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Alterations Induced in Heme Pathway Enzymes and Monooxygenases by Gold

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SUMMARY

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In this study, the effects of the gold compound, gold sodium thiomalate, on the heme biosynthetic pathway, on cytochrome P-450-dependent monooxygenases, and on heme catabolism were examined. The addition of the gold compound, *in vitro,* resulted in the inhibition of hepatic δ-aminolevulinic acid dehydratase, NADPH-cytochrome c reductase, and ethylmorphine N-demethylase activities. There was also a slight decrease in cytochrome P-450 content. Gold was a noncompetitive inhibitor of both δ-aminolevulinic acid dehydratase and ethylmorphine N-demethylase activities.

Gold sodium thiomalate, administered acutely, altered heme biosynthetic pathway enzymes in erythrocytes, liver, and kidney. Erythrocyte δ-aminolevulinic acid dehydratase activity was decreased with a concomitant increase in protoporphyrin content. In the liver δ-aminolevulinic acid dehydratase and ferrochelatase activities were significantly inhibited and the microsomal heme content was significantly decreased. In the kidney, the major site of gold deposition, the activities of δ-aminolevulinic acid synthase, δ-aminolevulinic acid dehydratase, and ferrochelatase were markedly inhibited and total porphyrin content was markedly decreased.

After acute gold treatment, monooxygenase activities in liver and kidney were decreased. Cytochrome P-450 content of both tissues decreased significantly and ethylmorphine N-demethylase and benzo(a)pyrene hydroxylase activities were both inhibited. NADPH-cytochrome c reductase activity, however, was not altered. In contrast to its inhibitory effects on the heme biosynthetic pathway and cytochrome P-450-dependent monooxygenases, gold caused a 1.5- and 8-fold induction in the liver and kidney, respectively, of microsomal heme oxygenase activity, the rate-limiting enzyme in the catabolism of heme.

There was no change in any of the parameters in the liver or erythrocytes after chronic treatment with gold. In the kidney, δ-aminolevulinic acid dehydratase activity and total porphyrins were significantly decreased. However, as in the liver, cytochrome P-450

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content was not significantly altered. These results indicate that an adaptive response develops during chronic gold treatment which prevents the depression of heme biosynthesis and the formation of cytochrome P-450.

INTRODUCTION

Gold salts are heavy metal compounds that are presently administered systemically in therapy of rheumatoid arthritis. Two widely used gold compounds in the treatment of this disease state are gold sodium thiomalate and gold sodium thioglucose. Gold, like other heavy metals, has been shown to inhibit numerous sulfhydryl enzymes (1–3). The inhibition of lysosomal enzymes by gold may in part explain its therapeutic efficacy. Since some sulfhydryl enzymes in heme metabolism are sensitive to heavy metals, it was of interest to determine if gold may have an inhibitory effect on the heme biosynthetic pathway, resulting in a decrease in heme and consequently in the hemeprotein, cytochrome P-450. Certain other heavy metals have previously (4–7) been shown to be potent inducers of microsomal heme oxygenase, the rate-limiting enzyme that catalyzes the breakdown of heme to bilirubin. The relationship between heme biosynthesis, cytochrome P-450, and heme degradation is shown in Fig. 1.

Enzymes in the heme biosynthetic pathway that are particularly sensitive to the effects of heavy metals are the two sulfhydryl-containing enzymes, δ-aminolevulinic acid (ALA)[2] dehydratase, which catalyzes the formation of porphobilinogen from two molecules of ALA, and the terminal enzyme, ferrochelatase, which catalyzes the formation of heme from protoporphyrin IX and ferrous ion. For example, lead causes marked inhibition of ALA dehydratase in erythrocytes and inhibition of this enzyme is used as a tool for the clinical diagnosis of lead poisoning (8). Lead and certain other divalent cations have been shown to inhibit ferrochelatase activity *in vitro* (9). Inhibition of these pathway enzymes by gold could result in decreased heme content in tissues where gold accumulates.

About 60% of the heme synthesized in the liver serves as the prosthetic group for cytochrome P-450 (10), the terminal oxidase of the hepatic monoxygenase systems which catalyze the metabolism of drugs, carcinogens, and other environmental chemicals, and certain endogenous substrates such as steroid hormones. Heavy metals, such as lead (11, 12) and cadmium (13, 14), have previously been shown to inhibit the formation of cytochrome P-450 and decrease the activities of cytochrome P-450-dependent enzymes. Since the kidney and the liver are primary sites of gold deposition (15, 16), it was of importance to examine whether gold has a direct effect on the components of the monooxygenase system in these tissues. An inhibitory effect of gold on the formation of cytochrome P-450 would be reflected by decreased metabolism of prototype substrates.

In the present studies, the effects of gold administration to experimental animals on heme biosynthesis and catabolism and on the functional capacity of cytochrome P-450 were examined. The effects of *in vitro* addition of gold on the kinetics of ALA dehydratase activity and on ethylmorphine

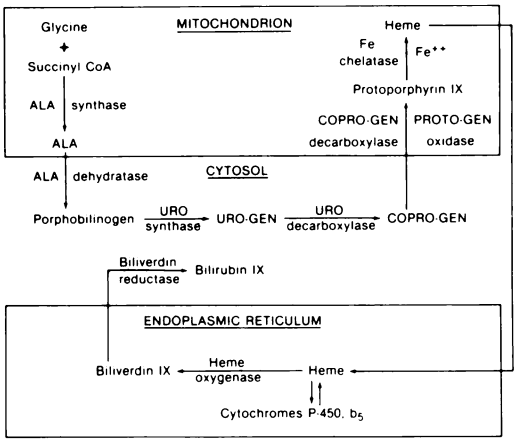

FIG. 1. *Relationship between the heme biosynthetic pathway, microsomal hemeproteins and heme catabolism*

Abbreviations: ALA, δ-aminolevulinic acid; URO, URO-GEN, uroporphyrinogen; COPRO-GEN, coproporphyrinogen; PROTO-GEN, protoporphyrinogen.

