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Sn-protoporphyrin inhibition of fetal and neonatal brain heme oxygenase. Transplacental passage of the metalloporphyrin and prenatal suppression of hyperbilirubinemia in the newborn animal.

G S Drummond, A Kappas

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Research Article

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Sn-Protoporphyrin Inhibition of Fetal and Neonatal Brain Heme Oxygenase

Transplacental Passage of the Metalloporphyrin and Prenatal Suppression of Hyperbilirubinemia in the Newborn Animal

George S. Drummond and Attallah Kappas
The Rockefeller University Hospital, New York 10021

Abstract

Sn(tin)-protoporphyrin, a potent competitive inhibitor of heme oxygenase, can suppress hyperbilirubinemia in animal neonates and significantly reduce plasma bilirubin levels in animals and man. To further explore the biological actions and metabolic disposition of Sn-protoporphyrin, we have examined its effect in the suckling neonate when administered to the mother either 24–48 h before or immediately after birth. Sn-protoporphyrin, when administered before birth, crossed the placental membranes, inhibited fetal heme oxygenase, and suppressed the transient hyperbilirubinemia that occurs in the neonate after birth in a dose-dependent manner. Tissue heme oxygenase activity in the neonate was also lowered in a dose-dependent manner. The blood-brain barrier of the neonate was permeable to Sn-protoporphyrin for a period of between 20–28 d of postnatal life. Sn-protoporphyrin, however, was not retained in brain, but left the brain space with a $t_{1/2}$ of 1.7 d. In addition, Sn-protoporphyrin administered once at birth to neonates inhibited brain heme oxygenase in a dose-dependent manner.

The results of this study demonstrate that Sn-protoporphyrin can cross the placental membranes, inhibit tissue heme oxygenase activity in the fetus, and can also, following such prenatal treatment, suppress the hyperbilirubinemia of the newborn animal.

Introduction

The synthetic heme analogue Sn-protoporphyrin is a potent inhibitor of the activity of heme oxygenase (1, 2), the rate-limiting enzyme in the degradation of heme to bile pigment (3). This inhibition is competitive in nature as has been demonstrated in studies of the metalloporphyrin in various animal and human microsomal preparations as well as in a reconstituted heme oxidation system utilizing homogenously purified heme oxygenase in vitro (1, 2, 4, 5). We have demonstrated that the administration of Sn-protoporphyrin shortly after birth prevents the transient increase in serum bilirubin levels which occurs in the rat (1, 2). This effect of the metalloporphyrin has been confirmed in the rhesus neonate (6); the compound also blocks the development of starvation-induced hyperbilirubinemia in the squirrel monkey, a form of jaundice which is considered to be a model of Gilbert's syndrome in man (7). Sn-protoporphyrin also decreases plasma bilirubin levels in adult mice with congenital forms of severe hemolytic anemia (8); in the 7–14-d-old suckling rat with hyperbilirubinemia resulting from administration of the heme precursor, δ-aminolevulinic acid, or of heme itself (9); in the bile duct-ligated rat (10); and in humans with sustained high levels of serum bilirubin due to primary biliary cirrhosis (10, 11). Sn-protoporphyrin reduces the in vivo production of bilirubin (12) as well as of carbon monoxide from the degradation of endogenous as well as exogenous heme (9, 10, 13). It also stimulates the excretion of heme into the bile of bile duct-cannulated rats (14). We have developed a fluorometric method for measuring Sn-protoporphyrin in tissues (15) and have found that the metalloporphyrin is rapidly cleared from plasma and promptly and markedly inhibits heme oxygenase activity in tissues, such as the liver, kidney, and spleen (16).

The present study was designed to examine the disposition of Sn-protoporphyrin in the fetus and the neonate when this synthetic metalloporphyrin was administered to the mother both before and after birth in order to explore the potential use of Sn-protoporphyrin in infants who have high levels of serum bilirubin at birth due to hemolysis in utero. In addition, we examined the ability of Sn-protoporphyrin to cross the blood-brain barrier in the suckling neonate. The results of these experiments indicate that Sn-protoporphyrin freely crosses placental membranes before birth, that the compound can significantly inhibit heme oxygenase in the fetus, and that it is capable of suppressing subsequent postnatal jaundice in the newborn of prenatally-treated dams. Sn-protoporphyrin also crosses the blood-brain barrier in the neonate, which is known to be permeable to many chemicals in the immediate postnatal period, and can thereby also inhibit heme degradation to bilirubin directly in sites in the brain where heme metabolism to this bile pigment could take place. Unlike the prolonged localization of Sn-protoporphyrin in tissues such as liver or spleen (16), the localization of Sn-protoporphyrin in the brain is transient and the compound leaves the brain space with a $t_{1/2}$ of 1.7 d.

