0090-9556/99/2710-1214–1219$02.00/0
DRUG METABOLISM AND DISPOSITION
Vol. 27, No. 10
Printed in U.S.A.

SELECTIVE INHIBITION OF HEME OXYGENASE, WITHOUT INHIBITION OF NITRIC OXIDE SYNTHASE OR SOLUBLE GUANYLYL CYCLASE, BY METALLOPORPHYRINS AT LOW CONCENTRATIONS

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(Received April 13, 1999; accepted June 23, 1999)

This paper is available online at http://www.dmd.org

ABSTRACT:

Studies on the physiological role of heme oxygenase (HO) require an inhibitor that will selectively inhibit HO activity without inhibiting the activity of either nitric oxide synthase (NOS) or soluble guanylyl cyclase (sGC). The objective of this study was to test a series of metalloporphyrins that have previously been shown to inhibit HO activity, for their ability to inhibit HO without inhibiting NOS or sGC activities. Measurement of activity of HO in rat brain microsomes and NOS in rat brain cytosol was made for samples incubated with metalloporphyrins (0.15–50 μM), including zinc protoporphyrin IX, zinc deuteroporphyrin IX 2,4-bis-ethylene glycol (ZnBG), chromium mesoporphyrin IX (CrMP), tin protoporphyrin IX, and zinc *N*-methylprotoporphyrin IX. CrMP and ZnBG were found to be the most selective inhibitors of HO activity (i.e., caused the greatest inhibition of HO activity, 89 and 80%, respectively, without inhibition of NOS activity). Based on these results, sGC activity in rat lung cytosol incubated with CrMP or ZnBG (0.15–15 μM) was measured. ZnBG did not affect basal sGC activity but did potentiate *S*-nitroso-*N*-acetylpenicillamine (SNAP)-induced sGC activity. CrMP did not affect either basal or SNAP-induced activity. It was concluded that of the five metalloporphyrins studied, CrMP, at a concentration of 5 μM, was a selective inhibitor of HO activity and was the most useful metalloporphyrin for the conditions tested. Thus, CrMP would appear to be a valuable chemical probe in elucidating the physiological role of HO.

It has been proposed that carbon monoxide (CO),[1] which is formed endogenously during heme oxygenase (HO) catabolism of heme, plays a role in the regulation of cell function and communication (Marks et al., 1991; Verma et al., 1993). The biological role proposed for CO is similar to that of another endogenously formed gaseous molecule, namely, nitric oxide (NO), which is produced by NO synthase (NOS) from L-arginine (Moncada et al., 1991). Both gaseous molecules can mediate a physiologic effect, such as blood vessel relaxation, by activating soluble guanylyl cyclase (sGC) (Vedernikov et al., 1989; Furchgott and Jothianandan, 1991; Moncada et al., 1991; Hussain et al., 1997). However, CO has a lower potency than NO as a vasodilator (Furchgott and Jothianandan, 1991).

Inhibitors of enzymatic activity are useful chemical probes in establishing a physiological role for specific enzymes, such as the role N^{ω}-nitro-L-arginine methyl ester (L-NAME) has played in elucidating the biological roles of NOS (Moncada et al., 1991). Metalloporphyrins have been shown to inhibit HO, and their potency is affected by the metal cation associated with the porphyrin ring as well by different ring substituents (Vreman et al., 1993). Inhibition of HO activity has been demonstrated for each of the metalloporphyrins used in this study, specifically zinc protoporphyrin IX (ZnPP), tin protoporphyrin IX (SnPP), chromium mesoporphyrin IX (CrMP) (Vreman et al., 1993; Cook et al., 1995; Marks et al., 1997), zinc deuteroporphyrin IX 2,4-bis-ethylene glycol (ZnBG) (Chernick et al., 1989; Vallier et al., 1991; Vreman et al., 1992), and zinc *N*-methylprotoporphyrin IX (ZnMePP) (De Matteis et al., 1985). ZnPP has been exploited therapeutically to reduce hyperbilirubinemia in the neonate (Qato and Maines, 1985; Valaes et al., 1994). More recently, metalloporphyrins have been used to test the hypothesis that CO has a physiological role. SnPP and ZnPP have been used to investigate a possible role for CO as a vasodilator (Zakhary et al., 1996). ZnPP also has been used to demonstrate an apparent role for CO in long-term potentiation (Zhuo et al., 1993) and the inhibition of depolarization-induced glutamate release (Shinomura et al., 1994).

