Biochemical Pharmacology, Vol. 38, No. 21, pp. 3767–3771, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

MODULATION OF THE INDUCTION OF RAT HEPATIC CYTOCHROMES P-450 BY SELENIUM DEFICIENCY*

STEVEN A. WRIGHTON†‡ and BARBARA ELSWICK§

†Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226; and §Division of Clinical Toxicology and Environmental Medicine, Medical College of Virginia, Richmond, VA 23298, U.S.A.

(*Received* 22 *August* 1988; *accepted* 23 *March* 1989)

Abstract—The induction by phenobarbital of liver microsomal cytochrome P-450 has been demonstrated to be impaired in rats fed a selenium-deficient diet. Cytochrome P-450 isozyme specific immunologic and molecular techniques were used in the present study to better define the role of selenium in the induction of cytochrome P-450 by phenobarbital. Phenobarbital treatment of the selenium-deficient rats resulted in an increase in the level of total cytochrome P-450 50% of that observed with control rats and in a 10-fold increase in microsomal heme oxygenase. Quantitative immunoblot analyses demonstrated that the levels of cytochromes P-450b + e and P-450p in the phenobarbital-treated selenium-deficient rats were approximately 50% of those found in the phenobarbital-treated control rats. Finally, RNA hybridization studies using cDNA probes to cytochromes P-450b + e or P-450p demonstrated that the accumulations of the RNAs encoding these cytochromes P-450 were unaffected by the selenium status of the rats. These studies suggest that the impaired phenobarbital induction of the cytochromes P-450 in the selenium-deficient rats is the result of an increase in the degradation of the cytochromes P-450 or a decrease in the translation of the mRNAs coding for them.

The cytochromes P-450 (P-450s)‖ are a superfamily of microsomal hemoproteins that are the terminal oxidases of the hepatic mixed-function oxidase system [1, 2]. An important property of many of the isozymes of the hepatic P-450s is that they are inducible [1–3]. For example, in rats treated with phenobarbital (PB) several different hepatic isozymes accumulate including P-450p, P-450k, P-450a and the major PB-inducible forms P-450b and P-450e [1–4]. The mechanism by which PB stimulates the expression of these isozymes is currently poorly understood.

The induction by PB of total CO-binding P-450 has been demonstrated to be impaired in rats fed a selenium-deficient diet for 4 months [5, 6]. In addition, the effect of selenium deficiency was shown to be specific for the PB-inducible isozymes since the induction of P-450 by another classic inducer, 3-methylcholanthrene, is unaffected by the selenium status of the rats [6]. Further investigations into the specific effect of selenium deficiency on the induction of P-450 by PB demonstrated that the synthesis of the P-450s induced by PB treatment in the selenium-deficient rats is not affected and suggested that the accumulation of the apo-P-450 is not affected [7, 8]. Instead, it appeared that selenium deficiency impairs the incorporation of the heme into apo-P-450 [9]. That is, PB treatment of control rats was shown to increase heme synthesis, apo-P-450 synthesis, and incorporation of heme into apo-P-450 resulting in no increase in heme catabolism [9]. However, in selenium-deficient rats, PB treatment results in an increase in heme synthesis and an increase in apo-P-450 synthesis without a concomitant increase in the incorporation of heme into the apo-P-450 which results in an accumulation of heme in the hepatocyte and thus an induction of microsomal heme oxygenase (MHO), the rate-limiting enzyme in the heme degradation pathway [9–12]. Finally, studies using immunochemical methods which specifically determined the rate of synthesis of the major PB-inducible P-450s indicated that the addition of selenium to the medium of PB-treated primary cultures of adult rat hepatocytes results in a significant increase in the *de novo* synthesis of the major PB-inducible forms [13]. Thus, the role of selenium in the induction of P-450 by PB is still in question.

In the following study, we used P-450 isozyme specific immunologic and molecular techniques to further define the role of selenium in the mechanism of induction by PB of the P-450s. Unlike previous studies which indicated that the accumulation of the PB-inducible P-450s is not impaired, the studies presented here indicate that the accumulation of the P-450s induced by PB was reduced significantly in selenium-deficient rats. However, the accumulation of the mRNAs encoding these proteins was unaffected by the selenium status of the rats. The data presented also demonstrate that the reduced accumulation of total CO-binding P-450 in the selenium-deficient rats was the result of decreased levels of the isozymes induced by PB and not the result of impaired heme incorporation into apo-P-450 as previously believed. Furthermore, the effect of selenium deficiency on PB induction was on both the

* This research was supported by Grants ES-03699 and GM-37498 to S. A. W. from the National Institutes of Health.