[2] The abbreviations used are ALA, δ-aminolevulinic acid; URO, uroporphyrinogen; AuTM, gold sodium thiomalate.

N-demethylase activity were also examined.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 100–150 g, were used in these studies. The animals were fasted for 12 hr prior to sacrifice. Gold sodium thiomalate and sodium thiomalate were purchased from ICN Pharmaceuticals, Plainview, N. Y. These salts were administered as aqueous solutions.

Tissue preparation. Livers and kidneys were perfused *in situ* with 50 ml of 1.15% KCl solution, excised, blotted, and weighed. They were then homogenized in 1.15% KCl solution, such that each milliliter contained the equivalent of 300 mg of liver or kidney, wet weight. The tissue homogenate was centrifuged at 9000 × *g* for 20 min. The supernatant was removed and an aliquot was saved for benzo(a)pyrene hydroxylase determination. The remaining supernatant was centrifuged at 105,000 × *g* for 1 hr and the resulting microsomal pellet was resuspended in 0.1 M phosphate buffer, pH 7.4. For the erythrocyte studies, a cardiac puncture was performed on ether-anesthetized rats. Blood was collected in heparin-coated tubes, mixed thoroughly, and stored on ice. The hematocrit was determined and blood samples were quick frozen in liquid nitrogen for 10 min to lyse the cells.

ALA synthase assay. ALA synthase activity, measured in whole homogenates, was determined by the method of Sassa as described by Rifkind *et al.* (17). ALA formed was reacted with 2,4-pentanedione to convert ALA to 2-methyl-3-acetyl-4-(3-propionic acid) pyrrole, which was determined by colorimetric techniques using modified Ehrlich reagent (18). A difference spectrum of the color salt was obtained in the wavelength range of 450 to 650 nm using an Aminco DW-2 spectrophotometer in the split beam mode. ALA formed was determined, using a molar extinction coefficient of 58,000 between 552 and 650 nm for a 1 cm light path.

ALA dehydratase assay. ALA dehydratase activity was determined by the method of Granick *et al.* (19) using 5 μl of blood or tissue homogenate equivalent to 2 mg of tissue, wet weight. Concentration of porphobilinogen formed was determined between 555 nm and 650 nm using a molar extinction coefficient of 61,000 for a 1 cm light path.

Uroporphyrinogen-I synthase assay. URO synthase activity was determined by the micromethod assay of Sassa *et al.* (20) using 5 μl of blood or tissue homogenate equivalent to 2 mg tissue, wet weight. Fluorescence was compared with a URO standard to determine the amount of URO formed.

Total porphyrins assay. Total porphyrin content of tissues was determined fluorometrically by the method of Granick *et al.* (21) using an excitation wavelength of 400 nm and scanning the emission spectrum between 580 and 680 nm.

Ferrochelatase assay. Ferrochelatase activity was assayed by a modification of the method of Porra and Jones (22) using mesoporphyrin and ferrous citrate as substrates. The reaction mixture containing 50 μl of tissue homogenate equivalent to 16.7 mg, wet weight, 1 ml of 0.1 M Tris buffer, pH 8.4, 100 μl of mesoporphyrin (400 nmoles/ml) and 100 μl of ferrous citrate (4 nmoles/ml) was incubated anaerobically for 30 min at 37°C in a shaking water bath. The reaction was terminated with 0.3 ml of 0.1 mM iodoacetamide. Mesoheme formed was measured by the pyridine-hemochromogen method as described by Falk (23), between 547 and 531 nm using a molar extinction coefficient of 21,700 for a 1 cm light path.

Cytochrome P-450. Cytochrome P-450 content was determined using microsomes from liver and kidney, equivalent to 100 and 200 mg of tissue, wet weight, respectively. The hemeprotein was assayed by the method of Omura and Sato (24) between 450 and 490 nm, using a molar extinction coefficient of 91,000 for a 1 cm light path.

NADPH-cytochrome c reductase assay. NADPH-cytochrome c reductase activity was determined by the method of Phillips and Langdon (25) using a molar extinction coefficient of 21,000 for a 1 cm light path to calculate the amount of cytochrome c reduced.

Ethylmorphine N-demethylase assay. The composition of the incubation mixture

for the N-demethylase assay was similar to that described previously (26), except that nicotinamide was omitted from the incubation mixture. Formaldehyde formed from the N-demethylation reaction was measured by the method of Nash (27), as modified by Anders and Mannering (28).

Benzo(a)pyrene hydroxylase assay. Benzo(a)pyrene hydroxylase activity was determined using the post-mitochondrial fraction as described previously (29) and the product formed was measured by the method of Nebert and Gelboin (30). Four milligrams of tissue, wet weight, were employed for the assay, and incubation times of 5 min for liver and 10 min for kidney were used to maintain the linearity of reaction rates.