Methods

Materials

15-d pregnant Sprague-Dawley rats supplied by Holtzman Co. (Madison, WI) were used throughout this study. Pregnancy was synchronized in dams so that sufficient numbers of newborn could be studied within the same postnatal time period. Metalloporphyrins were purchased from Porphyrin Products (Logan, UT). All other chemicals were of the highest grade obtainable from either Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific Co. (Pittsburg, PA).

Animal treatment

Metalloporphyrins [Sn(tin)-, Zn(zinc)- and Mn(manganese)-protoporphyrin] were administered subcutaneously in the nuchal region at two dose levels, 10 and 50 μmol/kg body wt, at the times indicated in the

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legends to figures and tables. To prepare metalloporphyrin solutions, the metalloporphyrin was dissolved in a small volume of 0.5 M NaOH (0.2 ml/1.0 ml of final volume of metalloporphyrin solution) adjusted to pH 7.4 with 1 M HCl, and made up to final volume with 0.9% NaCl (saline). All procedures were carried out under subdued light conditions. Control animals were administered an equivalent volume of 0.9% NaCl. Neonates remained with their mothers throughout the studies described below. Groups of neonates (6–30 per group, depending on age) were killed at the times indicated. Animals were housed in The Rockefeller University Laboratory Animal Research Center and were maintained in a controlled environment with a 12 h:12 h light/dark cycle in accordance with the applicable portions of the Animal Welfare Act and the guidelines prescribed in the Department of Health and Human Services publication, *Guide for the Care and Use of Laboratory Animals.*

Laboratory animals

Tissue preparation. Livers were perfused in situ with ice cold 0.9% NaCl and homogenized in 3 vol of 0.1 M potassium phosphate buffer, pH 7.4, containing 0.25 M sucrose, and the microsomal fractions were prepared as previously described for the determination of heme oxygenase activity (1). Spleen, brain, and kidney microsomal fractions were prepared in an identical manner. The cytosolic fraction from the liver of control animals served as the source of biliverdin reductase.

Assays. The activity of heme oxygenase in all tissues was determined as previously described (1). Bilirubin formation was calculated by using an absorption coefficient of 40 $mM^{-1}cm^{-1}$ between 464 and 530 nm. Spectral studies were carried out on an Aminco Chance DW2A spectrophotometer (American Instrument Co., Inc., Silver Springs, MD) in the split beam mode. Total serum bilirubin was estimated fluorometrically by the method of Roth (17); the variation of replicate assays was within 5%. Metalloporphyrins did not interfere with the determination of serum bilirubin in this assay. Sn-protoporphyrin levels in tissue were determined fluorometrically by the method of Simionatto et al. (15). Fluorometric determinations were carried out on an Hitachi MPF III fluoresence spectrophotometer (Hitachi Corp., Allendale, NJ) equipped with a R928 photomultiplier tube; the variation in replicate samples was <5%. Protein content was determined by the method of Lowry et al. (18) using crystalline bovine serum albumin as standard. The data were analyzed by the standard *t* test and the *P* value < 0.05 was regarded as significant.

Results

Effect of Sn-protoporphyrin on heme oxygenase levels in tissues of 21-d pregnant rats and in fetuses of such treated dams. Sn-protoporphyrin (50 μmol/kg body wt) was administered to 20-d pregnant rats, and the levels of heme oxygenase was measured in liver, kidney, and spleen of these animals at 24 h later. Sn-protoporphyrin administration resulted in a significant decrease in heme oxygenase activity in all three tissues examined (Table I). Sn-protoporphyrin produced a comparable decrease in fetal hepatic heme oxygenase; although this decrease (39%) was significant (Table II), it was not as marked as the decrease (64%) noted in hepatic heme oxygenase activity in the dam (Table I). Decreases in enzyme activity similar to those in fetal liver were observed in fetal brain (20%), spleen (30%), and kidney (44%) heme oxygenase (results not shown). Sn-protoporphyrin was detectable in small amounts in fetal liver, which supports the enzyme data (Table II) and confirms that the metalloporphyrin had crossed the placental membranes and entered the fetal circulation. The amount (4.10±0.15 nmol/g) of Sn-protoporphyrin in fetal liver represented 2.2% of the content (185.85±38.55 nmol/g) of Sn-protoporphyrin present in the liver of the dam. Sn-protoporphyrin was also detectable in trace amounts in fetal spleen and kidney, but not in brain (data not shown). These

