

Acute Effect of Amitriptyline, Phenobarbital or Cobaltous Chloride on δ -Aminolevulinic Acid Synthetase, Heme Oxygenase and Microsomal Heme Content and Drug Metabolism in Rat Liver

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Accepted March 22, 1989

Abstract—Cobaltous chloride (CoCl_2) caused very marked decreases of cytochrome P-450, b_5 and total heme contents and an increase of heme oxygenase activity. On the contrary, phenobarbital (PB) increased hepatic drug-metabolizing enzymes, but the total heme content remained unchanged. On the other hand, amitriptyline (AMT) caused a marked increase of δ -aminolevulinic acid (δ -ALA) synthetase activity at 12 and 24 hr. In addition, the contents of total heme and cytochrome b_5 and the activities of aminopyrine (AM) N-demethylase and aniline (AN) hydroxylase at 24 hr were also increased by AMT, whereas cytochrome P-450 content did not change. This may be explained by the fact that AMT would increase hepatic heme synthesis through the prolonged induction of δ -ALA synthetase, but it may not cause an increase in cytochrome P-450 heme because there are increases in the contents of cytochrome b_5 and total heme.

We previously reported that repeated administration of AMT (15 mg/kg) caused a significant increase of hepatic microsomal AM N-demethylase, AN hydroxylase, NADPH cytochrome *c* reductase and UDP-glucuronyltransferase activities in rats (1, 2).

Maines and Kappas (3, 4) reported that the administration of CoCl_2 to rats caused a decrease in the microsomal content of heme with a significant decrease in cytochrome P-450 content and an increase in the activity of δ -ALA synthetase.

Matsuura et al. (5) and Shedlofsky et al. (6) have recently demonstrated that many chemicals which induce hepatic drug-metabolizing enzymes also induce δ -ALA synthetase, and this activity exhibits a reciprocal relationship to that of heme oxygenase (4). Such a marked increase of drug-metabolizing enzyme activity by AMT is thought to change hepatic heme level and related enzymatic activity. In this study, therefore, we have compared the effects of CoCl_2 , PB or AMT on hepatic heme and drug metabolism.

In this connection, we have determined the activities of δ -ALA synthetase and heme ox-

ygenase, and have made an attempt to clarify the relationship between the contents of microsomal cytochrome P-450, b_5 and total heme and the enzyme activities in the heme metabolic pathway.

Materials and Methods

Animals: Jcl:Sprague Dawley strain male rats (4–5 weeks old) were purchased from Clea Japan, Inc., Tokyo. The animal room was maintained at $23 \pm 1^\circ\text{C}$, with $50 \pm 5\%$ relative humidity and on a 12 hr light-dark cycle (lights on 06:00 to 18:00). They were freely given water and commercial laboratory chow (MF; Oriental Yeast Co., Japan) for at least one week before use.

Treatment of animals: Rats were orally given AMT at a single dose of 600 mg/kg. The control animals received an equivalent volume of saline. PB (intraperitoneally) and CoCl_2 (subcutaneously) were injected at a single dose of 80 and 60 mg/kg, respectively. Within 12 hr, the hepatic drug-metabolizing enzyme activity and heme level by AMT did not change. Therefore, animals were killed either 12 or 24 hr after acute administration of the

drug; and the hepatic homogenate and the microsomal and cytosolic fractions were prepared as reported previously (2).

Chemicals: Hemin was obtained from Wako Pure Chemicals Co., Ltd., Tokyo, Japan. δ -ALA hydrochloride, AMT and cytochrome c were purchased from Sigma Chemical Company, St. Louis, U.S.A. All other chemicals used were of reagent grades available commercially.

Enzyme assay: Heme oxygenase activity was assayed by the method of Maines and Kappas (4) by determining the formation of bilirubin in the presence of cytosolic biliverdin reductase with the addition of an NADPH generating system. δ -ALA synthetase activity was assayed by the method of Marver et al. (7) using a total liver homogenate as the enzyme source. The ALA produced was estimated colorimetrically after condensation with acetylacetone and isolation of the pyrrole compound formed on a Dowex-1-acetate column. Total microsomal contents of heme were determined by the pyridine hemochromogen method of Paul et al. (8). Cytochrome P-450 and b_5 contents in the hepatic microsomal suspension were measured by the method of Omura and Sato (9) and Takesue and Omura (10), respectively. AM N-demethylase and AN hydroxylase activities were determined by the methods of Nash (11) and Imai et al. (12), respectively. Protein was determined using the method of Lowry et al. (13).