‡ Address all correspondence to: Dr Steven A. Wrighton, Eli Lilly & Company, Lilly Corporate Center, Indianapolis, IN 46285.

‖ Abbreviations: P-450s, cytochromes P-450; PB, phenobarbital; MHO, microsomal heme oxygenase; DEX, dexamethasone; TAO, triacetyloleandomycin; and BNF, β-naphthoflavone.

major and minor forms induced and was specific to the induction by PB since the induction of the P-450s by dexamethasone (DEX), triacetyloleandomycin (TAO), or β-naphthoflavone (BNF) was not affected by the selenium status of the rats.

MATERIALS AND METHODS

Materials. The selenium-deficient and control diets were prepared by Teklad (Madison, WI). Guanidine isothiocynate, PB, DEX and BNF were purchased from Sigma Chemical Co. (St Louis, MO). Sepharose 4B was purchased from Pharmacia (Piscataway, NJ). TAO was a gift from Pfizer, Inc. (New York, NY). All other chemicals were reagent grade or better.

Animals and treatments. Male rats (21 days old) were obtained from Flow Laboratories (Dublin, VA). The rats were allowed free access to tap water and fed *ad lib*. a semi-synthetic diet with Torula yeast and tocopherol stripped lard as the protein and fat sources respectively [6]. The control diet was supplemented with 0.2 ppm sodium selenite, and both diets contained 500 units/g vitamin E acetate and 3 g/kg DL-methionine. The rats were maintained on these diets for at least 4 months.

Treated rats were injected intraperitoneally with 80 mg/kg phenobarbital sodium in saline for 4 days, 200 mg/kg DEX sonicated in water, 200 mg/kg TAO in corn oil for 5 days or 80 mg/kg BNF in corn oil for 3 days. After an overnight fast, the rats were killed, their livers were perfused with phosphate-buffered saline, and microsomes were isolated by the method of van der Hoeven and Coon [14].

In agreement with others [6], selenium-dependent glutathione peroxidase levels in the cytosol fraction of the liver of the rats fed the selenium-deficient diet were found to be less than 5% of those determined for the rats fed the control diet. Glutathione peroxidase levels were determined by the method of Lawrence and Burk [15].

Analytical methods. Total CO-binding P-450 concentration was determined by the method of Omura and Sato [16]. Protein concentration of the microsomal samples was determined colorimetrically [17]. The ability of the various microsomal samples to form the TAO metabolite–cytochrome P-450 complex was determined as previously described by Wrighton *et al.* [18]. Microsomal heme oxygenase activity was determined as previously described [12].

Immunochemical procedures. Cytochrome P-450p was purified and a monoclonal antibody against P-450p was prepared as previously described [19]. Cytochrome P-450b was purified by the method of West *et al.* [20]. Antibodies to cytochrome P-450b were raised in goats as previously described [18] and rendered specific for the related isozymes P-450b and P-450e by immunoabsorption against microsomes isolated from BNF-treated male rats and then microsomes isolated from untreated male rats bound to CNBr activated Sepharose 4B [18].

Immunoblot analyses were performed as described in detail elsewhere [19] using peroxidase-conjugated rabbit anti-mouse IgG to detect the monoclonal antibody against P-450p and rabbit anti-goat IgG followed by goat peroxidase anti-peroxidase IgG to detect the goat antibody against P-450b + e.

RNA isolation and analysis. Total liver RNA was isolated by the guanidine isothiocyanate method [21]. Dot blot analyses were used to quantify the amount of RNA hybridizable to cDNA probes corresponding to either P-450p [22] or P-450b + e [23]. For each treatment group a range of RNA concentrations was used such that, upon determining the intensity of the dots on the autoradiograph by scanning densitometry, a linear relationship between intensity and RNA concentration was obtained. The slopes of these relationships for the untreated rats were compared to those of the treated rats to determine the fold-increase in the accumulation of hybridizable RNA.

RESULTS

The effects of xenobiotic treatment on male rats fed a selenium-deficient or control diet for 4 months from weaning on liver weight and several hepatic enzymes are shown in Table 1. Although a general trend towards greater liver weight in the selenium-deficient rats was noted, only the PB-treated selenium-deficient rats showed a statistically significant increase over matched controls (Table 1). The selenium status of the rats did not alter significantly the content of total CO-binding P-450 in microsomes isolated from untreated or DEX-, TAO- and BNF-treated rats (Table 1). However, PB treatment of the rats fed the control diet resulted in a significantly greater increase in P-450 content as compared to the increase observed after PB treatment of the selenium-deficient rats (Table 1). That is, treatment of control rats with PB resulted in a 84% increase in P-450 but in only a 30% increase in the selenium-deficient rats.