Several investigators have shown that metalloporphyrins are not only specific inhibitors of HO but also inhibit NOS and sGC (Luo and Vincent, 1994; Meffert et al., 1994; Grundemar and Ny, 1997). Based on these findings, the conclusions reached by investigators using metalloporphyrins to establish a physiological role for CO in biological systems, in which NOS and sGC are also active, have been criticized. In contrast, Zakhary et al. (1996) reported that SnPP was 10

This study was supported by the Heart and Stroke Foundation of Ontario (Grant T-3448) and by the National Institutes of Health (Grant HD 14426).

[1] Abbreviations used are: CO, carbon monoxide; HO, heme oxygenase; NO, nitric oxide; NOS, nitric oxide synthase; sGC, soluble guanylyl cyclase; L-NAME, N^{ω}-nitro-L-arginine methyl ester; ZnPP, zinc protoporphyrin IX; SnPP, tin protoporphyrin IX; CrMP, chromium mesoporphyrin IX; ZnBG, zinc deuteroporphyrin IX 2,4-bis-ethylene glycol; ZnMePP, zinc *N*-methylprotoporphyrin IX; SNAP, *S*-nitroso-*N*-penicillamine; MSI, maximum selective inhibitory; NANC, nonadrenergic, noncholinergic.

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times more potent in inhibiting HO-2 than NOS or sGC and based on this finding have used SnPP to study CO-induced vasodilation.

In this study, the objective was to test five metalloporphyrins that have been shown previously to inhibit HO activity, for their ability to selectively inhibit HO relative to NOS and sGC activities. For each metalloporphyrin, a concentration was determined that inhibited HO activity, without inhibiting NOS activity. The two most selective inhibitors, CrMP and ZnBG, were further studied to determine whether they affected basal or *S*-nitroso-*N*-acetylpenicillamine (SNAP)-induced sGC activity. It was found that CrMP, at a concentration of 5 μM, was a selective inhibitor of HO activity and appeared to be the most useful HO inhibitor based on the studies conducted.

Materials and Methods

Drugs and Solutions. EDTA disodium salt, hemin, ethanolamine, BSA, HEPES, L-arginine, leupeptin, Amberlite IRP-69, L-NAME, heparin, cGMP, NADPH, GTP, and SNAP were obtained from Sigma Chemical Co. (St Louis, MO). Tris·HCl, benzamidine HCl, and 3-isobutyl-1-methylxanthine were purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). CrMP, ZnBG, ZnPP, SnPP, and ZnMePP were purchased from Porphyrin Products, Inc. (Logan, UT). Neutral alumina was obtained from EM Science (Gibbstown, NJ). All other chemicals were at least reagent grade and were obtained from BDH Inc. (Toronto, Ontario, Canada). Stock solutions of methemalbumin (1.5 mM hemin and 0.15 mM BSA) and of each of the five metalloporphyrins (1.0 mM) were prepared as described previously (Vreman et al., 1993). Briefly, hemin or metalloporphyrin was dissolved in 0.5 ml of 10% (w/v) ethanolamine. BSA dissolved in 2 ml of deionized water was added to the hemin solution only. The volume was made up to 7 ml and slowly adjusted to pH 7.4 with 1 M HCl and vigorous stirring. The final volume for each stock solution was adjusted to 10 ml with deionized water. The metalloporphyrin vehicle was prepared as described above without the addition of any metalloporphyrin. The methemalbumin and metalloporphyrin stock solutions were prepared with the laboratory lights turned off and were stored at −20°C for up to 1 month.

Preparation of Subcellular Fractions of Rat Brain and Lung. Adult male Sprague-Dawley rats (300–350 g) were obtained from Charles River Canada, Inc. (Montreal, Quebec, Canada). Rats were given ad libitum access to Ralston Purina Laboratory Chow (5001; Ren's Feed and Supplies, Ltd., Oakville, Ontario, Canada) and water. All animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care and the experimental protocol was approved by the Queen's University Animal Care Committee. For measuring HO and NOS activity, each rat was sacrificed by decapitation and its brain was excised and weighed. For measuring sGC activity, the rat was injected i.p. with heparin (3 mg/kg b.wt.) and then 45 min later anesthetized with 93 mg/kg b.wt. sodium pentobarbital (MTC Pharmaceuticals, Cambridge, Ontario, Canada). The lungs were perfused with 40 ml of ice-cold phosphate-buffered saline by inserting a syringe into the pulmonary artery through the right ventricle. The left atrium was cut to allow outflow of perfusate. During perfusion, the lungs were continuously inflated and deflated with an air-filled syringe inserted into the trachea. Perfusion was continued until the lungs were cleared of all blood and appeared white. The lungs were excised and rinsed with 10 ml of ice-cold phosphate-buffered saline.

