substrate specificity of heme oxygenase may extend to the conditions in which the enzyme, in a structurally intact cellular environment, is exposed to the metalloporphyrins. As shown, catalytically nonactive Zn · and Sn · metalloporphyrin complexes, when administered to rats, also caused inhibition of the enzyme activity when heme oxygenase activity was assessed in vitro (Table I, and text). The observed limited extent of the activity of Zn · protoporphyrin in the enzyme systems presently tested is perhaps of biological and experimental relevance. As noted, this protoporphyrin complex did not interfere with the activities of various heme biosynthesis enzymes, and the cellular functions which depend on hemoproteins (Table III and text). Also Zn · protoporphyrin treatment did not alter the cellular levels of heme and hemoproteins. The finding that the activity of δ-aminolevulinate synthetase which was responsive in the spleen to Fe · protoporphyrin and Co · protoporphyrin, but was essentially refractory to the Zn complex, further suggests the selectivity of this metalloporphyrin for heme oxygenase.

A major concern in utilizing metalloporphyrin complexes for regulating heme oxygenase activity is the potential alteration of cellular functions, particularly the heme-related activities by the complexes and/or their metal moiety, should they be released in the body. However, it appears that this concern is not pertinent to Zn · protoporphyrin. As shown in the present report, Zn · protoporphyrin was not degradated by heme oxygenase, thus the possibility of the enzymatic release of Zn and the porphyrin moieties are remote. However, should Zn be released via nonenzymatic means, little biological activity could be expected. Zn, unlike Co, Sn, Fe and other metal ions, is a rather ineffectual trace element in altering heme degradation and hematological parameters [37, 42]. Our previous studies have shown that the administration of Zn, at doses as

high as 250 μmol/100 g body weight to rats did not alter heme metabolism activity in the kidney [37]. These studies also have shown that higher doses were required to moderately increase heme oxygenase activity in the liver. Other investigators have shown that the half-life of the erythrocytes of growing sheep was not significantly changed when fed for 4 weeks on a diet of 400 ppm zinc oxide [42]. Moreover, although the ingestion of several grams of Zn can produce disturbances of the gastrointestinal tract, the ingestion of 600 mg zinc sulfate/day to promote wound healing in man is thought to be safe [43]. In addition, the protoporphyrin moiety of Zn · protoporphyrin is a normal cellular component, and Zn · protoporphyrin itself neither causes photosensitivity nor photohemolysis [44].

Thus, it is plausible to employ the principle of specific suppression of heme oxygenase as an experimental tool to obviate the post-parturation surge of increase in plasma bilirubin levels, and diffuse the rate of hemoglobin heme degradation in the newborn animals. This principle may also be applicable to other experimental conditions in which the suppression of the enzyme activity is desirable.

Acknowledgments

I am grateful to Miss May Szeto for able technical assistance. This study was supported by U.S.P.H. ES-2180.

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