The activity of MHO, the rate-limiting enzyme in heme degradation, in the various microsomal samples is shown in Table 1. No statistically significant differences in the activity of MHO were observed between the selenium-deficient and control untreated and DEX-, TAO-, or BNF-treated rats. However, PB treatment of selenium-deficient rats resulted in a 10.4-fold increase in the activity of MHO as compared to untreated controls. These observations, along with the other data presented in Table 1, demonstrate that our selenium-deficient rats responded to xenobiotic treatment in a fashion similar to that observed by others [5–13].

Several isozymes of hepatic P-450 including the related forms P-450b and P-450e and the glucocorticoid-inducible P-450p are induced in rats treated with PB. To determine if the lack of induction by PB of total P-450 in the selenium-deficient rats resulted from a decrease in the expression of all or some of these isozymes, we determined the levels of these forms in the various microsomal samples by quantitative immunoblot analyses. The level of P-450b + e was found to be reduced significantly in the microsomes isolated from the PB-treated selenium-deficient rats (Table 2). Specifically, the level of P-450b + e in the selenium-deficient PB-treated rats was 52% of that detected in PB-treated control rats.

Table 1. Effect of xenobiotic treatment on liver weight and several hepatic enzymes in selenium-deficient and control rats

Treatment	Diet*	N	Liver weight (g/100 g body wt)	Cytochrome P-450 (nmol/mg protein)	Microsomal heme oxygenase (nmol bilirubin/ mg protein/10 min)
None	+	6	4.1 ± 0.9†	1.16 ± 0.24	0.12 ± 0.05
None	−	6	4.3 ± 0.3	1.27 ± 0.28	0.10 ± 0.05
PB	+	6	5.2 ± 0.8	2.13 ± 0.38	0.09 ± 0.05
PB	−	5	6.9 ± 0.6‡	1.65 ± 0.34‡	1.04 ± 0.35§
DEX	+	6	7.1 ± 0.9	1.68 ± 0.25	0.21 ± 0.22
DEX	−	6	7.0 ± 0.8	1.88 ± 0.48	0.25 ± 0.17
TAO	+	3	4.9 ± 0.5	2.99 ± 0.22	0.41 ± 0.07
TAO	−	3	5.6 ± 0.8	2.71 ± 0.19	0.69 ± 0.38
BNF	+	3	5.3 ± 0.5	1.67 ± 0.17	0.28 ± 0.19
BNF	−	3	5.8 ± 0.2	1.42 ± 0.19	0.17 ± 0.08

Liver microsomes and cytosol were isolated, as described in Materials and Methods, from male rats fed a selenium-deficient or control diet for at least 4 months. Total CO-binding cytochrome P-450 content and MHO activity were determined in the various samples, as described in Materials and Methods.

* Selenium supplemented (+) or deficient (−) diet.

† Values are means ± SD.

‡ Different from PB-treated control rats, $P < 0.025$.

§ Different from control rats, $P < 0.005$.

Table 2. Effect of selenium deficiency on the accumulation of cytochromes P-450b + e and P-450p

Treatment	Diet*	N	P-450b + e (nmol/mg protein)	P-450p (nmol/mg protein)	TAO–P-450p complex (nmol/mg protein)
None	+	6	BD†	0.04 ± 0.01‡	0.05 ± 0.02
None	−	6	BD	0.03 ± 0.01	0.03 ± 0.01
PB	+	6	1.49 ± 0.46	0.37 ± 0.12	0.30 ± 0.11
PB	−	5	0.77 ± 0.20§	0.16 ± 0.04§	0.11 ± 0.03§
DEX	+	6	BD	1.03 ± 0.29	0.85 ± 0.17
DEX	−	6	BD	1.25 ± 0.46	0.86 ± 0.21
TAO	+	3	BD	1.56 ± 0.26	2.08 ± 0.49
TAO	−	3	BD	1.66 ± 0.20	1.59 ± 0.17

Antibodies against P-450b + e and P-450p, prepared as described in Materials and Methods, were used in immunoblot analyses performed as previously described [19] on the various microsomal samples. The ability of the microsomal samples to form the TAO metabolite–P-450p complex was determined as described by Wrighton *et al.* [18].

* Selenium supplemented (+) or deficient (−) diet.

† Below limit of detection.

‡ Values are means ± SD.