Microsomal Fraction of Rat Brain for Measuring HO Activity. A homogenate (15%, w/v) of brains pooled from four rats was prepared in ice-cold HO homogenizing buffer (20 mM KH_2PO_4, 135 mM KCl, and 0.10 mM EDTA; adjusted to pH 7.4 at 4°C with 1 M KOH) using a Potter-Elvehjem homogenizing system with a Teflon pestle. The microsomal fraction of the rat brain homogenate was obtained by centrifugation at 10,000*g* for 20 min at 4°C, followed by centrifugation of the supernatant at 100,000*g* for 60 min at 4°C. The 100,000*g* pellet (microsomes) was resuspended in 100 mM KH_2PO_4 buffer (adjusted to pH 7.4 with 1 M KOH) with a Potter-Elvehjem homogenizing system. The rat brain microsomal fraction was divided into equal aliquots, placed into microcentrifuge tubes, and stored at −80°C for up to 2 months. Protein concentration of the microsomal fraction was determined by the biuret method (Gornall et al., 1949), which was modified as described previously (Marks et al., 1997).

Cytosolic Fraction of Rat Brain for Measuring NOS Activity. Brains from four rats were pooled and homogenized in ice-cold NOS homogenizing buffer (50 mM HEPES, 1 mM EDTA, and 10 μg/ml leupeptin; adjusted to pH 7.4 at 4°C with 1.0 M NaOH) with a Potter-Elvehjem homogenizing system, in a ratio of 1 g of tissue/1.1 ml of homogenizing buffer. The cytosolic fraction was obtained by centrifugation of the homogenate at 10,000*g* and then 100,000*g*, as described above for preparation of the microsomal fraction of rat brain. The 100,000*g* supernatant (cytosol) was divided into equal aliquots, placed into microcentrifuge tubes, and stored at −80°C for up to 2 months. Protein concentration of the cytosolic fraction was determined by the biuret method as described above.

Cytosolic Fraction of Rat Lungs for Measuring sGC Activity. Lungs from one rat were homogenized in ice-cold sGC homogenizing buffer (50 mM Tris·HCl (pH 7.4), 5 mM $MgCl_2$, 1 mM EDTA, and 5 mM benzamidine HCl) to produce a 20% (w/v) homogenate. The cytosolic fraction was obtained by differential centrifugation of the homogenate at 10,000*g* and then 100,000*g*, as described above for the preparation of the microsomal fraction of rat brain. The 100,000*g* supernatant (cytosol) was divided into equal aliquots, placed into microcentrifuge tubes, and stored at 4°C for <24 h. Protein concentration was determined with bicinchoninic acid (Smith et al., 1985), which was obtained as part of a protein assay kit from Pierce Chemical Co. (Rockford, IL).

Measurement of HO Enzymatic Activity in Microsomal Fraction of Rat Brain. HO activity in the microsomal fraction of rat brain homogenate was determined by measuring the rate of CO formation during the NADPH-dependent oxidation of heme (Vreman and Stevenson, 1988) and modified as described by Cook et al. (1996). For each ZnPP, CrMP, ZnBG, SnPP, and ZnMePP sample, a reaction mixture consisting of 100 mM KH_2PO_4, 0.2 mg of microsomal protein, and methemalbumin (final concentration of 25 μM hemin and 2.5 μM BSA) was pipetted into six 3.5-ml amber glass vials (Chromatographic Specialties, Inc., Brockville, Ontario, Canada). The following concentrations of metalloporphyrin were added to individual vials: 0.15, 0.5, 1.5, 5, 15, and 50 μM. Each vial was sealed with a silicon-Teflon septum and a screw cap (Chromatographic Specialties, Inc.) and then was preincubated for 5 min in the dark at 37°C in a shaking water bath. NADPH (0.5 mM) then was added to each vial, the headspace gas was displaced with CO-free air, and the incubation was continued for another 15 min. The reaction was stopped by placing the vial on pulverized dry ice (−78°C), where it remained for 30 min until the headspace gas was analyzed. For each metalloporphyrin reaction set, CO production was corrected for the CO produced in a blank reaction vial that contained a metalloporphyrin concentration of 50 μM, but no NADPH. To determine total HO activity in the microsomal fraction of rat brain, a reaction vial was prepared that did not contain metalloporphyrin or vehicle. (There was no measurable inhibition of HO activity in a reaction set containing metalloporphyrin vehicle equivalent to a range of 0.15 to 50 μM metalloporphyrin.)