Microsomal heme content. Microsomal heme content was measured by the pyridine hemochromogen method of Falk (23). Microsomes equivalent to 100 mg liver, wet weight, or 200 mg kidney, wet weight, were used for the determinations. The absorbance difference between 557 and 541 nm was used to calculate the protoheme content, using a molar extinction coefficient of 20,700 for a 1 cm light path.

Heme oxygenase assay. Microsomal heme oxygenase activity was measured using a modification of the method of Maines and Kappas (31). For the assay, 3.6 ml of 0.1 M Na_2HPO_4-KH_2PO_4 buffer, pH 7.4, 1 ml of microsomes containing 7–9 mg protein, 1 ml of 105,000 × *g* supernatent containing 10 mg protein, 0.1 ml of glucose-6-phosphate dehydrogenase (3 units) and 0.1 ml of $MgCl_2$ (12 μmoles) were added to a 10 ml vial and stored on ice. Twenty μl of hemin (17 μM) were added and the samples were preincubated in the dark for 5 min at 37°. After the 5 min preincubation, 2.9 ml of the reaction mixture was transferred to another vial. To the contents of this sample vial, 0.1 ml containing 3 μmoles NADP and 78 μmoles glucose-6-phosphate was added. Buffer, 0.1 ml, was added to the blank vial. The samples were incubated for 10 min at 37° in a shaking water bath, and the reactions were terminated by placing the samples on ice. The difference spectrum between the contents of the two vials was recorded immediately between 400 and 550 nm. Bilirubin formation was calculated, using a molar extinction coefficient of 40,000 for a 1 cm light path, between 464 and 530 nm.

Protein assay. Protein content of the various cell fractions was determined by the method of Lowry *et al.* (32) using crystalline bovine serum albumin as a standard.

Statistical analysis. The Student's *t*-distribution was used as a test of the null hypothesis, using a level of significance of p equal to or less than 0.05.

RESULTS

In vitro effects of gold on hepatic ALA dehydratase activity and microsomal monooxygenase system. The *in vitro* inhibitory effects of gold sodium thiomalate on ALA dehydratase, ethylmorphine N-demethylase and NADPH-cytochrome c reductase activities are shown in Fig. 2. The activity of each enzyme was reduced in a concentration-dependent manner. ALA dehydratase activity was very sensitive to the inhibitory effects of gold, with 50% inhibition occurring at a final gold concentration of about 45 μM. In the electron transport system of liver microsomes, NADPH-cytochrome c reductase activity was much more sensitive to the inhibitory effects of gold than cytochrome P-450. A 50% inhibition of NADPH-cytochrome c reductase activity occurred at a gold concentration of 35 μM. The addition of gold to liver microsomes did not markedly affect cytochrome P-450 content; at the highest gold concentration tested, cytochrome P-450 content decreased by only 27% (Fig. 2). This decrease in cytochrome P-450 content was accompanied by a corresponding increase in cytochrome P-420 content of the liver microsomes. The inhibition of ethylmorphine N-demethylase activity paralleled that of NADPH-cytochrome c reductase. If instead of gold sodium thiomalate, sodium thiomalate was added to liver cell fractions, no inhibitory effects of the enzymic activities or on cytochrome P-450 content was observed, indicating the inhibitory effects were due to the heavy metal, gold, and not to sodium thiomalate.

The kinetics of gold inhibition of ALA dehydratase activity show that gold is a

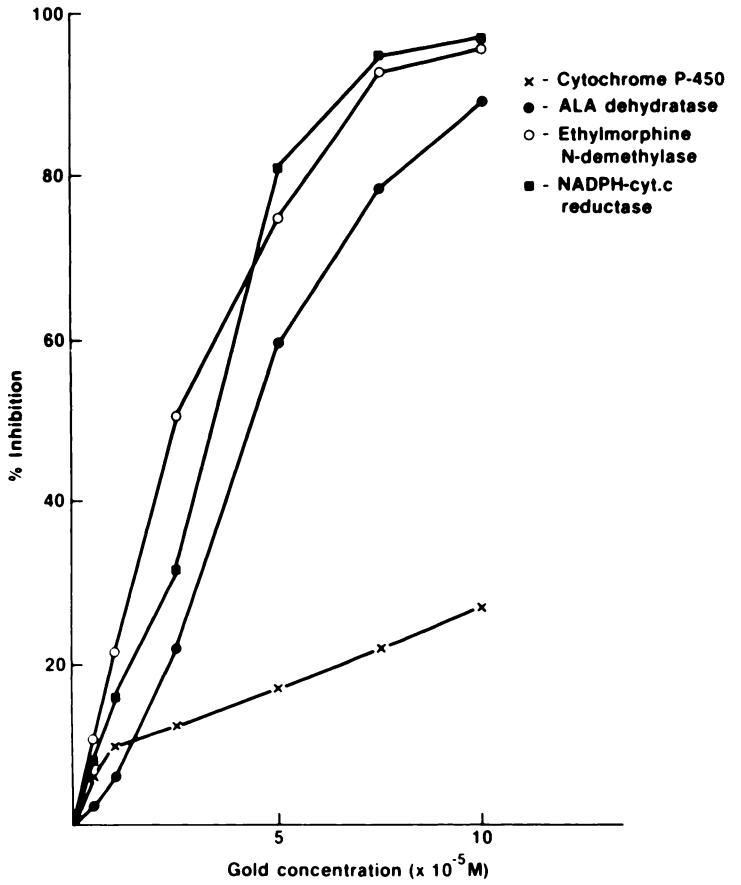

FIG. 2. *Effects of varying concentrations of gold on hepatic δ-aminolevulinic acid (ALA) dehydratase and monooxygenase activities*