Table I. Effect of Sn-Protoporphyrin on Tissue Heme Oxygenase Activities in 21-d Pregnant Rats

	Heme oxygenase		
Tissue	Liver	Kidney	Spleen
	nmol bilirubin/mg/h		
Control	3.40±0.28	1.11±0.15	14.17±1.94
Sn-protoporphyrin‡	1.23±0.10*	0.25±0.21*	5.09±1.31*

* $P < 0.05$.
‡ Sn-protoporphyrin, 50 μmol/kg body wt, administered subcutaneously to 20-d pregnant rats; the animals were sacrificed 24 h later.

results prompted us to examine the effect of prenatal Sn-protoporphyrin administration to the dams on the increase in serum bilirubin that normally occurs in the newborn of these animals during the first 48 h postnatally (1, 2). In addition, the developmental changes in tissue heme oxygenase activity as well as the distribution of Sn-protoporphyrin in the developing neonate were examined after prenatal administration of the metalloporphyrin.

Effect of administration of Sn-protoporphyrin to the dam before birth on neonatal hyperbilirubinemia, and on tissue heme oxygenase activity and Sn-protoporphyrin disposition in the developing neonate. A single injection of Sn-protoporphyrin (10 or 50 μmol/kg body wt) was administered to pregnant rats 24–48 h before birth. Control dams were administered an equal volume of saline. Sn-protoporphyrin administration to the dam before birth prevented the increase in serum bilirubin levels that occurs 24 h after birth in control neonates (Fig. 1). Serum bilirubin levels in control neonates were 0.56±0.04 mg/dl compared with significantly lower levels of 0.40±0.04 and 0.34±0.04 mg/dl (means±SEM, $n = 6–8$, $P < 0.02$) in the neonates of dams administered 10 and 50 μmol/kg body wt, respectively. Thus, the effect of Sn-protoporphyrin on serum bilirubin levels in neonates appears to be dose-dependent. At 48 h after birth, the levels of serum bilirubin in neonates born of saline- and Sn-protoporphyrin-treated dams were not significantly different. Sn-protoporphyrin administration prenatally to the dam produced a dose-dependent decrease in hepatic heme oxygenase activity in the developing neonate 24 and 48 h after birth; by 96 h, there was no significant difference between the control and metallo-

Table II. Effect of Sn-Protoporphyrin Administration on Heme Oxygenase Activity and Disposition of Sn-Protoporphyrin in Fetal Liver

Treatment	Heme oxygenase	Sn-protoporphyrin content
	nmol bilirubin/mg/h	*nmol/g*
Control	5.46±0.72	0
Sn-protoporphyrin‡	3.34±0.11*	4.10±0.15*

* $P < 0.05$.
‡ Sn-protoporphyrin, 50 μmol/kg body wt, administered subcutaneously to 20-d pregnant rats; the animals were sacrificed 24 h later.

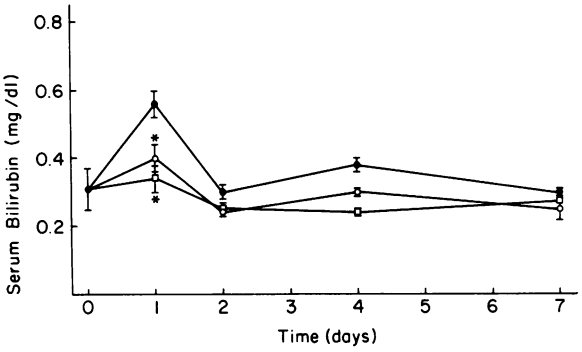

Figure 1. Effect of Sn-protoporphyrin administration to the dam before birth on hyperbilirubinemia in the neonate. Sn-protoporphyrin was administered subcutaneously to the dam 24–48 h before birth in a dose of either 10 (○) or 50 (□) μmol/kg body wt. Control dams (●) were administered saline. Neonates were sacrificed at the times indicated. Each time point represents the mean±SEM of between three and nine litters. $*P < 0.02$ when compared with control values.