CO in the headspace gas was quantitated by gas-solid chromatography and a spectrophotometric detector (RGA3; Trace Analytical, Inc., Menlo Park, CA) set at 254 nm to quantitate the mercury vapor formed from the reaction of CO with HgO, as described by Odrcich et al. (1998). The amount of CO in the headspace gas was determined by interpolating the peak area of the chromatographic signal on the linear CO standard curve (10–170 pmol of CO), which had a correlation coefficient of 0.999 (n = 9 determinations). The rate of formation of CO in the microsomal fraction of rat brain homogenate was expressed as nanomoles of CO formed per milligram of protein per hour. NADPH-dependent formation of CO was calculated by subtracting the value for CO produced in samples not containing NADPH (blank) from the value for CO formed in samples containing NADPH.

Measurement of NOS Enzymatic Activity in Cytosolic Fraction of Rat Brain. NOS activity in the cytosolic fraction of rat brain homogenate was determined with a modification of an established procedure (Bredt and Snyder, 1990) that was optimized for the hippocampus of the guinea pig (Brien et al., 1995; Kimura et al., 1996). A 100-μl volume of reaction buffer (50 mM HEPES, 1 mM EDTA, 1.25 mM $CaCl_2$, and 2 mM NADPH; adjusted to pH 7.4 at 37°C with 1.0 M NaOH) and a 35-μl volume of cytosol containing 0.525 mg of protein were added to six test tubes. The following metalloporphyrin concentrations, 0.15, 0.5, 1.5, 5, 15, and 50 μM, were added to individual test tubes in a volume of 100 μl. The samples were preincubated for 5 min at 37°C, after which a 35-μl aliquot of an aqueous solution containing 35,000 dpm L-[^{14}C] arginine (New England Nuclear-Mandel, Guelph, Ontario, Canada) and 180 μM nonradiolabeled L-arginine was added. The samples were incubated for 15 min, and the reaction was stopped by the addition of ice-cold

"stop" buffer (20 mM HEPES and 2 mM EDTA; adjusted to pH 5.5 at 4°C with 1.0 M NaOH). Experimental blanks were prepared by adding the stop buffer to a sample containing reaction buffer, cytosolic protein, and a metalloporphyrin concentration of 50 μM, before the addition of L-[^{14}C] arginine and incubation at 37°C for 15 min. To determine total NOS activity, samples were prepared that did not contain metalloporphyrin or vehicle. (There was no measurable inhibition of NOS activity in samples containing metalloporphyrin vehicle equivalent to a concentration range of 0.15 to 50 μM metalloporphyrin.)

NOS activity was measured with a radiometric assay that monitored the conversion of L-[^{14}C] arginine to L-[^{14}C] citrulline (Brien et al., 1995; Kimura et al., 1996). NOS activity was expressed as nanomoles of L-[^{14}C]citrulline formed/mg protein/h. In a preliminary experiment, incubation of rat brain cytosol with 1 mM L-NAME, an inhibitor of NOS, inhibited Ca^{2+}-dependent formation of L-[^{14}C] citrulline in rat brain cytosol by 97.6 $\pm$ 0.4% (N = 4).