Results

Effects of AMT, PB or CoCl_2 on the activities of hepatic δ -ALA synthetase and heme oxygenase: Table 1 shows the activities of δ -ALA synthetase and heme oxygenase either 12 or 24 hr after the administration of AMT, PB or CoCl_2 . CoCl_2 produced a marked increase of heme oxygenase activity, by 8.8 and 23.9 times, respectively, when measured 12 and 24 hr after the administration. Moreover, at 12 and 24 hr, the δ -ALA synthetase activity was increased 1.3- and 3.3-fold by CoCl_2 . On the other hand, PB caused a significant increase of hepatic δ -ALA synthetase (213%) and heme oxygenase (202%) activities when measured 12 hr after the administration, but by 24 hr, the enhancement of both enzymatic activities by PB was suppressed or returned to control levels. Similarly, a single administration of AMT produced a steady sustained increase in δ -ALA synthetase activity, which reached levels approximately 2.2- and 2.3-fold higher than the control levels at 12 and 24 hr. However, the activity of heme oxygenase by AMT remained unchanged.

Effects of AMT, PB or CoCl_2 on hepatic microsomal contents of protein, cytochromes and drug-metabolizing enzyme activities: As shown in Table 2, a single administration of CoCl_2 resulted in a marked decrease of the contents of cytochrome P-450, b_5 , microsomal protein and total heme (but not by 12 hr).

Table 1. Effects of a single administration of AMT, PB or CoCl_2 on the activities of hepatic δ -ALA synthetase and heme oxygenase in rats

Treatment	N	Time (hr)	δ -ALA synthetase (nmol/g liver/hr)	Heme oxygenase (nmol/mg protein/hr)
Control	(8)	12	54.90 \pm 2.67	2.31 \pm 0.42
AMT	(6)		120.20 \pm 21.16**	2.87 \pm 0.32
PB	(6)		116.87 \pm 10.70***	4.66 \pm 0.50**
CoCl_2	(6)		71.70 \pm 5.84*	20.36 \pm 1.04***
Control	(7)	24	58.10 \pm 5.85	2.34 \pm 0.23
AMT	(5)		135.90 \pm 9.73***	2.83 \pm 0.45
PB	(5)		107.06 \pm 12.04**	1.77 \pm 0.29
CoCl_2	(5)		190.04 \pm 20.31***	55.98 \pm 18.69*

Doses of AMT, PB and CoCl_2 were 600, 80 and 60 mg/kg, respectively. Animals were killed 12 and 24 hr after drug administration. Each value represents the mean \pm S.E.M. Numbers in parenthesis are the numbers of animals used. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control.

Table 2. Effects of a single administration of AMT, PB or CoCl₂ on the contents of microsomal cytochrome P-450, *b*₅, total heme and protein in rat liver

Treatment	N	Time (hr)	Cytochrome P-450 (nmol/mg protein)	Cytochrome <i>b</i> ₅ (nmol/mg protein)	Microsomal heme (nmol/mg protein)	Microsomal protein (mg/g liver)
Control	(9)		0.75±0.03	0.30±0.02	1.08±0.06	16.74±0.59
AMT	(6)	12	0.74±0.03	0.36±0.02	1.21±0.04	14.21±1.29
PB	(6)		1.10±0.06***	0.41±0.03**	1.17±0.04	15.42±0.79
CoCl ₂	(6)		0.50±0.04***	0.11±0.02***	0.93±0.08	10.70±1.18***
Control	(8)		0.84±0.05	0.30±0.02	1.34±0.05	17.60±0.99
AMT	(6)	24	0.99±0.06	0.38±0.01**	1.64±0.01**	15.12±1.05
PB	(6)		1.16±0.05***	0.37±0.04	1.45±0.11	18.23±1.50
CoCl ₂	(5)		0.22±0.04***	0.05±0.02***	0.81±0.10***	7.84±0.89***

Doses of AMT, PB and CoCl₂ were 600, 80 and 60 mg/kg, respectively. Animals were killed 12 and 24 hr after drug administration. Each value represents the mean±S.E.M. Numbers in parenthesis are the numbers of animals used. **P<0.01, ***P<0.001 vs. control.