Liver homogenate was used to assay for ALA dehydratase activity and microsomes were used to assay for cytochrome P-450, NADPH-cytochrome c reductase and ethylmorphine N-demethylase activities in the absence and presence of gold sodium thiomalate. Reactions were initiated by the addition of the substrate in the enzymic assays. Values on the abscissa represent final concentrations of gold in the incubation mixture. Control values, in absence of gold, were: ALA dehydratase: 6.02 nmoles porphobilinogen formed/mg protein/hr; NADPH-cytochrome c reductase: 113.5 nmoles cytochrome c reduced/mg protein/min; cytochrome P-450: 0.837 nmoles/mg protein; and ethylmorphine N-demethylase: 297 nmoles HCHO formed/mg protein/hr. Each value represents the mean of values obtained from livers of 4 untreated rats.

noncompetitive inhibitor of the enzyme (Fig. 3). The apparent Michaelis-Menton constant, K_m, obtained in the absence of gold was 2.22 mM. This is comparable to the K_m determined by Doyle and Schimke (33) for ALA dehydratase in mouse liver homogenates. As in the case of ALA dehydratase, gold added to liver microsomes resulted in a noncompetitive inhibition of ethylmorphine N-demethylase activity (Fig. 4). The inhibition constant, K_i, obtained for the N-demethylation of ethylmorphine in the presence of gold, was 30 μM.

Effects of gold on heme biosynthetic pathway in erythrocytes. In these experiments, male rats were administered gold sodium thiomalate, 75 mg/kg intraperitoneally 24 hr prior to sacrifice. This dose is equivalent to administering a dose of 37.5 mg/kg of the metal itself. The residual erythrocyte enzymes ALA dehydratase and URO-I synthase activities, and protoporphyrin content were determined and the

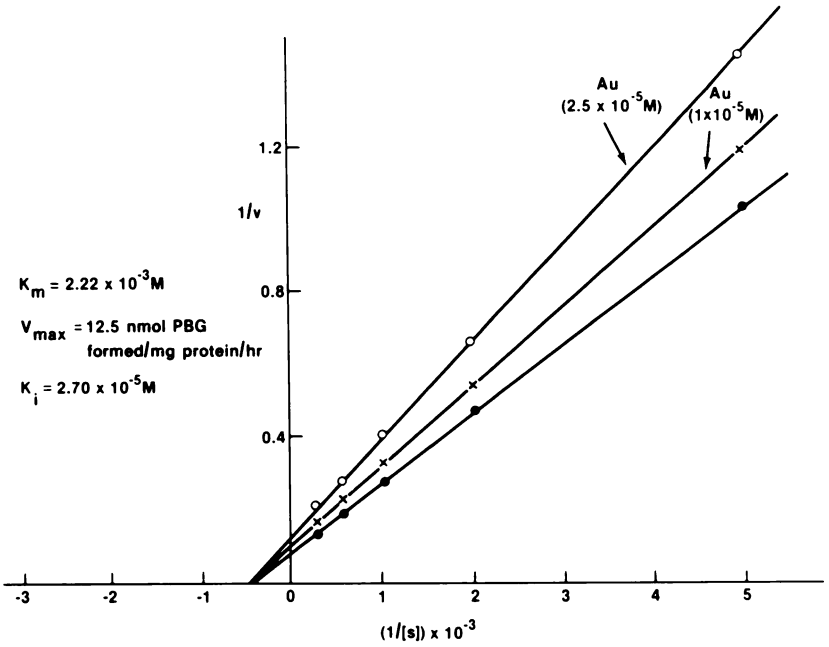

FIG. 3. *Effect of gold on the kinetics of δ-aminolevulinic acid (ALA) dehydratase activity*

Gold was added in the form of gold sodium thiomalate. Concentrations of gold shown are the final concentrations of the metal in the incubation mixture. In the gold experiments, reactions were initiated by the addition of ALA to the reaction mixture. Each point represents the mean of 5 experiments; each experiment was done with a different rat.

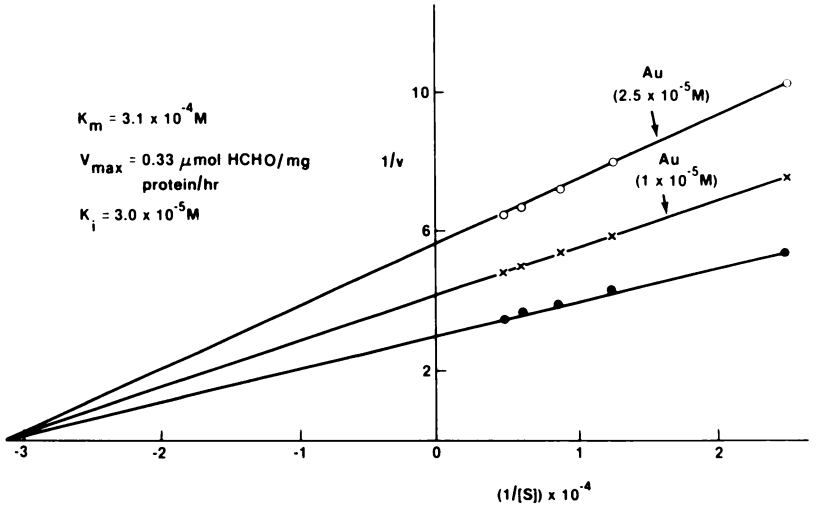

FIG. 4. *Effect of gold on the kinetics of ethylmorphine N-demethylase activity*

Gold was added in the form of gold sodium thiomalate. Concentrations of gold shown are the final concentrations of the metal in the incubation mixture. Reactions were initiated by the addition of microsomes to the reaction mixture. Each point represents the mean of 5 experiments; each experiment was done with a different rat.

results are shown in Fig. 5. Erythrocyte ALA dehydratase activity was significantly decreased in gold-pretreated rats; however, URO-I synthase activity was unaffected. There was a 2-fold increase in erythrocyte protoporphyrin content. These observations are similar to those seen in erythrocytes from lead-poisoned animals and patients (8, 12, 34, 35), although the extent of the inhibition of ALA dehydratase by gold is not as large as that elicited by lead.