Measurement of sGC Enzymatic Activity in Cytosolic Fraction of Rat Lung. sGC activity was measured in the cytosolic fraction of rat lung homogenate with a modification of the procedure described by Liu et al. (1993). Rat lung cytosolic fraction (25 μl) and 50 μl of a reaction mixture [100 mM Tris·HCl (pH 7.4), 6 mM $MgCl_2$, 2 mM 3-isobutyl-1-methylxanthine, 10 mM cGMP, and 1 mg of BSA/ml] were added to each of five test tubes. The following metalloporphyrin concentrations, 0.15, 0.5, 1.5, 5, and 15 μM, were added to individual test tubes in a final volume of 10 μl. For the basal sGC activity experiments, the reaction was started by the addition of 25 μl of an aqueous solution containing 500,000 dpm of [^{3}H]GTP (New England Nuclear-Mandel) and 200 μM nonradiolabeled GTP. The samples were incubated at 30°C for 30 min, and the reaction was stopped by adding 100 μl of ice-cold 10% (w/v) trichloroacetic acid and putting the tube on ice. For the stimulated sGC activity experiments, the reaction was started by adding 100 μM SNAP, followed by 25 μl of [^{3}H]GTP solution for a final volume of 120 μl. The reaction was carried out as described for the basal sGC activity experiments. Experimental blanks were prepared by adding rat lung cytosolic fraction that had been boiled for 5 min, reaction mixture, and a metalloporphyrin concentration of 15 μM to a vial before starting the reaction. To determine total sGC activity, samples were prepared that did not contain metalloporphyrin. After incubation was completed, ~1,000 dpm of [^{14}C]cGMP (New England Nuclear-Mandel) was added to each sample to provide an index of column efficiency. The precipitated proteins were removed from the acidified sample by centrifugation at 12,000*g* for 3 min. The supernatant was subjected to ion-exchange chromatography and [^{3}H]cGMP and [^{14}C]cGMP were subsequently quantitated by liquid scintillation spectrometry as described by Liu et al. (1993). The amount of cGMP formed during the incubation was calculated from the specific radioactivity of the [^{3}H]GTP (26.9 Ci/mmol) and corrected for experimental blank. sGC activity was expressed as picomoles of cGMP formed per milligram of protein per hour.

Data Analysis. The activity of HO, NOS, and sGC following incubation with each concentration of metalloporphyrin was expressed as a percentage of total activity, measured in the absence of metalloporphyrin. The data are presented as group means $\pm$ S.D. of four tissue preparations from different animals, unless otherwise stated. Parametric statistical analysis of the data was conducted by repeated-measures, one-way ANOVA. For a statistically significant F statistic ($p < .05$), a post hoc Newman-Keuls test was conducted to determine which experimental groups were statistically different ($p < .05$).

Results and Discussion

The metalloporphyrins used in this study have been shown previously to inhibit HO activity (De Matteis et al., 1985; Chernick et al., 1989; Vreman et al., 1992, 1993). The use of metalloporphyrins in investigating a physiological role for HO has been criticized because some metalloporphyrins have been shown also to inhibit NOS and sGC activity (Lou and Vincent, 1994; Meffert et al., 1994; Grundemar and Ny, 1997). In contrast, Zakhary et al. (1996), in a study demonstrating HO-derived CO as a vasodilator, reported a dose of SnPP that appeared to selectively inhibit HO relative to NOS. Thus, the potential exists for finding a metalloporphyrin and/or a metalloporphyrin concentration that would inhibit HO activity without inhibiting NOS or sGC activity. In the present study, five metalloporphyrins were investigated to determine whether there is a metalloporphyrin and/or a metalloporphyrin concentration that can be used as a selective inhibitor of HO relative to NOS and sGC.

HO activity in rat brain microsomes and NOS activity in rat brain cytosol were 3.2 $\pm$ 0.3 nmol of CO formed/mg protein/h (N = 6) and 5.0 $\pm$ 1.1 nmol of L-citrulline formed/mg protein/h (N = 4), respectively. All five metalloporphyrins (CrMP, ZnBG, SnPP, ZnPP, and ZnMePP) inhibited both HO and NOS activity (Fig. 1). However, there was a concentration for each metalloporphyrin at and below which only HO activity was inhibited. The metalloporphyrin vehicle did not affect HO or NOS activity (data not shown).

In comparing the metalloporphyrins as selective inhibitors of HO, the first step was to determine a maximum selective inhibitory (MSI) concentration for each metalloporphyrin, at or below which there is inhibition of HO, with no effect on NOS or sGC activity (Table 1). The next step was to compare the percentage of inhibition of HO activity at the MSI concentration for each metalloporphyrin. Also, the percentage of inhibition of HO activity at concentrations 3- and 10-fold lower than the MSI concentration was compared (Table 1). This latter comparison was made because in an experiment, a concentration lower than the MSI concentration, at which the metalloporphyrin becomes nonselective, would normally be used. Based on these criteria, the inhibition of HO activity was lowest and/or declined most rapidly with metalloporphyrin concentration for ZnMePP, ZnPP, and SnPP (Table 1). Thus, it was concluded that CrMP and ZnBG at concentrations at and below 5 μM were selective inhibitors of HO activity relative to NOS activity in rat brain.

