THE ROLE OF HEME SYNTHESIS DURING THE INDUCTION OF HEPATIC MICROSOMAL
CYTOCHROME P-450 AND DRUG METABOLISM PRODUCED BY BENZPYRENE

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Summary

δ-Aminolevulinic acid synthetase, the initial and rate limiting step in
hepatic heme synthesis, is induced by both benzpyrene and phenobarbital.  In-
duction of this enzyme by benzpyrene results in the stimulation of glycine-2-
$^{14}C$ incorporation into hepatic microsomal heme in vivo and in the induction of
cytochrome P-450 and N-demethylase activity.  3-Amino-1,2,4-triazole, an in-
hibitor of the second step in hepatic heme synthesis, prevents the stimulation
of hepatic heme synthesis and the induction of P-450 and N-demethylase activity.
It is suggested that induction of δ-aminolevulinic acid synthetase leading to
increases in hepatic heme synthesis may be the mechanism by which benzpyrene
induces cytochrome P-450 and certain hepatic microsomal oxidations.

Pretreatment of animals with 3,4-benzpyrene (BP) and other polycyclic

aromatic hydrocarbons such as 3-methylcholanthrene results in increased hepatic

microsomal levels of cytochrome P-450 and in the induction of the hepatic

microsomal oxidations of certain drugs and carcinogens (1-5).  Although the

exact mechanism by which induction occurs remains to be elucidated, stimulation

of both heme synthesis and protein synthesis are undoubtedly essential to the

induction process since, at least during the initial stages of induction, there

appears to be a good correlation between increases in P-450 and certain micro-

somal oxidations (10).

Hepatic mitochondrial δ-aminolevulinic acid synthetase (ALA synthetase)

is the initial and rate limiting step in hepatic heme synthesis (6,7).  Pheno-

barbital (PB) has been demonstrated to induce ALA synthetase in chick embryo

liver cells in culture and in the livers of experimental animals (6,8,9).  Pre-

vious studies in this laboratory (10) have suggested that PB might exert its

stimulatory effect on drug metabolism by increasing the rate of hepatic heme

synthesis which directs the increased synthesis of cytochrome P-450.  Since the

administration of BP also results in increases in the level of cytochrome P-450

in hepatic microsomes, stimulation of hepatic heme synthesis via induction of

ALA synthetase is also a possible mechanism for the induction of the hepatic

microsomal drug-metabolizing system by BP.

The herbicide 3-amino-1,2,4-triazole (AT), an inhibitor of the second

step in hepatic heme synthesis, has been employed to inhibit hepatic heme syn-

thesis and to prevent PB from stimulating hepatic heme synthesis (10). AT has

also been demonstrated to inhibit the induction of certain hepatic microsomal

oxidations produced by either PB (10,11,12) or by methylcholanthrene (11).

The present report describes the stimulatory effect of BP on rat hepatic

ALA synthetase, hepatic heme synthesis, cytochrome P-450 synthesis and on the

N-demethylation of 3-methyl-4-monomethylaminoazobenzene (3-$CH_3$-MAB). The

inhibitory effect of AT on these inductions, and the effects of both BP and AT

on the microsomal levels of cytochrome $b_5$ and NADPH-cytochrome c reductase

activity are also described.

## Methods

Male Holtzman rats weighing 130 to 170 g were used throughout these studies.

Drugs were administered intraperitoneally in the following doses: BP, 20 mg/kg

in corn oil; AT, 3 g/kg in saline; and PB, 40 mg/kg in saline. Control animals

received an equal volume of saline and/or corn oil. Rats were fasted for 24 hr

prior to sacrifice but were given water ad libitum. In all experiments, the

livers from two rats which received identical treatments were pooled upon

homogenization. ALA synthetase activity was determined in hepatic homogenates

by a modification of the method of Marver et al. (13). Methods for the

preparation of microsomes and measurements of the N-demethylation of 3-$CH_3$-MAB,

the activity of NADPH-cytochrome c reductase, the content of cytochromes P-450

and $b_5$ and the incorporation of isotopic precursor into hepatic microsomal heme

have been described previously (10).