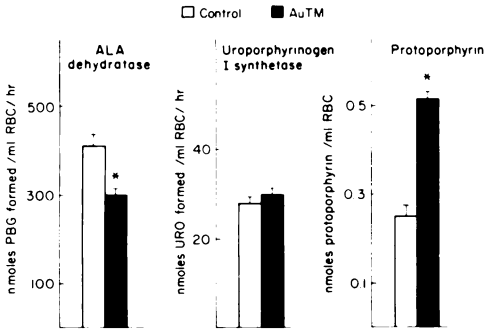

FIG. 5. *Effect of gold pretreatment of rats on erythrocyte heme biosynthetic pathway*

Male Sprague-Dawley rats were administered gold sodium thiomalate (AuTM) 75 mg/kg, intraperitoneally and sacrificed 24 hr later. The dose of gold itself was 37.5 mg/kg. δ-aminolevulinic acid (ALA) dehydratase (19), uroporphyrinogen-I synthetase (20) activities and protoporphyrin (21) contents of erythrocytes were determined as described previously. Each bar represents the mean ± SE for 5 rats. Asterisk denotes value significantly different from the respective control value ($p < 0.05$). Abbreviations: PBG, porphobilinogen; URO, uroporphyrinogen.

Effect of gold on heme biosynthetic pathway in the liver. The effect of gold sodium thiomalate, 75 mg/kg, on the heme biosynthetic pathway in livers of rats pretreated for 24 hr is shown in Fig. 6. The activity of the rate-limiting enzyme, ALA synthase, was not significantly altered, nor was the total liver porphyrin content. However, ALA dehydratase and ferrochelatase activities were significantly decreased: 22% and 40%, respectively. These decreases were accompanied by a significant decrease in microsomal heme content of these livers. These data demonstrate an impairment of heme synthesis in the livers of rats following a single injection of gold.

Effect of gold on the hepatic monooxygenases. The effect of gold sodium thiomalate on components of the monooxygenase system and cytochrome P-450-dependent oxidations is shown in Table 1. Following the acute treatment of rats with the gold salt, cytochrome P-450 content of liver microsomes was significantly decreased by 22%. NADPH-cytochrome c reductase activity was decreased by 14%, but this decrease was not statistically significant. Comcomitant with the decrease in cytochrome P-450 levels, the cytochrome P-450-dependent enzymic activities, ethylmorphine N-demethylase and benzo(a)pyrene hydroxylase, were significantly decreased

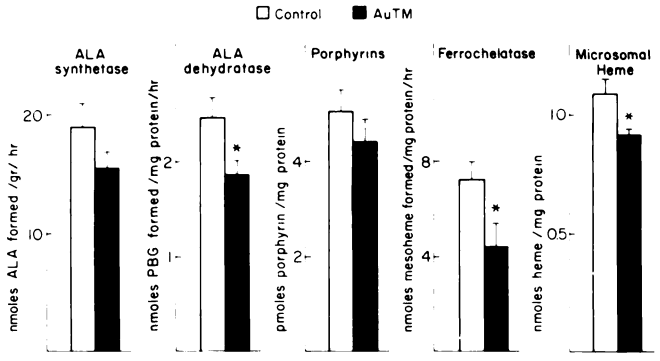

FIG. 6. *Effect of gold pretreatment of rats on hepatic heme biosynthetic pathway.*

Male Sprague-Dawley rats were administered gold sodium thiomalate (AuTM), 75 mg/kg, intraperitoneally and sacrificed 24 hr later. Heme pathway enzymic activities in liver homogenates and microsomal heme content were determined as described in the MATERIALS AND METHODS section. Each bar represents the mean ± SE for 5 rats. Asterisk denotes value significantly different from the respective control value ($p < 0.05$). Abbreviations: ALA, δ-aminolevulinic acid; PBG, porphobilinogen.

TABLE 1

The acute effect of gold pretreatment on hepatic and renal drug metabolizing enzyme systems

Male Sprague-Dawley rats were administered gold sodium thiomalate, 75 mg/kg, intraperitoneally and rats were sacrificed 24 hr later. Enzymic assays on individual livers or kidneys pooled from each rat were determined as described in MATERIALS AND METHODS. To determine the hexobarbital sleeping time, sodium hexobarbital was administered intraperitoneally at a dosage of 100 mg/kg, and the duration of loss of the righting reflex was determined. Each value represents mean ± SE for 5 rats.