It is interesting to compare the above data (Fig. 1) with that of Meffert et al. (1994). These workers concluded that CrMP and ZnPP, but not tin mesoporphyrin or ZnBG, inhibited NOS in the rat hippocampus. Based on these findings, it was emphasized that some metalloporphyrins are nonselective and would therefore not be useful in biological studies involving CO. The concentration of metalloporphyrin used in these studies ranged from 10 to 100 μM, whereas in the present study metalloporphyrin concentrations from 0.15 to 50 μM were used. The results of Meffert et al. (1994), showing that CrMP and ZnPP inhibited NOS at concentrations of 10 μM and higher are in agreement with the results of the present study. In contrast, it was found in this study that ZnBG inhibits NOS at concentrations >5 μM. Tin mesoporphyrin was not tested in the present study. The message that emerges in comparing the data is that to achieve selectivity of metalloporphyrin-induced inhibition of HO versus NOS, the concentration used is critically important and must be kept ≤ 5 μM.

To characterize further the selectivity of CrMP and ZnBG as inhibitors of HO, the effect of the two metalloporphyrins on basal and SNAP-induced sGC activity was determined for metalloporphyrin concentrations similar to those used for the study of HO and NOS activity. The basal and SNAP-induced sGC activities in the rat lung cytosol were 121 $\pm$ 56 (N = 6) and 2059 $\pm$ 821 (N = 3) pmol of cGMP formed/mg protein/h, respectively. Thus, the addition of 100 μM SNAP produced an ~17-fold increase in sGC activity from basal level. Neither CrMP nor ZnBG had any effect on basal sGC activity (Fig. 2) at the concentrations tested. However, ZnBG elevated SNAP-induced sGC activity, which is consistent with reports from investigators who found that certain metalloporphyrins, such as cobalt protoporphyrin, enhance NO-induced sGC activity (Dierks et al., 1997). CrMP had no effect on SNAP-induced sGC activity for the concentration range tested, a range that included the MSI concentration of 5 μM, at and below which CrMP selectively inhibits HO activity, with no effect on NOS activity. Thus, CrMP was found to be the most

