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HEPATIC CYTOCHROME P-450 IN CHRONICALLY HYPOXEMIC RATS

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SUMMARY

The basal level of hepatic cytochrome P-450 and its inducibility by phenobarbital pretreatment have been found to be enhanced by chronic hypoxemia. Pentobarbital sleeping times were decreased in parallel to changes in levels of cytochrome P-450. The increase in level of hepatic cytochrome P-450 in chronically hypoxemic rats occurred despite the increased levels of hepatic heme oxygenase which previously were associated with the chronic hemoglobinemia of the hypoxemic state. Chronically hypoxemic rats may provide a useful model for study of control of hepatic heme and hemoprotein metabolism.

INTRODUCTION

Levels of hepatic cytochrome P-450 can be induced by numerous drugs and chemicals, a prototype of which is phenobarbital (1,2). Administration of other agents leads to decreases in the levels of hepatic cytochrome P-450 (3). Acute administration of hematin or hemoglobin are among the treatments that have been found to decrease P-450 in normal rats (4,5). These treatments also increase activity of hepatic heme oxygenase (5), and it has been proposed that decreases in cytochrome P-450 are due to increases in heme oxygenase (6).

Rats chronically exposed to hypoxic conditions (simulating 18,000 ft above sea level) develop hemoglobinemia (free plasma Hgb = 60 to 90 mg/dl) and a 5-fold increase in activity of hepatic heme oxygenase (7). Despite this increase in heme oxygenase, the rate of bilirubin production from hepatic heme is not detectably increased (8). Since the heme of cytochrome P-450 makes up the major fraction of hepatic heme turnover in rats (9), the previous results in chronically hypoxemic rats suggested that induction of hepatic heme

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oxygenase is not necessarily associated with increased rates of turnover of the heme of cytochrome P-450. In the present study, we show that, in chronically hypoxemic rats, the basal levels and inducibility of hepatic cytochrome P-450 are greater than those in normoxemic controls.

MATERIALS AND METHODS

36 male Sprague-Dawley rats, housed at Hanover, NH (altitude = 500 ft), weighing 280-300 g were divided into two groups. One group was assigned as the non-treatment group, and the other as phenobarbital treatment group. Each group was further subdivided into 3 subgroups: one served as sea level control, the other two subgroups were exposed to reduced atmospheric pressures simulating altitudes of 12,000 and 18,000 ft, respectively, for 30 days (7).

The groups designed for the study of cytochrome P-450 induction received phenobarbital injection i.p. daily on days 26-29. Doses were 60 mg/kg body wt on day 26 and 80 mg/kg on days 27-29. For injections rats were briefly removed from the special chambers used to simulate high altitude.

On the 30th day, the animals were sacrificed by exsanguination through the vena cava under ether anesthesia, and the liver was perfused through the portal vein with 50 ml cold saline. A piece of tissue (1 g) from the left lobe was excised and homogenized for 1 min. with 3 volumes of a 0.1 M sodium phosphate - 20% glycerol - 0.1 mM menadione buffer (pH. 7.4). The homogenate was frozen in acetone-dry ice mixture (-70°C) and stored at (-80°C) until analyzed within 7 days. Cytochrome P-450 in liver homogenate was determined by a modification (J.F. Sinclair, et al., submitted) of the method of Schoene et al. (10). Sleeping times were determined at sea level pressure by the method of Rupe et al. (11). Proteins (12) and hemoglobin (Hgb) in the whole blood (13) and plasma (14) were determined spectrophotometrically.

RESULTS

Table 1 summarizes the liver weights, liver protein concentration and plasma hemoglobin of the various groups of animals. In rats not given phenobarbital, hypoxemia had no effect upon the liver weight and liver protein content. For the sea level and 12,000 ft groups, there was no detectable plasma hemoglobin; but in the 18,000 ft group, a moderate degree of hemoglobinemia (65 mg/dl) developed as previously reported (7,8). Phenobarbital treatment for 4 days resulted in increases of liver weight and liver protein per 100 g body weight (absolute liver weights also increased significantly after phenobarbital treatment). It is interesting to note that liver protein content per 100 g body weight in chronically hypoxemic rats was higher than in the sea level controls; the difference between controls and the rats maintained at 18,000 ft is statistically significant. This finding is consistent with the

Table 1. Liver weights, liver proteins and plasma hemoglobin.