Table 3. Effects of a single administration of AMT, PB or CoCl₂ on hepatic microsomal drug metabolizing enzyme activities in rats

Treatment	N	Time (hr)	Aminopyrine N-demethylase (nmol/mg protein/20 min)	Aniline hydroxylase (nmol/mg protein/20 min)
Control	(9)		88.07± 5.39	13.98±0.66
AMT	(6)	12	84.37± 5.91	15.53±0.92
PB	(6)		116.55± 3.66**	20.68±1.73**
CoCl ₂	(5)		55.96± 4.01**	7.87±0.47***
Control	(8)		79.58± 0.44	13.37±0.34
AMT	(6)	24	102.93± 5.86**	19.78±1.68**
PB	(6)		144.71±12.11***	21.44±1.68***
CoCl ₂	(5)		37.49± 1.09***	5.61±1.12***

Doses of AMT, PB and CoCl₂ were 600, 80 and 60 mg/kg, respectively. Animals were killed 12 and 24 hr after drug administration. Each value represents the mean±S.E.M. Numbers in parenthesis are the numbers of animals used. **P<0.01, ***P<0.001 vs. control.

Moreover, a significant decrease of the activities of AM N-demethylase and AN hydroxylase was also observed by CoCl₂ (Table 3). On the contrary, the treatment of rats with PB alone caused a marked increase of the contents of cytochrome P-450 and *b*₅ (but not by 24 hr) and of the activities of AM N-demethylase and AN hydroxylase, but microsomal heme content remained unchanged. Similarly, AMT alone led to a significant increase of the contents of cytochrome *b*₅ (127%) and heme (122%) and of the activities of AM N-demethylase (129%) and AN hydroxylase (148%) when measured at 24 hr after the administration, although AMT had no effect on

these activities at 12 hr.

Discussion

The present investigation has revealed that hepatic drug-metabolizing enzyme and δ -ALA synthetase activities and total heme content at 24 hr were significantly increased by AMT.

In our experiments, heme oxygenase activity by CoCl₂ at 12 and 24 hr remarkably increased, while an increase in this enzyme resulted in a marked decrease of cytochrome P-450, *b*₅ and total heme contents (but not by 12 hr) (Tables 1 and 2). However, at 12 and 24 hr in the CoCl₂ treated rats, the

enhancement of δ -ALA synthetase, a rate limiting enzyme in heme synthesis, was observed to increase to the same degree as in the PB- or AMT-treated rats. These results suggest that the induction of heme oxygenase would be correlated to the decrease of cytochrome P-450, b_5 and total heme contents, as described by others (3, 5, 14, 15).

In general, it is known that after repeated PB administration, centrilobular hepatocytes led to hypertrophy due to proliferation of smooth endoplasmic reticulum (16, 17). In this study, however, a significant increase of microsomal protein and liver weight (data not shown) by PB or AMT was not observed within 24 hr, as suggested by Orrenius et al. (18). However, during the 12 to 24 hr-period, PB resulted from the increase of drug-metabolizing enzymes (Tables 2 and 3). Moreover, at 12 hr after PB, a marked induction of δ -ALA synthetase and heme oxygenase was observed, but by 24 hr, the enhancement of these activities was suppressed; The total heme content remained unchanged. On the other hand, AMT caused a significant increase of δ -ALA synthetase activity at 12 hr, and the increment of this enzyme was sustained markedly for at least 24 hr, although heme oxygenase activity was not affected (Table 1). In addition, the contents of total heme and cytochrome b_5 and the activities of AM N-demethylase and AN hydroxylase at 24 hr were also increased by AMT, whereas during this period, the content of cytochrome P-450 did not change.

Bissell and Hammaker (19) have shown that PB might stimulate primarily the synthesis of apocytochrome P-450, which draws heme from the regulatory heme pool, causing secondary derepression of the heme synthetic pathway. Accordingly, the inhibition of PB-induced increase of hepatic δ -ALA synthetase activity may be due to a negative feedback control by heme (5, 20–22). Moreover, our results may indicate that at 12 hr after PB, the induction of heme oxygenase is associated with major depletions of total heme contents. In this connection, Bock and Siekevitz (23) reported that heme exchange was taking place between cytochrome b_5 and a heme pool. Therefore, it may be explained by the fact that the enhancement of cytochrome b_5

and total heme contents by AMT fails to increase the amount of cytochrome P-450 in the liver.

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