Treatment	Cytochrome P-450[a]		NADPH-cyt. c reductase[b]		Ethylmorphine N-demethylase[c]		Benzo(a)pyrene hydroxylase[d]		Hexobarbital sleeping time[e]
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	
Control	0.926 ±0.048	0.095 ±0.029	123.9 ±4.2	32.0 ±6.0	314.9 ±35.5	N.D.[f]	3.87 ±0.91	0.622 ±0.070	90 ±5
Gold	0.725[g] ±0.032	0.049[g] ±0.007	106.8 ±7.8	25.6 ±5.8	234.7[g] ±29.8	N.D.	2.20[g] ±0.52	0.229[g] ±0.014	113[g] ±8
% change	−21.7	−48.4	−13.8	−20.0	−25.5	—	−43.2	−63.1	+25.6

[a] nmoles/mg protein.

[b] nmoles cytochrome c reduced/mg protein/min.

[c] nmoles HCHO formed/mg protein/hr.

[d] nmoles hydroxybenzo(a)pyrene formed/mg protein/hr.

[e] min.

[f] Not detectable.

[g] Value significantly different from the respective control value ($p < 0.05$).

by 26% and 43%, respectively. Pretreatment of rats with gold resulted in a significant prolongation of hexobarbital-induced sleeping times. Thus the decreased capacity of liver microsomes of rats pretreated with gold to metabolize ethylmorphine and benzo(a)pyrene *in vitro* was accompanied by an enhanced pharmacological action of hexobarbital *in vivo*. If instead of gold sodium thiomalate, an equimolar dosage of sodium thiomalate was administered to rats, no inhibitory effects on any of the above parameters measured were observed. These data indicate that the observed inhibitory effects were due to gold administered and not due to sodium thiomalate.

Effect of gold on heme metabolism in the kidney. Since the kidney is the major site of gold deposition (15, 16) and is the organ most susceptible to toxicity by heavy metals, it was of importance to determine the effect of gold on heme metabolism in the kidney. Indeed, the effects of acute gold treatment were more pronounced in the kidney than in the liver. As shown in Fig. 7, following the administration of a single dose of gold sodium thiomalate, the activities of ALA synthase, ALA dehydratase and ferrochelatase were inhibited 13%, 59%, and 69%, respectively, in the kidney. Total porphyrin content decreased to 50% of the control values. These data demonstrate that acute gold administration decreases heme synthesis in the kidney.

The effect of gold sodium thiomalate on components of the renal monooxygenase system and on benzo(a)pyrene hydroxylase activity are shown in Table 1. With the analytical technique used in the present studies, ethylmorphine N-demethylase activity was not detectable in kidney microsomes. Acute gold treatment decreased cytochrome P-450 content by 48%. Although NADPH-cytochrome c reductase activity was decreased by 20%, this decrease was not statistically significant. As expected, benzo(a)pyrene hydroxylase activity was markedly inhibited in gold-pretreated rats. The inhibitory effects of gold were significantly greater in the kidney when compared to those observed with liver microsomes (Table 1).

Effects of acute gold treatment on microsomal heme oxygenase activities in liver and kidneys. Twenty-four hours following the intraperitoneal injection of 75 mg/kg of gold sodium thiomalate, rats were sacrificed and heme oxygenase activity was determined in liver and kidney microsomes. As shown in Fig. 8, a 1.5-fold increase in heme oxygenase activity was observed in the liver. An 8-fold increase in this enzymic

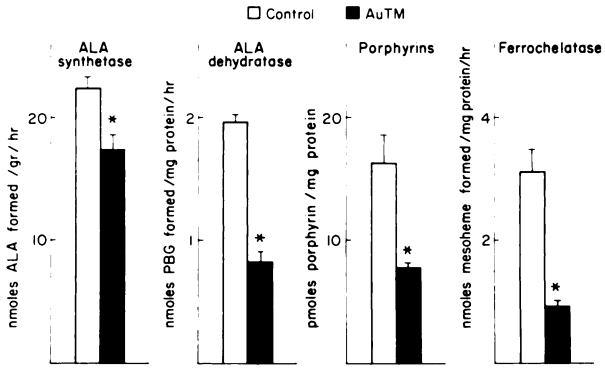

FIG. 7. *Effect of gold pretreatment of rats on the heme biosynthetic pathway enzymes in the kidneys*

Male Sprague-Dawley rats were administered gold sodium thiomalate (AuTM), 75 mg/kg, intraperitoneally and sacrificed 24 hr later. Heme biosynthetic pathway enzymic activities and total porphyrin contents were determined in kidney homogenates as described in the MATERIALS AND METHODS section. Each bar represents the mean ± SE for kidneys of 5 rats, the kidneys from each rat being pooled for each determination. Asterisk represents value which is significantly different from the respective control value ($p < 0.05$). Abbreviations: ALA, δ-aminolevulinic acid; PBG, porphobilinogen.

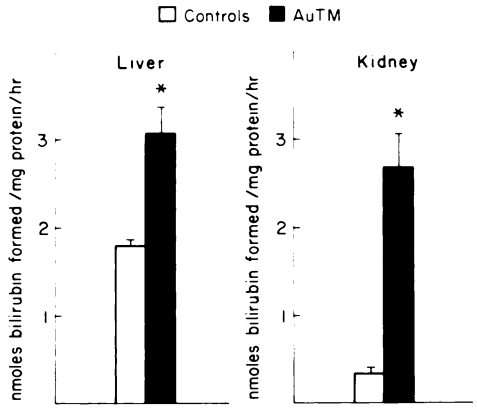

FIG. 8. *Effect of gold pretreatment of rats on microsomal heme oxygenase activities in the liver and kidneys.*

Male Sprague-Dawley rats were administered gold sodium thiomalate (AuTM), 75 mg/kg, intraperitoneally and sacrificed 24 hr later. Microsomal heme oxygenase activity was determined as described in the MATERIALS AND METHODS section. Each bar represents mean ± SE for 5 rats. The kidneys of each rat were pooled for each determination. Asterisk represents value which is significantly different from the respective control value ($p < 0.05$).

activity was observed in kidney microsomes.