## Results

BP administration results in the induction of microsomal cytochrome

Table 1.    Effects of benzpyrene (BP) and aminotriazole (AT) on the incorporation
of glycine-2-^{14}C into hepatic microsomal heme _in vivo_. [1]

| Pretreatment | Percent of control [2] |
|---|---|
| BP | 131.8 $\pm$ 1.7 [3] |
| AT | 86.1 $\pm$ 2.8 [3] |
| BP + AT | 109.0 $\pm$ 2.1 |

[1]Rats received intraperitoneally a 45-min.
pulse-dose of 30 µC of glycine-2-[14]C 16 hr.
after the administration of drugs.  The
control value was 196.9 cpm/mµmole of
protoheme.

[2]Values represent the mean $\pm$ S.E.M. of at
least three experiments.

[3]$p < 0.05$.

P-450 and the induction of certain microsomal oxidations (1,4,5).  If this

phenomenon is related to an increase in the synthesis of cytochrome P-450, a

stimulation of hepatic microsomal heme synthesis should be observed after the

administration of BP to rats.  Table 1 shows that there is a 32% stimulation

of the _in vivo_ incorporation of glycine-2-^{14}C into hepatic microsomal heme 16

hr after BP administration.  This stimulation is prevented by AT, an inhibitor

of ALA dehydratase (10,14), the second step in hepatic heme synthesis.  AT has

previously been observed to inhibit the PB-induced increase in hepatic heme

synthesis (10).

    The mechanism for the induction of heme synthesis and cytochrome P-450

may be related to an increase in ALA synthetase activity.  Table 2 shows that

both BP and PB produce a marked induction of hepatic ALA synthetase activity

16 hr after administration to rats.  The induction is not affected by AT, nor

does AT alone alter the level of activity of this enzyme.  This indicates that

both BP and PB induce cytochrome P-450 by stimulating hepatic microsomal heme

synthesis via induction of ALA synthetase.  PB has also been shown to stimulate

Table 2.  Effects of benzpyrene (BP), phenobarbital (PB), and aminotriazole (AT) pretreatments on hepatic ALA synthetase activity. [1]

| Pretreatment | mμmoles ALA formed/ g of liver/hr | Percent of control |
|---|---|---|
| Control | $15.3 \pm 0.4$ [2] | |
| AT | $16.0 \pm 0.5$ | $104.9 \pm 1.7$ |
| BP | $79.8 \pm 9.7$ | $522.4 \pm 15.8$ [3] |
| BP + AT | $74.3 \pm 12.1$ | $519.2 \pm 23.6$ [3] |
| PB | $225.2 \pm 28.7$ | $1449.0 \pm 87.1$ [3] |
| PB + AT | $193.8 \pm 15.8$ | $1281.5 \pm 52.6$ [3] |

[1]Drugs were administered 16 hr. prior to sacrifice.

[2]Values represent the mean $\pm$ S.E.M. of at least three experiments.

[3]$p < 0.05$.

synthesis of microsomal heme (10) and AT inhibits this stimulation.  Treatment of rats with actinomycin D (3 mg/kg) at the same time as either BP or PB completely prevented the induction of ALA synthetase.

Table 3 shows that BP induces cytochrome P-450 and the N-demethylation of $3-CH_3-MAB$.  That these effects are due to an enhancement of hepatic microsomal heme synthesis is indicated from the observation that AT significantly antagonizes the increases of both P-450 content and $3-CH_3-MAB$ N-demethylase activity.  The effects of AT do not result from either interference with the assay or from a direct inhibition (10).  However AT begins to lose its effect after the first 24 hr.  This is likely due to increased levels of endogenous ALA which would reverse the inhibition.

