THE EFFECT OF COBALT CHLORIDE ADMINISTRATION ON THE SYNTHESIS OF HEPATIC MICROSOMAL CYTOCHROME P-450

T. R. Tephly and P. Hibbeln

Department of Pharmacology, The University of Michigan Medical School
Ann Arbor, Michigan 48104

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Summary

Cobalt chloride administration to rats stimulates erythropoesis but inhibits the synthesis of hepatic microsomal P-450 and ethylmorphine N-dealkylase activity by hepatic microsomes. No effect on NADPH-cytochrome c reductase activity was observed. Phenobarbital induction of P-450 and ethylmorphine N-demethylation are prevented by simultaneous administration of cobalt chloride but phenobarbital induction of NADPH-cytochrome c reductase activity is not affected. Hexobarbital oxidation by the perfused rat liver is also inhibited by prior administration of cobalt chloride. Since cobalt treatment of rats has no effect on hepatic protein synthesis over the course of these experiments, cobalt administration may be valuable as a tool for studying the role(s) of P-450 in various biological reactions.

A previous report from this laboratory showed that a cobalt-protoporphyrin IX complex was formed enzymatically in hepatic mitochondria through the mediation of an enzyme that differed from ferrochelatase (1). It has long been known that cobalt salts have a stimulatory effect on bone marrow function which results in increased red cell production (2). Indeed, cobalt therapy has been used for the treatment of bone marrow aplasia (3). It was of interest in this laboratory to examine the effects of cobalt administration *in vivo* in order to learn whether the formation of cobalt-protoporphyrin could have either an inhibitory or stimulatory influence on hepatic hemoproteins. It has been shown that certain substances such as phenobarbital and benzpyrene have a rapid and dramatic effect on the heme biosynthetic pathway and that the levels of the microsomal hemoprotein, cytochrome P-450, increases as a result of this stimulation (4). The following report documents a profound effect of cobaltous chloride administration *in vivo* on cytochrome P-450 and certain reactions dependent upon that pigment.

Methods and Materials

Male, Sprague-Dawley rats weighing 150-200 g were used throughout the study. They were routinely fasted for 24 hours prior to sacrifice. Sodium phenobarbital was administered intraperitoneally at a dose of 40 mg/kg of body weight and cobaltous chloride ($CoCl_2 \cdot 6H_2O$) was injected subcutaneously at doses of 40 or 60 mg/kg of body weight. After decapitation, livers were perfused with iced 0.9% NaCl solution. Homogenates of liver were subjected to differential centrifugation as described previously (4). Cytochrome P-450, cytochrome b_5, ethylmorphine N-demethylation and NADPH-cytochrome c reductase activity were measured as described by Baron and Tephly (4). Studies with isolated, perfused rat livers were performed according to the method of Stitzel et al (5).

Results

Effect of Cobaltous Chloride Administration on Microsomal Cytochrome P-450 and Ethylmorphine N-dealkylation. The subcutaneous injection of cobaltous chloride (60 mg/kg) has been shown to produce a marked stimulation of erythropoesis in rats (1). This was confirmed in this laboratory. However, a single injection of cobaltous chloride (60 mg/kg) 24 hours prior to sacrifice exerts a profound decrease in microsomal levels of cytochrome P-450 (Table 1) and microsomal ethylmorphine N-demethylation. Two injections of cobalt at 48 and 24 hours prior to sacrifice produce even greater effects on P-450 and ethylmorphine oxidation. A moderate effect is seen on microsomal cytochrome b_5 levels and no effect on NADPH-cytochrome c reductase activity was observed.

Inhibition of Phenobarbital Induction of Cytochrome P-450 and Ethylmorphine N-Demethylation by Cobaltous Chloride. When 40 mg/kg of cobaltous chloride was injected subcutaneously either 24 or 48 and 24 hours prior to sacrifice, no, or only slight, effects on cytochrome P-450 and ethylmorphine oxidation were observed (Table 2). Phenobarbital exerts its expected increase on both. Simultaneous administration of cobalt with phenobarbital drastically curtails the effects of phenobarbital (Table 2) on cytochrome P-450 and ethylmorphine N-demethylation. However, cobalt administration has no effect on the induction of the flavoprotein, NADPH-cytochrome c reductase. When the relation between P-450 and ethylmorphine

Table 1

EFFECT OF COBALTOUS CHLORIDE ADMINISTRATION ON P-450 AND ETHYLMORPHINE N-DEMETHYLATION

Treatment	P-450 (nmoles/mg protein)	b_5 (nmoles/mg protein)	NADPH-Cytochrome c Reductase (nmoles cytochrome c reduced/mg protein/min)	Ethylmorphine N-Demethylation (nmoles HCHO formed/mg protein/min)
Control	0.6	0.6	103.1	3.1
$CoCl_2$ (60 mg/kg, 24 hrs before sacrifice)	0.3	0.4	98.3	1.6
$CoCl_2$ (60 mg/kg, 48 and 24 hrs before sacrifice)	0.1	0.3	90.6	0.3

Rats were injected with cobaltous chloride (60 mg/kg) subcutaneously either 24 hours or 48 and 24 hours before sacrifice. Control animals were injected with 0.9% NaCl solution. Measurements were made as described by Baron and Tephly (4). Each value is the mean of 3 experiments.