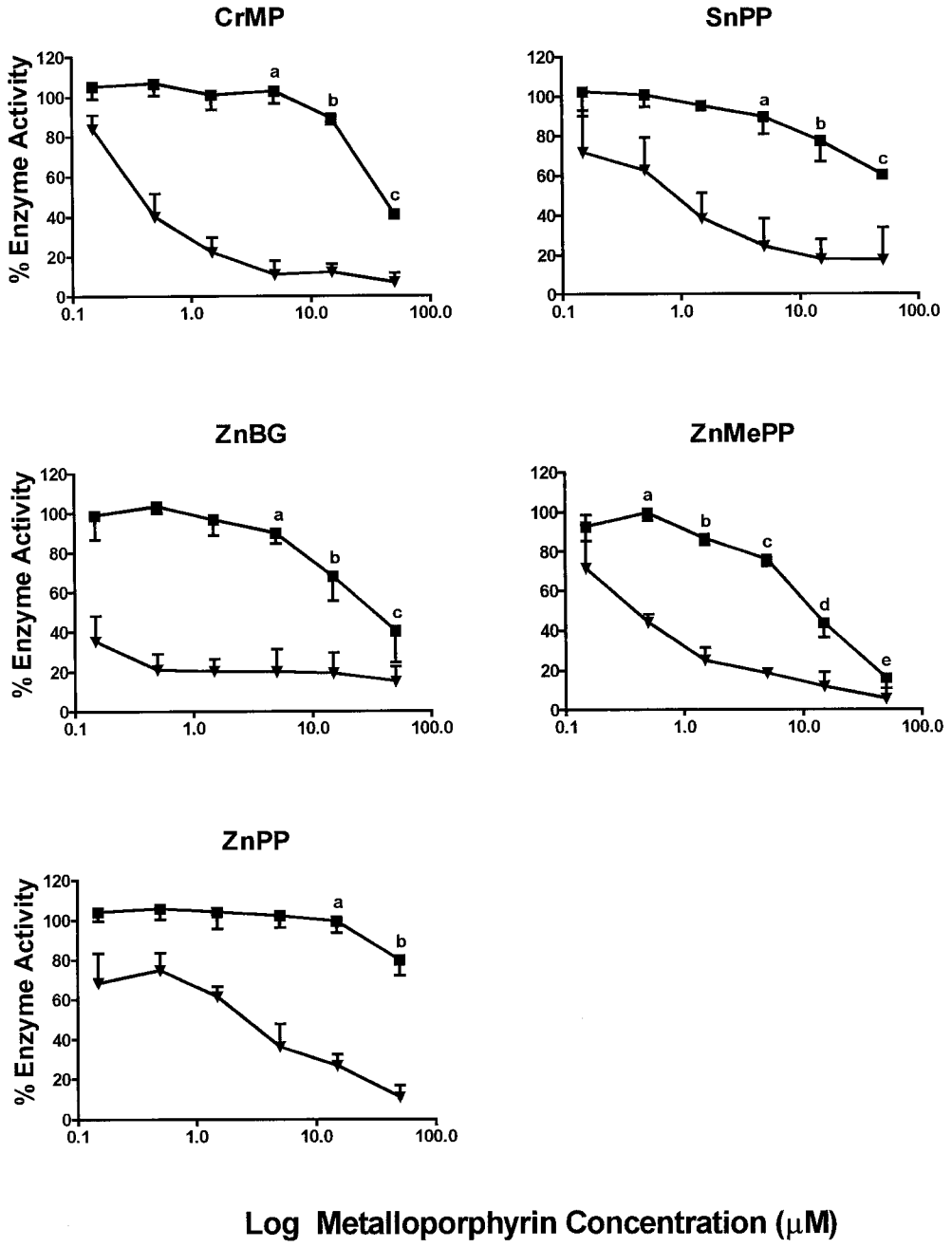

FIG. 1. *Inhibition of HO and NOS activity by CrMP, SnPP, ZnBG, ZnMePP, and ZnPP.*

Concentration-response curves for HO (▼) and NOS (■) activity in rat brain microsomes and cytosol, respectively, were obtained in the presence of CrMP, SnPP, ZnBG, ZnMePP, and ZnPP. The data are presented as group means ± S.D. ($N = 4$). a, the MSI concentration at or below which there is selective inhibition of HO activity, with no effect on NOS activity; NOS activity for lower concentrations of metalloporphyrins were not different from the MSI concentration. Group means with different letters are statistically different from each other, $p < .05$.

selective and useful inhibitor of HO activity compared with NOS and sGC activities in rat brain and lung.

To our knowledge, no particular structural feature of metalloporphyrins has been identified that allows the prediction of the efficacy of the compounds to inhibit HO or NOS. For sGC, a mechanism to explain, at least in part, the interactions of metalloporphyrins with this enzyme has been proposed by Serfass and Burstyn (1998). These investigators postulate that a key requirement for sGC activation by metalloporphyrins is the absence of a bond between a proximal protein-histidine and the metal in the porphyrin. This proposed mechanism is based on the observation that activation of sGC by NO has been attributed to binding of NO to heme iron with concomitant breaking of a bond between a proximal protein-histidine and iron. In their study, the minimal activation of sGC by ZnPP is attributed to the likelihood that the bond between a proximal histidine and Zn atom is intact. However, the marked activation of sGC by SnPP is attributed to the absence of a bond between a proximal histidine and the Sn atom. Activation of sGC by SnPP, as demonstrated by Serfass and Burstyn (1998), provides further rationale for selecting CrMP, from among the metalloporphyrins tested, as the most selective metalloporphyrin to elucidate the physiological role of HO.

TABLE 1

Comparison of metalloporphyrin inhibition of HO

Metalloporphyrin	MSI[a] Concentration	Inhibition of HO Activity (%)		
		At MSI Concentration	At ⅓ MSI Concentration	At 1/10 MSI Concentration
	μM			
ZnMePP	0.5	56 ± 4.2	29 ± 27.1	16 ± 20.9[b]
CrMP	5	89 ± 7.1	78 ± 7.5	60 ± 11.7
ZnBG	5	80 ± 11.3	80 ± 6.1	80 ± 8.1
SnPP	5	76 ± 13.9	62 ± 12.7	37 ± 16.4
ZnPP	15	73 ± 5.6	64 ± 11.5	38 ± 5.2

$N = 4$.
[a] MSI concentration, concentration at or below which there is selective inhibition of HO activity with no effect on NOS activity.
[b] Note: the data for this concentration are not shown on the ZnMePP graph in Fig. 1.