Effects of chronic treatment with gold on heme metabolism in erythrocytes, liver and kidneys of rats. Since gold salts are administered chronically to patients with rheumatoid arthritis, the effect of chronic administration of a low dose, 7.5 mg/kg, of gold sodium thiomalate to rats was examined. The gold salt was administered to rats intraperitoneally at a dose of 7.5 mg/kg/day for 20 days, and 24 hr after the last injection the rats were sacrificed. Chronic pretreatment with gold resulted in no change in erythrocyte ALA dehydratase and URO-I synthase activities or protoporphyrin contents (Fig. 9). Similarly, heme biosynthetic pathway enzymes and monooxygenase activities were not affected in the liver (Table 2). In the kidney, although ALA dehydratase activity and porphyrin content were decreased, cytochrome P-450 content and NADPH-cytochrome c reductase and benzo(a)pyrene hydroxylase activities were not altered.

DISCUSSION

Gold compounds in use today in therapy of rheumatoid arthritis are complexes of aurous gold with different sulfur-containing ligands, such as thiomalic acid, thioglucose, and sodium thiomalate. Because currently used gold drugs are heavy metal mercaptides, it has been frequently suggested that their biological activity is caused by reactivity with sulfhydryl groups. Previous studies have shown that the sulfhydryl reactivity of gold compounds plays a role in their inhibition of lysosomal enzyme activity (2). Since certain key enzymes in the

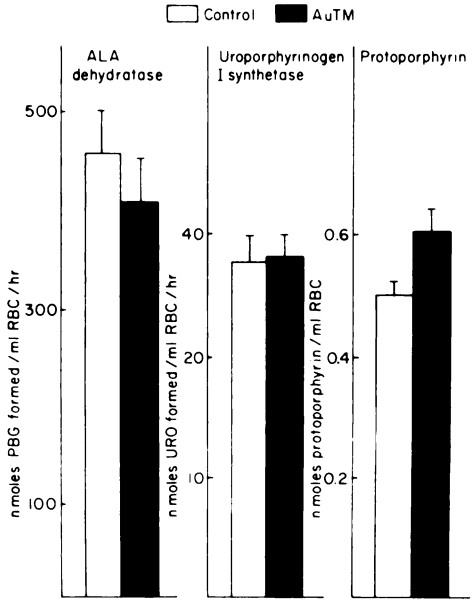

FIG. 9. *Effect of chronic gold treatment of rats on erythrocyte heme biosynthetic pathway*

Male Sprague-Dawley rats were administered gold sodium thiomalate (AuTM), 7.5 mg/kg/day, for 20 days and sacrificed 24 hr after the last injection. δ-aminolevulinic acid (ALA) dehydratase (19), uroporphyrinogen-I synthetase (20) activities and protoporphyrin (21) content of erythrocytes were determined as described previously. Each bar represents the mean ± SE for 5 rats. Abbreviations: PBG, porphobilinogen; URO, uroporphyrinogen.

heme biosynthetic pathway are sulfhydryl-containing enzymes and can be inhibited by heavy metals, it was of importance to determine the effects of gold on these enzymic activities. The present studies demonstrate that gold sodium thiomalate added *in vitro* caused a marked noncompetitive type of inhibition of hepatic ALA dehydratase. The mechanism of this inhibition is probably due to the interaction of the metal with sulfhydryl groups. Following the acute administration of gold to rats, alterations were observed in the heme biosynthetic pathway in erythrocytes, liver and kidney. ALA dehydratase activity was inhibited in all tissues examined. Ferrochelatase was inhibited in both the liver and the kidneys. A 2-fold increase in protoporphyrin content was observed in erythrocytes. Since ferrochelatase is a mitochondrial enzyme, the increase in erythrocyte protoporphyrin content is probably due to inhibition of this sulfhydryl enzyme in bone marrow erythroblasts. While the protoporphyrin levels observed in erythrocytes were increased, porphyrin contents of liver and kidney cells were decreased, the observed decrease being much greater in the kidney than in the liver. Indeed, in the kidney there was a decrease in all the heme biosynthetic pathway enzymes examined, including ALA synthase activity. The kidney is a major site of gold deposition following the parenteral administration of gold salts to experimental animals (15, 16). In the present studies, all kidneys of rats pretreated with gold for 24 hr exhibited an abnormally pale color at autopsy. Studies by Deter and Liebelt (36) have shown that cellular degeneration in the proximal and distal tubules of the kidney occurs following the administration of gold thioglucose to rats.