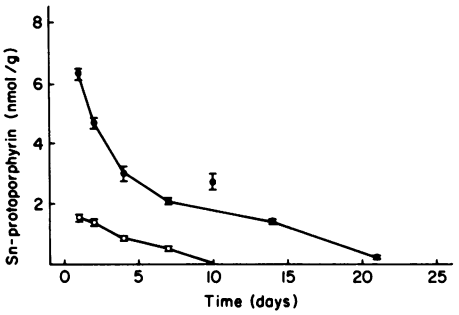

Figure 3. Effect of Sn-protoporphyrin administration to the dam before birth on the content of the metalloporphyrin in the neonatal liver. Sn-protoporphyrin was administered subcutaneously to the dam 24–48 h before birth in a dose of either 10 (○) or 50 (●) μmol/kg body wt. Each time point represents the mean±SEM of at least three litters. Since the bilirubin values for the treated animals paralleled those for the controls, the latter data are not shown.

porphyrin-treated groups with respect to heme oxygenase activity in this organ (Fig. 2 *A*). Sn-protoporphyrin administration retarded the previously described (1, 19) postnatal, developmental increase in splenic heme oxygenase activity (Fig. 2 *B*), while only the higher dose of Sn-protoporphyrin (50 μmol/kg body wt) significantly lowered neonatal renal heme oxygenase activity when administered prenatally (data not shown). The content of Sn-protoporphyrin in neonatal liver was found to be dose-dependent, to persist for varying periods, and to decline with time (Fig. 3). Sn-protoporphyrin was detectable in neonatal kidney only after the higher dose (50 μmol/kg body wt) was administered to the dam before birth of the neonates. A similar effect was noted in spleen with trace amounts of the metalloporphyrin being detectable only after the 50 μmol/kg body wt dose. These findings confirm that the metalloporphyrin can cross the placental membranes in a functionally intact form and can suppress neonatal hyperbilirubinemia in the newborn rat.

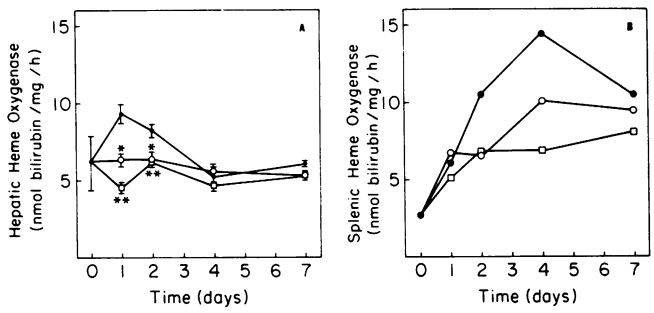

Figure 2. Effect of Sn-protoporphyrin administration to the dam before birth on hepatic and splenic heme oxygenase activity in the neonate. Sn-protoporphyrin was administered subcutaneously to the dam at 24–48 h before birth in a dose of either 10 (○) or 50 (□) μmol/kg body wt. Control dams (●) were administered saline. Neonates were sacrificed at the times indicated. Each time point for hepatic heme oxygenase represents the mean±SEM of between three and nine litters. $*P < 0.02$ and $**P < 0.001$ when compared with control values. Each time point for splenic heme oxygenase represents the average of duplicate determination of spleens bulked from between three and nine litters.

Effect of administration of Mn- and Zn-protoporphyrin to the dam before birth on neonatal hyperbilirubinemia and tissue heme oxygenase activity in the developing neonate. A single injection of either Mn-protoporphyrin or Zn-protoporphyrin (50 μmol/kg body wt) to pregnant rats 24–48 h before birth did not prevent the transient increase in serum bilirubin that occurs in the neonate 24 h after birth (Table III). In addition, neither metalloporphyrin was effective in lowering heme oxygenase activity in liver (Table III), kidney (data not shown), or spleen (data not shown) in neonates born to dams treated prenatally with these compounds in contrast with the effect produced by prenatal Sn-protoporphyrin administration (Fig. 2 *A*).