Although BP pretreatment results in an enhancement of hepatic heme synthesis and an increase in cytochrome P-450 levels, there was no observable effect by either BP or AT on the hepatic microsomal level of cytochrome $b_5$ during the first 48 hr of treatment (Table 3).  This is similar to the effect

Table 3.  Effects of benzpyrene (BP) and aminotriazole (AT) pretreatments on microsomal hemoproteins, $3-CH_3$-MAB N-demethylase and NADPH-cytochrome c reductase.[1]

| Measurement | Duration of Treatment | Percent of control[2] | | |
|---|---|---|---|---|
| | | BP | AT | BP + AT |
| P-450[3] | 24 | 164 + 13* | 70 + 5* | 114 + 8 |
| | 48 | 229 + 19* | 78 + 3* | 162 + 15* |
| Cytochrome $b_5$[4] | 24 | 123 + 23 | 101 + 15 | 126 + 19 |
| | 48 | 127 + 22 | 117 + 31 | 124 + 16 |
| $3-CH_3$-MAB N-demethylase[5] | 24 | 151 + 17* | 76 + 5* | 115 + 18 |
| | 48 | 212 + 21* | 76 + 10* | 153 + 12* |
| NADPH-cytochrome c reductase[6] | 24 | 113 + 10 | 116 + 10 | 119 + 9 |
| | 48 | 121 + 13 | 113 + 13 | 138 + 17 |

[1] Drugs were administered every 24 hr.

[2] Values represent the mean + S.E.M. of at least three experiments.

[3] Control value was 0.023 $\Delta$OD 450-490 m$\mu$/mg protein.

[4] Control value was 0.096 m$\mu$moles cytochrome $b_5$/mg protein.

[5] Control value was 0.69 m$\mu$moles HCHO formed/mg protein/min.

[6] Control value was 42 m$\mu$moles cytochrome c reduced/mg protein/min.

* $p < 0.05$.

of PB (10,15).  Unlike PB, BP does not induce NADPH-cytochrome c reductase activity (Table 3), the microsomal flavoprotein which may be responsible for the reduction of cytochrome P-450.  This may indicate that in those oxidations induced by BP, NADPH-cytochrome c reductase is not a rate limiting component. On the other hand, NADPH-cytochrome c reductase activity may not be representative of NADPH-cytochrome P-450 reductase activity (16).

Discussion

        Within 24 hr after the administration of BP to rats there is an increase in the level of microsomal cytochrome P-450 and an induction of certain hepatic microsomal oxidations (1,4,5).  The proximal mechanism by which BP and related compounds produce this effect remains to be elucidated.  However, the present

studies suggest that, like PB (10), BP produces a stimulation of hepatic microsomal heme synthesis through the enhancement of ALA synthetase activity. This leads to an increased synthesis of cytochrome P-450 and the increase in certain microsomal oxidations.  However, the species of cytochrome P-450 induced by BP and related compounds may be different from that induced by PB (2,3, 17) which may reflect the fact that certain protein moieties confer specificity to P-450.  BP and PB may also influence the synthesis of these apocytochromes.

PB has been demonstrated to induce the rate controlling step in hepatic heme synthesis, ALA synthetase (6,8,9), and has also been demonstrated to stimulate hepatic microsomal heme synthesis (9,10).  The present studies have demonstrated that BP similarly produces an induction of ALA synthetase and a stimulation of hepatic microsomal heme synthesis.  The latter event is abolished by AT, although AT does not prevent the induction of ALA synthetase.  Thus, it appears that both PB and BP stimulate hepatic heme synthesis through the induction of ALA synthetase.

Further support for the postulate that induction of the drug-metabolizing system is dependent upon heme synthesis is seen from the data on the induction of cytochrome P-450 and $3-CH_3-MAB$ N-demethylation by BP.  When AT is adminis-tered together with BP, the stimulatory effects of BP are significantly reduced or prevented.  Since AT exerts its effect on heme synthesis exclusive of an effect on protein synthesis and since any direct inhibitory effect of AT on microsomal drug metabolism is minimal (10), these results suggest that the stimulation of hepatic heme synthesis may be the mechanism by which BP induces the hepatic microsomal drug-metabolizing system.

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