	LIVER WEIGHT (mg/100 g body wt)	LIVER PROTEIN (mg/100 g body wt)	Plasma [Hgb] mg/dl
SEA LEVEL			
Control	3.89 ± 0.23	0.69 ± 0.03	0
PB**	4.86 ± 0.40	0.88 ± 0.04	---
P	0.01	0.01	
12,000 ft			
Control	4.18 ± 0.35	0.75 ± 0.12	0.5 ± 0.8
PB	4.92 ± 0.33	0.94 ± 0.04	---
P	0.01	0.02	
18,000 ft			
Control	3.75 ± 0.29	0.67 ± 0.02	63.7 ± 24.6
PB	4.99 ± 0.28	0.95 ± 0.07*	---
P	0.01	0.01	

* $P < 0.05$ when compared to sea-level phenobarbital-treated group. Each group has 5 animals except 18,000 ft phenobarbital group which has 8 animals. Values = means ± 1 S.D.

** Phenobarbital pre-treatment.

greater increase of hepatic cytochrome P-450 produced by phenobarbital in this hypoxemic group as will be noted subsequently. No attempt was made to determine the plasma hemoglobin in the phenobarbital-treatment groups. However, the intensity of the pink color of the plasma from these rats was similar to those of rats not given phenobarbital.

In rats not given phenobarbital, hepatic cytochrome P-450 contents per g of wet tissue, per mg of protein or per 100 g body weight were significantly higher (30%) in the 18,000 ft group than the sea level controls (Figure 1-A). Phenobarbital increased cytochrome P-450 levels in normoxemic and in both hypoxemic groups (Figure 1-B). If the cytochrome P-450 level of the appropriate non-treatment group is taken as the "basal" cytochrome P-450 level, the increase of cytochrome P-450 by phenobarbital can be estimated. When the data are treated in this way, it can be noted that chronic hypoxia significantly potentiated the increase of cytochrome P-450 produced by phenobarbital in the 18,000 ft group (Figure 1-C).

Increased levels of cytochrome P-450 are associated with increased rates of metabolism of drugs by the microsomal mixed-function oxidase system and with shorter

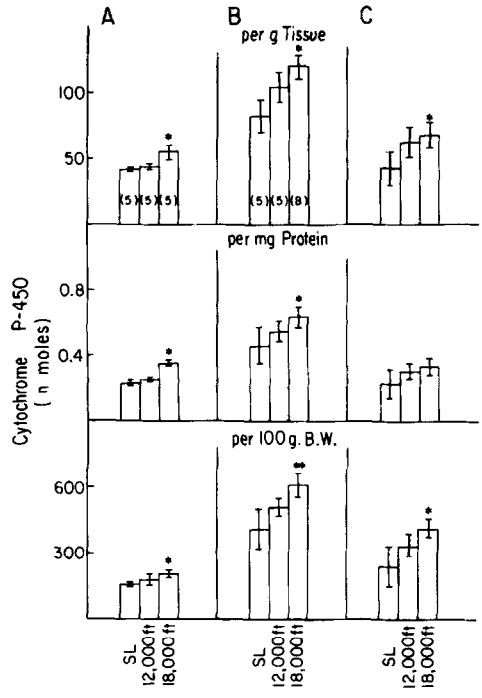

Figure 1. Effects of chronic hypoxia and phenobarbital treatment on levels of hepatic cytochrome P-450. A. Rats not given phenobarbitol; B. Rats treated with phenobarbital for 4 d prior to sacrifice; C. The increment in levels of cytochrome P-450 produced by phenobarbital treatment. C = B-A.
Each bar represents the mean ± S.D. Figures in parentheses represent the numbers of animals studied.

* < .05 for the comparison of 18,000 ft group with the sea level controls; ** P < .05 for the comparisons of 18,000 ft with either sea level controls or 12,000 ft group.
SL = sea level control.

duration of drug effects in the intact animal. Pentobarbital sleeping times were estimated in control and phenobarbital pre-treatment rats, maintained either at sea level or at a simulated altitude of 18,000 ft. As shown in table 2, rats maintained at 18,000 ft had shorter sleeping-times than those maintained at sea level pressure. The effect was particularly pronounced in those pretreated with phenobarbital. That the magnitude of the reduction in sleeping times does not appear to conform with that of the increase in cytochrome P-450 suggests that factors other than cytochrome P-450 may also contribute to the decrease in sleeping time.