Table 2

EFFECT OF COBALTOUS CHLORIDE ON PHENOBARBITAL INDUCTION

Treatment	P-450 (nmoles/mg protein)	b_5 (nmoles/mg protein)	NADPH-Cytochrome c Reductase (nmoles cytochrome c reduced/mg protein/min)	Ethylmorphine N-Demethylation (nmoles HCHO formed/mg protein/min)
Control	0.8	0.7	121.4	3.6
$CoCl_2$ (40 mg/kg, 24 hrs before sacrifice)	0.6	0.7	153.0	3.1
$CoCl_2$ (40 mg/kg, 48 and 24 hrs before sacrifice)	0.4	0.7	174.4	0.8
Phenobarbital (24 hrs before sacrifice)	1.6	0.8	210.1	11.5
Phenobarbital + $CoCl_2$ (24 hrs before sacrifice)	0.9	0.6	198.8	5.7
Phenobarbital (48 hrs and 24 hrs before sacrifice)	2.2	0.8	251.2	11.6
Phenobarbital + $CoCl_2$ (48 and 24 hrs before sacrifice)	0.9	0.6	223.7	5.2

Conditions were the same as described in Table 1. Animals were injected subcutaneously with cobaltous chloride (40 mg/kg). Sodium phenobarbital was injected intraperitoneally at a dose of 40 mg/kg. Each value is the mean of at least 2 experiments.

N-demethylation are plotted, as in figure 1, a straight line is obtained with a correlation coefficient of 0.96.

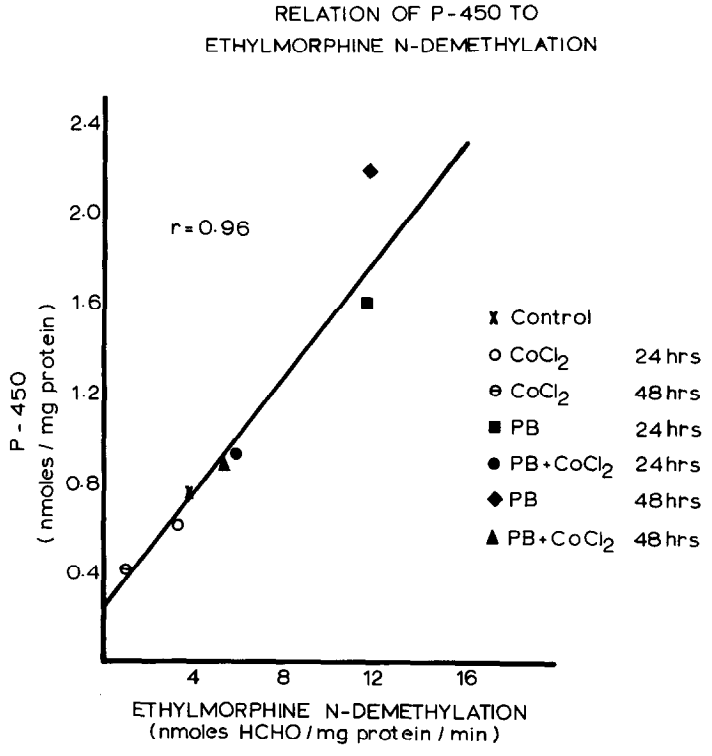

Figure 1. Data for the plot was obtained from Table 2. Twenty-four hours indicates that animals received only one injection of $CoCl_2$, phenobarbital or both, 24 hours before sacrifice. Forty-eight hours indicates that animals received 2 doses of the agent, either 24 hours or 48 and 24 hours before sacrifice. r = correlation coefficient.

Inhibition of Hexobarbital Metabolism in the Isolated, Perfused Rat Liver by Prior Administration of Cobaltous Chloride. Hexobarbital is a Type I substrate metabolized by microsomal hydroxylation and its rate of disappearance in vivo or in the isolated, perfused rat liver has often been used as a measure of P-450 dependent microsomal hydroxylation in these systems (5). Figure 2 shows that administration of cobalt strongly inhibits the oxidation of hexobarbital in the perfused rat liver.