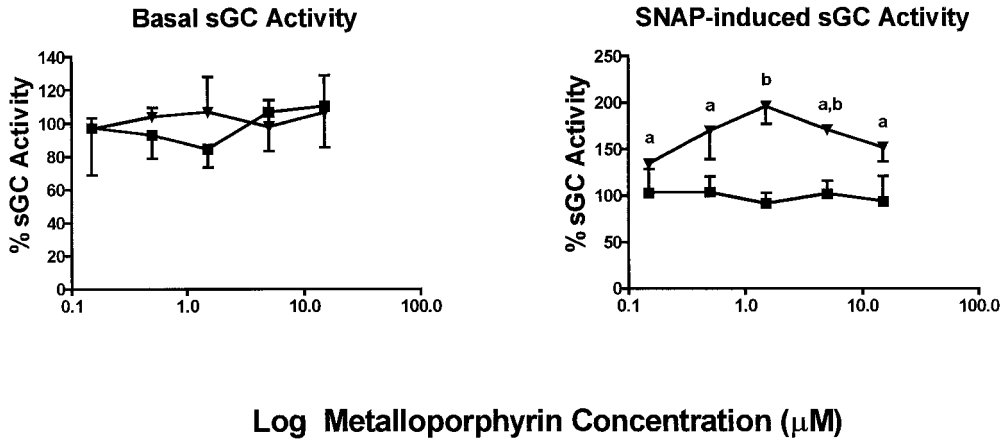

FIG. 2. *Effects of ZnBG and CrMP on basal and SNAP-induced sGC activity.*

Concentration-response curves for basal ($N = 4$) and SNAP-induced ($N = 3$) sGC activity were obtained for ZnBG (▼) and CrMP (■). The data are presented as group means ± S.D. Group means with different letters are statistically different ($p < .05$) from other group means within the same concentration-response curve.

Luo and Vincent (1994) and Grundemar and Ny (1997) concluded that ZnPP, SnPP, and ZnBG cannot be used to establish a messenger role for CO. This comment is based on the fact that these metalloporphyrins inhibit sGC in addition to HO. However, in their study, the concentration of these metalloporphyrins ranged from 10 to 100 μM for sGC inhibition. This conclusion requires reconsideration in light of our data that demonstrates that concentrations of metalloporphyrins <10 μM can inhibit HO activity without inhibiting NOS and sGC activities. Thus, careful exploration of concentration-response relationships with a variety of metalloporphyrins potentially can lead to the identification of an appropriate selective inhibitor for the biological model being used. This conclusion is reinforced by the results of Zakhary et al. (1997), who used HO-2 knockout mice and SnPP to demonstrate that CO plays a role in nonadrenergic, noncholinergic (NANC) relaxation evoked by electrical field stimulation of mouse ileal segments. In wild-type mice, SnPP partially inhibited NANC relaxation. However, in mice where the gene for HO-2 had been deleted, SnPP did not affect NANC transmission.

There is considerable interest in the use of metalloporphyrins to inhibit HO in the treatment of juvenile jaundice (Qato and Maines, 1985; Valaes et al., 1998). CrMP and ZnBG appear to be promising candidates because of their high potency to inhibit HO as demonstrated in the present study and other studies (Vreman et al., 1998), good oral absorption (Vallier et al., 1991, 1993), resistance to metabolism by HO and inability to up-regulate HO-1 in cell culture (W.S. Zhang, P.R. Contag, D.K.S., and C.H. Contag, personal communication). Moreover, CrMP has the additional advantages of not distributing across the blood-brain barrier and being photochemically inactive. Although ZnBG is a photosensitizer, the potential low doses required for therapeutic use, due to its high potency as a HO inhibitor, may restrict its photoreactivity. For the above reasons, therapeutic and toxicological studies of CrMP and ZnBG are warranted.

In summary, of the five metalloporphyrins tested, CrMP and ZnBG inhibited HO to the greatest extent at and below the concentration for which there was no measurable inhibition of NOS activity. Furthermore, CrMP was found to have no effect on basal or SNAP-induced sGC activity, unlike ZnBG, which enhanced SNAP-induced sGC activity. Thus, in this study, CrMP, at or below 5 μM, was found to be the most selective inhibitor of HO relative to NOS and sGC, in rat brain and lung. In other studies with different biological models, it will be necessary to determine the concentration of CrMP or other metalloporphyrins that will selectively inhibit HO activity without inhibiting NOS and sGC activities.

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