Effect of administration of Sn-protoporphyrin to the dam after birth on neonatal hyperbilirubinemia and tissue heme oxygenase activity in the developing neonate. Sn-protoporphyrin (50 μmol/kg body wt) was administered to dams immediately after birth and 24, 48, and 72 h later in order to determine if Sn-protoporphyrin could be transported via the dam's milk to the neonate and thereby prevent the increases in serum bilirubin levels and tissue heme oxygenase activity that occur in the postnatal period. Sn-protoporphyrin administration failed to prevent the transient increase in serum bilirubin that occurs in the neonate at 24 h after birth (Table IV). In addition no difference was found in the levels of hepatic heme oxygenase activity between neonates born of control or treated dams (Table IV). No significant dif-

Table III. Effect of Mn- and Zn-Protoporphyrin Administration to Dam Before Birth on Hepatic Heme Oxygenase Activity and Serum Bilirubin Levels in the Developing Neonates

	Heme oxygenase		Serum bilirubin	
Treatment	24 h	48 h	24 h	48 h
	nmol bilirubin/mg/h		*mg/dl*	
Control	10.26±0.37	8.95±0.26	0.48±0.02	0.33±0.02
Zn-protoporphyrin	11.72±0.79	9.54±0.76	0.43±0.01	0.31±0.01
Mn-protoporphyrin	11.93±0.59	12.16±0.27	0.42±0.02	0.31±0.03

Metalloporphyrins were administered subcutaneously to the dam at 24–48 h before birth at a dose of 50 μmol/kg body wt.

Table IV. Effect of Sn-Protoporphyrin Administration to the Dam After Birth on Hepatic Heme Oxygenase Activity and Serum Bilirubin Levels in the Suckling Neonate

	Heme oxygenase			Serum bilirubin		
Treatment	24 h	48 h	96 h	24 h	48 h	96 h
	nmol bilirubin/mg/h			*mg/dl*		
Control	8.87±0.32	8.69±1.0	8.80±0.54	0.54±0.02	0.37±0.02	0.28±0.01
Sn-protoporphyrin	8.96±0.13	9.26±0.31	8.89±0.54	0.52±0.01	0.36±0.01	0.32±0.04

Sn-protoporphyrin, 50 μmol/kg body wt, was administered to dams immediately after birth, and 24, 48, and 72 h later. Neonates were sacrificed at the times indicated. Each point represents SEM of three to six litters.

ference was found in the activities of splenic and renal heme oxygenase between the two groups. No detectable levels of Sn-protoporphyrin were observed in liver, kidney, and spleen of neonates of Sn-protoporphyrin-treated dams using a sensitive fluorometric assay (15). It was not technically feasable to collect enough breast milk from the nursing dam to detect Sn-protoporphyrin. However, the findings presented above suggest that when Sn-protoporphyrin is administered to the dam after birth it does not enter breast milk in sufficient amounts to inhibit tissue heme oxygenase activity or to lower serum bilirubin levels in the suckling neonate.

Disposition of Sn-protoporphyrin in the brain of newborn rats. Sn-protoporphyrin was administered once to neonates immediately after birth (10 or 50 μmol/kg body wt) and the disposition of the metalloporphyrin in brain was followed with time. The highest levels of brain Sn-protoporphyrin were noted at 24 h after administration of the metalloporphyrin; the two doses of Sn-protoporphyrin, which differed by a fivefold factor, nevertheless declined in a parallel manner with time (Fig. 4). The mean $t_{1/2}$ of Sn-protoporphyrin was ~1.7 d (Fig. 4). The levels of Sn-protoporphyrin in the brain of neonates represented <0.5% of the administered dose. Thus, Sn-protoporphyrin when administered immediately after birth is able, like many other chemicals, to cross the blood-brain barrier which is known to be permeable in the newborn animal. The presence of the compound, in brain, is however transient and it disappears from the brain space with a $t_{1/2}$ of ~1.7 d.