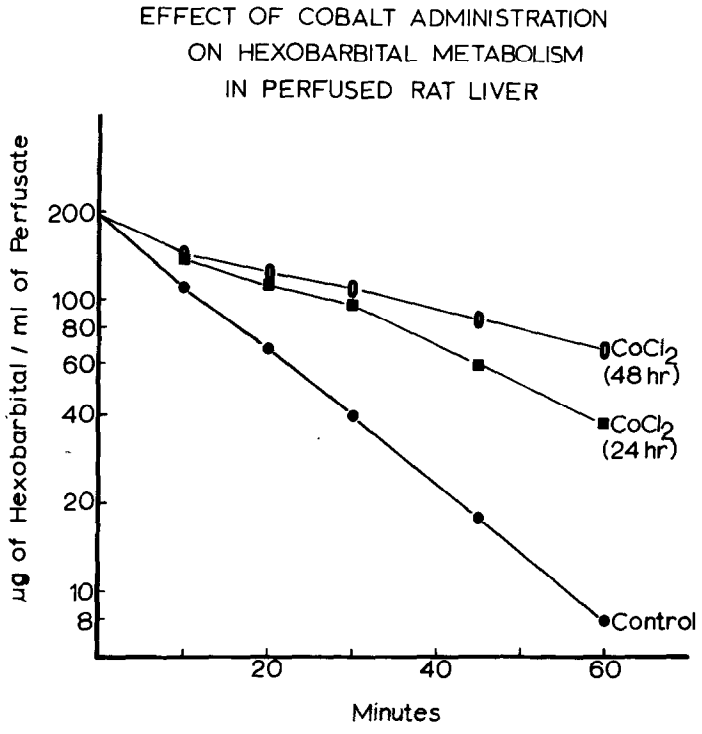

Figure 2. Hexobarbital disappearance from the perfusion fluid was determined as described previously (5). Animals were injected with $CoCl_2$ as described in Table 1.

Discussion

Baron and Tephly (6) have shown that 3-amino-1, 2, 4-triazole (AT) inhibited the synthesis of microsomal heme, cytochrome P-450 and oxidations dependent upon hepatic P-450. It also prevented the phenobarbital induction of cytochrome P-450 and dependent reactions but had no effect on the induction of NADPH-cytochrome c reductase activity produced by phenobarbital. Cobalt chloride appears to have effects similar to AT. However, the effects of AT are shortlived, whereas, the action of cobalt is not. Furthermore, cobalt produced marked decreases in P-450 and ethylmorphine N-demethylation while AT could not produce decreases greater than 50%. In this respect, cobalt chloride administration may be a valuable tool for studies where one wishes to obtain a desired level of hepatic P-450 without exerting great effects on NADPH-cytochrome c reductase. NADPH-cytochrome c reductase activity is not decreased by cobalt and phenobarbital induction is not altered. NADPH-P-450 reductase activities have been measured by the method of

Gigon et al (7). Only when low levels of P-450 were obtained was this activity affected. This suggests that the measurement of P-450 reductase activity depends in part upon the levels of its substrate, P-450. Furthermore, cobalt treatment does not prevent the ethylmorphine stimulation of NADPH-cytochrome P-450 reductase activity.

Although there is a good correlation between the level of P-450 and ethylmorphine N-demethylation in hepatic microsomes the line does not go through the origin. One possible explanation for this may be that a certain amount of P-450 is not functional in the N-demethylation of ethylmorphine.

It is interesting to note that the activities of P-450 and cytochrome b_5, remaining in the hepatic microsomes after treatment with 60 mg/kg of cobaltous chloride, are close to the value that one would predict on the basis of proposed half-lives. Greim et al (8) have reported that the half-life of P-450 is about 22 hours and that the half-life of cytochrome b_5 was 45 hours. Table 1 shows data consistant with these values. It may be suggested that cobalt chloride treatment could be a valuable tool for the measurement of hemoprotein turnover. However, more work is required before this can be stated with certainty. Studies have shown that under the conditions of the experiments reported in this study, no inhibition of microsomal protein synthesis occurs.

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References

1. Hasegawa, E., Smith, C. and Tephly, T. R. Biochem. Biophys. Res. Comm., 40, 517 (1970).
2. Waltner, K. and Waltner, K. Klin. Wochschr., 8, 313 (1929).
3. Voyce, M. A. Br. J. Haemat., 9, 412 (1963).
4. Baron, J. and Tephly, T. R. Arch. Biochem. Biophys., 139, 410 (1970).
5. Stitzel, R. E., Tephly, T. R. and Mannering, G. J. Mol. Pharmacol., 4, 502 (1968).
6. Baron, J. and Tephly, T. R. Mol. Pharmacol., 5, 10 (1969).
7. Gigon, P. L., Gram, T. E. and Gillette, J. R. Mol. Pharmacol., 5, 109 (1969).
8. Greim, H., Schenkman, J. B., Klotzbucher, M. and Remmer, H. Biochim. Biophys. Acta, 201, 20 (1970).