Derangements in heme metabolism induced by gold would result in alterations in hepatic monooxygenases. The data presented in this paper show that *in vitro* gold sodium thiomalate caused a marked inhibition of NADPH-cytochrome c reductase and ethylmorphine N-demethylase activities but only a slight decrease in cytochrome P-450 content of liver microsomes. At the highest gold concentration tested, the complete inhibition of NADPH-cytochrome c reductase would account for the loss of N-demethylase activity, in the presence of only a partial decrease in cytochrome P-450 content. The decrease in cytochrome P-450 content was accompanied by an increase in cytochrome P-420. Gold may exert a direct action on the electron transport components of the monooxygenase system inasmuch as there is a suggestion of involvement of sulfhydryl groups in the integrity and electron-transfer function of cytochrome P-450 (37) as well as NADPH-cytochrome c reductase activities (25, 38). Following the acute administration of gold sodium thiomalate to rats, a significant decrease in hepatic cytochrome P-450, with a concomitant decrease in cytochrome P-450-dependent drug oxidations, was observed. As with heme biosynthesis, a greater decrease was observed in cytochrome P-450 and benzo(a)pyrene hydrox-

TABLE 2

The effects of chronic gold pretreatment on heme biosynthetic pathway and on monooxygenases in liver and kidneys of rats

Male Sprague-Dawley rats weighing 80–100 g were administered, intraperitoneally, gold sodium thiomalate, 7.5 mg/kg/day for 20 days, and rats were sacrificed 24 hr after the last injection. Each value represents mean ± SE for 5 rats.

Assay	Liver		Kidney	
	Controls	Gold Treated	Controls	Gold Treated
ALA synthase, nmoles ALA formed/g liver/hr	18.25 ±2.37	25.70 ±4.92	6.93 ±1.26	8.12 ±1.88
ALA dehydratase, nmoles PBG formed/mg protein/hr	4.15 ±0.58	4.21 ±0.25	2.02 ±0.21	1.01[a] ±0.13
Total porphyrins, pmoles/mg protein	7.66 ±0.70	8.56 ±1.16	18.18 ±1.68	12.85[a] ±1.16
Ferrochelatase, nmoles mesoheme/mg protein/hr	5.28 ±0.54	5.58 ±0.18	3.12 ±0.30	3.12 ±0.30
Cytochrome P-450, nmoles/mg protein	0.811 ±0.024	0.905 ±0.047	0.030 ±0.004	0.023 ±0.003
NADPH-cyt. c reductase, nmoles cyt. c reduced/mg protein/min	89.99 ±6.31	95.22 ±6.75	35.66 ±2.83	40.06 ±4.79
Ethylmorphine N-demethylase, nmoles HCHO formed/mg protein/hr	228.1 ±23.5	220.4 ±10.2	N.D.[b]	N.D.
Benzo(a)pyrene hydroxylase, nmoles OHBP formed/mg protein/hr	4.30 ±0.31	3.82 ±0.14	0.315 ±0.047	0.242 ±0.027

[a] Value significantly different from the control value ($p < 0.05$).

[b] Not detectable.

ylase activity in kidney microsomes. The action of gold, when administered to rats, on NADPH-cytochrome c reductase activity was of a much lesser magnitude than when gold was added *in vitro* to liver microsomes. These differences could be due to concentration and distribution patterns of gold *in vivo* and *in vitro*. The enhanced effect of the metal on this enzymic activity *in vitro* may also be due to the unmasking of additional active sites of the reductase when endoplasmic reticulum membranes are disrupted. In data not shown, the inhibitory effects of gold sodium thiomalate on the monooxygenases were not observed if sodium thiomalate was administered, demonstrating that the inhibitory effects were due to the heavy metal rather than the ligand.

Heme oxygenase catalyzes the conversion of heme to biliverdin and serves as the rate-limiting step in heme catabolism (39). The present studies show that gold administration caused a 1.5- and 8-fold increase in heme oxygenase activity in liver and kidney microsomes, respectively. The decrease in heme content observed in rats pretreated with gold could be due to a decrease in heme biosynthesis and/or an increase in heme catabolism, since both phenomena were observed in the present studies. Bissell and Hammaker (40) suggest that stimulation of microsomal heme oxygenase is linked temporally and quantitatively to degradation of cytochrome P-450. A number of other metals, such as nickel, mercury, tin, cobalt, and cadmium (4, 5, 6, 9), also appear to cause a decrease in cytochrome P-450 and associated enzymic activities and induction of microsomal heme

oxygenase. The complex interaction between metals such as gold and heme biosynthesis and catabolism requires further study.

In contrast to the pronounced effects on heme metabolism elicited in rats acutely treated with gold sodium thiomalate chronic administration of the gold salt at a low dose did not cause any significant changes in the various parameters of heme metabolism in the liver, kidney, and erythrocytes. An initial loss of weight was observed in the chronically-treated animals, but after the first week body weight gains were similar to those observed in control animals. The lack of effect on heme metabolism following chronic treatment with gold may be due to the induction of an adaptive responsive in the tissues studied. Cortell and Richards (41) have previously reported that tolerance develops to the toxic effects of gold if rats are pretreated with small doses of the metal every other day for 3 weeks. The present findings with gold are also similar to those recently reported with rats chronically pretreated with cadmium (14, 42) or lead (12). These studies showed that, although acute treatment with cadmium (14, 42) or lead (12) produced a marked decrease in hepatic monooxygenases, chronic treatment of rats with these metals did not alter the monooxygenase activities. Similarly, antipyrine elimination rates in workers chronically exposed to lead were minimally affected when compared to unexposed individuals (12). Recent studies have shown that pretreatment of experimental animals with cadmium (43, 44), mercury (45), zinc (46) or copper (47) induces the synthesis in the liver and kidney of low molecular weight metal-binding proteins, known as metallothioneins. These cytoplasmic proteins have a high cysteine and metal content. It has been suggested that metallothioneins may have a protective effect against heavy metal toxicity. Induction of a similar low molecular weight protein with a high affinity for gold could explain the apparent development of tolerance. Preliminary data (48) indicate an increase in 10,000 molecular weight proteins in the soluble fraction of kidneys from rats chronically treated with gold. However, this protein(s) has not been further characterized.

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