Effect of Sn-protoporphyrin on heme oxygenase activity in neonatal brain. The activity of brain heme oxygenase in the rat neonate was low at birth and increased with time (Fig. 5). Adult levels (20) are not attained until 14–21 d after birth. Sn-protoporphyrin administered once at birth significantly lowered brain heme oxygenase activity in a dose-dependent fashion at 24 and 48 h after birth. By 7 d postnatally, no significant difference in brain heme oxygenase activity was found between control and Sn-protoporphyrin-treated animals. Thus, Sn-protoporphyrin administered once at birth can traverse the blood-brain barrier and inhibit brain heme oxygenase activity for a period of at least several days in duration.

Permeability of the blood-brain barrier to Sn-protoporphyrin in the developing neonate. Sn-protoporphyrin (10 μmol/kg body wt) was administered to suckling neonates 24 h before sacrificing the neonates at the times indicated (Fig. 6). The neonates were killed 24 h later and the levels of Sn-protoporphyrin determined in brain. At the dose of Sn-protoporphyrin examined (10 μmol/kg body wt), the metalloporphyrin was able to traverse the blood-brain barrier immediately after birth and the level of Sn-protoporphyrin detectable in brain represented <0.5% of the administered dose. Thereafter, increasingly smaller levels of Sn-protoporphyrin were detectable in brain after administration of the same dose of Sn-protoporphyrin until sometime between 20–28 d after birth when the metalloporphyrin became undetectable, which indicated that the compound could no longer cross the blood-brain barrier (Fig. 6).

Discussion

The results of this study demonstrate that Sn-protoporphyrin administered to pregnant rats 24–48 h before birth significantly lowers tissue heme oxygenase activity in these animals (Table

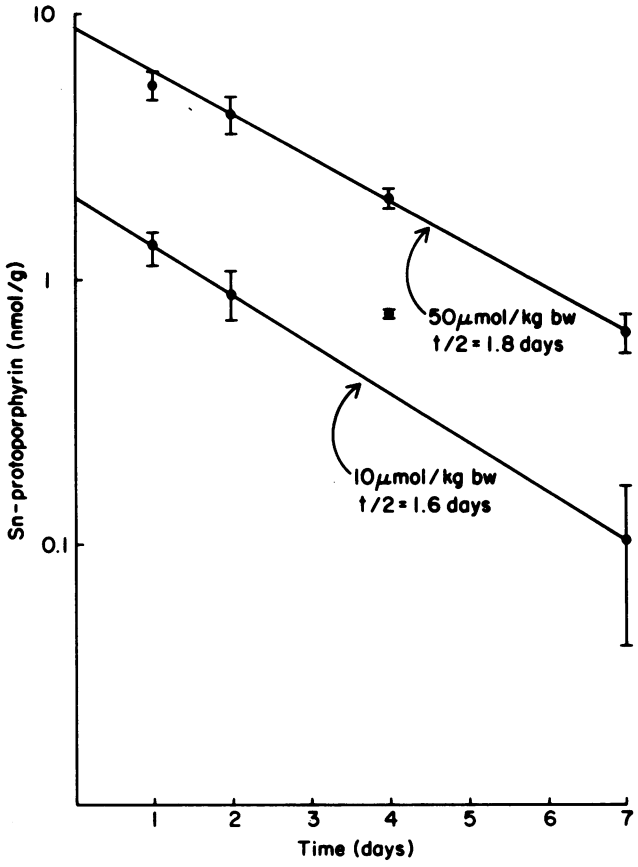

Figure 4. Effect of Sn-protoporphyrin administration on the disposition of the metalloporphyrin in the brain of the suckling neonate. Sn-protoporphyrin (10 or 50 μmol/kg body wt) was administered to neonates immediately after birth. Animals were sacrificed at the times indicated. Each time point represents the mean±SEM of at least three litters.

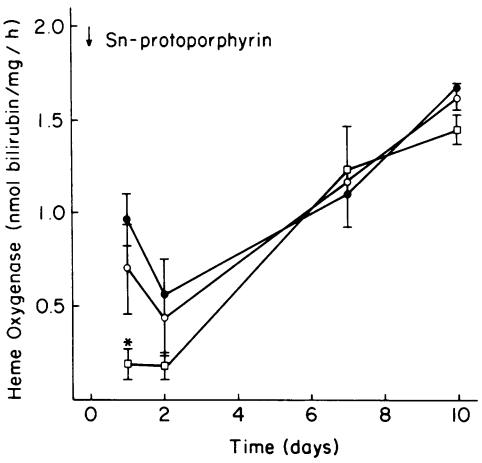

Figure 5. Effect of Sn-protoporphyrin administration on the developmental increase of brain heme oxygenase in the suckling neonates. Sn-protoporphyrin was administered subcutaneously immediately after birth at a dose of 10 (○) or 50 (□) μmol/kg body wt. Control neonates were administered an equal volume of saline (●). Animals were sacrificed at the times indicated. Each time point represents the mean±SEM of at least three litters. $*P < 0.01$ when compared with control values.