Table 2. Potentiation by chronic hypoxemia of the effects of phenobarbital to shorten sleeping times.

Pre-treatment	Sea level	18,000 ft	P
Control	120, 140 (2)	103.3 ± 2.8 (6)	---
Phenobarbital	83.3 ± 35 (3)	11.0 ± 10.0 (5)	< 0.025
P	---	< 0.001	

Figures in parentheses are the numbers of animals studied. Various groups of animals were pretreated for 4 d with phenobarbital as described in Methods. Controls received an appropriate volume of isotonic saline. 18 h after the last injection of phenobarbital or saline, rats were given pentobarbital (50 mg/Kg IP) and sleeping times recorded.

DISCUSSION

This study demonstrates that the level of hepatic cytochrome P-450 and its inducibility by phenobarbital are increased in chroncially hypoxemic rats. Several possible explanations for this finding may be considered: In hypoxemic rats, (i) the rate of degradation of cytochrome P-450 could be decreased; (ii) its rate of synthesis could be increased; or (iii) the saturation of apocytochrome P-450 with heme could be increased.

Decreased rate of degradation of cytochrome P-450 in hypoxemic rats:

There are no direct measurements of cytochrome P-450 turnover in liver of hypoxemic rats. In splenectomized hypoxemic rats, the rate of total bilirubin excretion is not increased (8). Therefore, there is not evidence for an increase in the rate of hepatic heme turnover to bilirubin, despite the increased levels of hepatic heme oxygenase (7) and cytochrome P-450 (Figure 1-A). The increased concentration of cytochrome P-450 with no detectable increase in bilirubin production from hepatic heme suggests that the degradation rate of heme of cytochrome P-450 is decreased in hypoxemic rats.

Increased synthesis and increased heme saturation of cytochrome P-450:

Chronically hypoxemic rats have increased levels of plasma free hemoglobin (65 mg/dl, Table 1), the heme of which is taken up and metabolized chiefly by hepatocytes (15). One might speculate that the rate of synthesis of apocytochrome P-450 is thereby enhanced as part of a more general stimulatory

effect of heme on hepatic protein synthesis (16). Furthermore, more cellular heme may associate with apo-cytochrome P-450, increasing levels of the holoenzyme. Reconstitution of hepatic cytochrome P-450 has recently been demonstrated in vivo (17,18) and in vitro (19,20). However, such reconstitution of cytochrome P-450 by heme has thus far been demonstrable only after hepatic heme has been depleted, by inhibiting its synthesis and/or increasing its degradation. In normal rats, synthesis of cytochrome P-450 appears to be limited by the rate of formation of apo-protein rather than synthesis of heme (21). Thus, a stimulatory effect of chronic hypoxemia upon synthesis of apo-protein(s) of cytochrome P-450 is perhaps more probable than that chronic hypoxemia increases heme saturation of the cytochrome.

Previously it has been shown that chronically hypoxemic hemoglobinemic rats have 5-fold increases of hepatic heme oxygenase (7). These previous results and the present findings provide further evidence against the concept that increases in heme oxygenase obligatorily produce decreases in hepatic cytochrome P-450 (22). Similarly, in mice with chronic hereditary hemolytic anemia, induction of hepatic heme oxygenase was accompanied by normal or increased levels of cytochrome P-450 (23). In the mouse model, plasma hemoglobin has not been determined. Since large numbers of erythrocytes are broken down in the mice with hemolytic anemia, it is possible that heme oxygenase activity is increased both in hepatocytes and in Kupffer cells in these animals (S. Sassa, personal communication). In contrast, in the hypoxemic rats, the increase of heme oxygenase activity probably occurs primarily in hepatocytes rather than in Kupffer cells (15), since free plasma hemoglobin is increased (Table 1) with normal red cell survival (7).

In the present study, the increases in liver cytochrome P-450 during chronic hemoglobinemia are in contrast to the variable decreases previously described after acute administration of heme or hemoglobin (4,5). Chronically hypoxemic rats thus provide a new model for studying regulation of hepatic heme and hemoprotein metabolism. Findings using this model may

also have clinical implications relating to hypoxemic patients and to effects of hematin therapy in patients with porphyria (24,25,26).

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