I). Furthermore, after treatment with the compound, Sn-protoporphyrin is detectable in fetal tissues and the levels of fetal heme oxygenase are significantly lowered following treatment of pregnant animals prenatally (Table II). Finally, such prenatal treatment with Sn-protoporphyrin is capable of suppressing the postnatal hyperbilirubinemia which subsequently develops (1, 2) in the newborn animal (Fig. 1).

The ability of Sn-protoporphyrin to cross the placental membranes was further explored with time in the newborn. The rat neonate experiences a transient increase in the levels of serum bilirubin immediately after birth (1, 2), which is presumably due to the induction of hepatic heme oxygenase (1, 19, 21) by heme derived from the lysis of fetal red cells, a developmental increase in the activity of the splenic enzyme (1, 19), and the immaturity of the conjugating mechanism for bilirubin (22, 23). Sn-protoporphyrin administered to the dam once, at 24–48 h before birth, prevented, in a dose-dependent manner, the transient increases in serum bilirubin levels which occur in the newborn animal immediately after birth (Fig. 1). In addition the increase in hepatic heme oxygenase activity which takes place in the newborn was suppressed and the developmental increase in splenic heme oxygenase activity was retarded (Fig. 2 *A, B*). A similar effect on heme oxygenase activity was noted in kidney. Thus administration of Sn-protoporphyrin to the dam before birth appears effective in suppressing postnatal jaundice and in markedly reducing the developmental perturbations in tissue heme oxygenase activity which occur in the newborn animal.

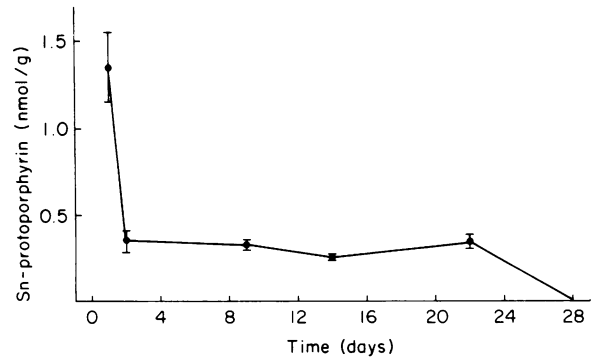

Figure 6. Permeability of the blood-brain barrier to Sn-protoporphyrin in the suckling neonate at different time periods postnatally. Sn-protoporphyrin (10 μmol/kg body wt) was administered subcutaneously to the neonate 24 h before sacrifice. Neonates were kept with their mothers during the experiment. Each time point represents the mean±SEM of a minimum of three litters.

Sn-protoporphyrin administered to the dam before giving birth crosses the placental membranes and can remain in neonatal tissue for varying periods of time (Fig. 3). The levels of Sn-protoporphyrin in the tissues of the neonate were found to be dependent on the dose administered to the mother at 24–48 h before birth. At the higher of the two doses examined, 50 μmol/kg body wt, Sn-protoporphyrin was detectable in the liver of neonates at 21 d after birth; at the lower dose, 10 μmol/kg body wt, the metalloporphyrin was undetectable after 10 d (Fig. 3). Trace amounts of Sn-protoporphyrin were detectable in kidney and spleen only after the higher dose (50 μmol/kg body wt) of metalloporphyrin. The $t_{1/2}$ of the total body burden of tin, as determined by appropriate atomic absorption spectroscopy techniques, injected in the whole neonate once at birth with the metalloporphyrin (doses of 10 and 50 μmol/kg body wt) was 2.35 d (results not shown). In contrast Sn-protoporphyrin administered to the mother after birth was not detectable in the tissues of the suckling neonates. Tissue heme oxygenase activity was not inhibited nor were serum bilirubin levels lowered in these newborn animals (Table III). Thus Sn-protoporphyrin administered to the dam after birth does not appear to reach tissue sites (liver, kidney, and spleen), does not inhibit heme oxygenase, and does not prevent hyperbilirubinemia in the suckling neonate. It is possible that, if some Sn-protoporphyrin is secreted in breast milk, it may be transported via the milk to the neonate where it fails to be absorbed from the gastrointestinal tract. This point remains to be clarified.

In other studies, we have demonstrated that essentially no Sn-protoporphyrin is able to cross the intact blood-brain barrier of adult rats (16). It has been shown, however, that the blood-brain barrier of neonates remains permeable to chemicals for several weeks after birth (24). Sn-protoporphyrin (10 μmol/kg body wt) administered to developing neonates is able to traverse the blood-brain barrier for up to ~21 d after birth. The blood-brain barrier, however, is most permeable to Sn-protoporphyrin immediately after birth; the levels of the metalloporphyrin that are detectable 24 h postnatally represent <0.5% of the administered dose (Fig. 6). Moreover, the presence of Sn-protoporphyrin in the brain is transient and the compound rapidly disappears from the brain space with a $t_{1/2}$ of ~1.7 d (Fig. 4). The ability of Sn-protoporphyrin to enter the brain space is thus age-dependent, as demonstrated by the data in Fig. 6 and by the fact that the compound has previously been shown not to enter adult brain (16). In the neonate the metalloporphyrin also readily leaves the brain and thus becomes available for excretion through biliary and urinary routes (11, 16).

We extended these studies in neonates to an examination of the ability of Sn-protoporphyrin to inhibit brain heme oxygenase (Fig. 5). The levels of heme oxygenase in brain are low at birth and gradually increase with time (Fig. 5), reaching adult levels at 14–21 d postnatally (20). Sn-protoporphyrin administered once at birth to rats substantially reduced brain heme oxygenase activity in a dose-dependent manner 24 and 48 h later; this effect did not persist beyond 7 d after a single injection of the compound at birth. The ability of Sn-protoporphyrin to cross

the blood-brain barrier and to inhibit brain heme oxygenase represent previously unidentified and potentially important properties of this compound. The amount of Sn-protoporphyrin detectable in brain at 24 h after birth is <0.5% of the administered dose; in addition, Sn-protoporphyrin rapidly disappears from brain with a $t_{1/2}$ of ~1.7 d. No acute toxicity of Sn-protoporphyrin in adult or neonatal rats and mice has been noted in studies involving over 5,000 animals; the half-maximal lethal dose (LD_{50}) of the compound exceeds the dose required to suppress hyperbilirubinemia in the neonate by ~150-fold i.e., 10 μmol/kg body wt (2) vs. 1,500 μmol/kg body wt. In addition, long-term weekly treatment of genetically anemic mice with hemolytic anemia (sph^{ha}/sph^{ha}) with Sn-protoporphyrin (100 μmol/kg body wt for 32 wk; cumulative dose, 3,200 μmol/kg body wt) did not alter hematological indices, histological findings, or enzyme activities related to heme biosynthesis, even though it resulted in sustained decreases in microsomal heme oxygenase activity in liver, kidney, and spleen and a prolonged decrease in serum bilirubin concentration. In addition, inhibition of heme oxygenase did not alter the levels of hepatic and renal cytochrome P-450 (25). Thus the presence of small amounts of Sn-protoporphyrin in brain in the period immediately after birth and its rapid disappearance postnatally, coupled with its innocuous nature (25), make it unlikely that the transient presence of the compound in brain would represent a significant hazard, especially in view of the common occurrence of intracranial bleeding in premature infants and thus the enhanced possibility of local formation of bilirubin in brain tissue.

The studies described in this report provide further confirmation of the potent ability of Sn-protoporphyrin to suppress elevated levels of serum bilirubin levels in the particular setting of the immediate prenatal-postnatal periods associated with gestation and parturition, in which exaggerated levels of heme degradation occur (26). The ability of Sn-protoporphyrin to traverse both the placental membranes in the pregnant animal, and thereby, to suppress, by such prenatal treatment, the development of postnatal jaundice in the newborn animal; and to traverse the blood-brain barrier in the developing neonate, and thus, to transiently suppress heme oxygenase activity in brain, are newly defined and potentially useful biological properties of this compound.

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