§ Different from PB-treated control rats, $P < 0.005$.

The levels of P-450b + e were below the limit of detection in all of the other microsomal samples.

The data presented in Table 2 also demonstrate that the induction of P-450p by PB was impaired significantly in the selenium-deficient rats. Specifically, the increase in the accumulation of P-450p in the PB-treated selenium-deficient rats was only 43% of that determined for PB-treated control rats (Table 2). In addition, the impaired expression of P-450p was specific to PB treatment since the levels of P-450p found in selenium-deficient and control untreated and DEX-, TAO- or BNF-treated rats were not significantly different (Table 2). A stable complex has been shown to be formed between TAO and P-450p [18]. Therefore, we measured the abilities of the various microsomal samples to form the TAO–P-450p complex to determine if the P-450p quantitated by the immunoblot analysis was functional (Table 2). The ability of the microsomal samples to form the TAO–P-450p complex and their corresponding level of immunoreactive P-450p were found to correlate well ($r = 0.96$ for selenium-deficient, $r = 0.98$ for control and $r = 0.92$ for all groups) suggesting that selenium-deficiency does not alter the ratio of total (apo and holo) P-450p (immunoreactive) to holo-P-450p (enzymatically active) in these samples.

Next we determined the effect of selenium status on the levels of cytoplasmic RNA hybridizable to a P-450b + e cDNA probe. Both the selenium-deficient and control rats responded to PB treatment with a greater than 40-fold increase in the accumulation of RNA hybridizable to the P-450b + e cDNA probe (Table 3). It is interesting to note that DEX treatment of both control and selenium-deficient rats

Table 3. Effect of selenium deficiency on the accumulation of RNA hybridizable to cytochrome P-450b + e or P-450p cDNA probes

Treatment	Diet*	N	P-450b + e mRNA†	P-450p mRNA†
PB	+	6	45.2 ± 7.8	10.6 ± 3.5
PB	−	5	42.5 ± 18.6	9.1 ± 2.4
DEX	+	6	10.9 ± 3.2	46.5 ± 12.0
DEX	−	6	12.2 ± 5.2	35.6 ± 18.8

Total cytoplasmic RNA was isolated and blotted with P-450b + e and P-450p cDNA probes as described in Materials and Methods.

* Selenium supplemented (+) or deficient (−) diet.

† Values are fold over appropriate untreated control (means ± SD).

resulted in approximately a 10-fold increase in the accumulation of P-450b + e message (Table 3) without an increase in P-450b + e protein (Table 2). Simons *et al.* [24] previously observed a similar effect of DEX treatment on the accumulation of P-450b + e protein and message.

Table 3 also demonstrates that PB treatment of control and selenium-deficient rats resulted in, respectively, 10.6- and 9.1-fold increases in the accumulation of RNA hybridizable to a P-450p cDNA probe despite the reduced level of P-450p protein in the selenium-deficient PB-treated rats (Table 2). In addition, the levels of RNA hybridizable with the P-450p cDNA were similar in the DEX-treated control and selenium-deficient rats (Table 3). These data indicate that the decreased accumulation of P-450b + e and P-450p after PB treatment of the selenium-deficient rats does not appear to be related to the accumulation of RNAs encoding these proteins.

DISCUSSION

Several studies have indicated that selenium plays a permissive role in the induction of P-450 by PB (reviewed in Ref. 10). In summary, these studies indicate that PB treatment of selenium-deficient and control rats results in the induction of heme synthesis and apo-P-450 synthesis. In the control rats, the apo-P-450 and heme are properly assembled and thus there is no accumulation of excess heme in the hepatocyte. However, in the selenium-deficient rats these reports suggest that there is a defect in the incorporation of heme into the apo-P-450 (both heme and P-450s are accumulating at normal rates), resulting in increased levels of heme in the hepatocyte which results in the induction of MHO. The studies presented here confirm that the induction of the P-450s by PB was impaired significantly in rats fed a selenium-deficient diet for 4 months. In addition, our results also confirm that MHO was induced in only the PB-treated selenium-deficient rats. Thus, our selenium-deficient rats appeared to respond to PB treatment in a fashion similar to that reported previously [5–13].

However, in our studies, specific antibody techniques were used to determine the effect of selenium status on the accumulation of three isozymes of P-450 (P-450b, P-450e and P-450p) normally induced by PB treatment. Our studies clearly demonstrate for the first time, and in opposition to what has been previously suggested, that the accumulation of these P-450s in response to PB treatment was reduced by approximately 50% (Table 2) in the selenium-deficient versus the control rats. Since the induction of the P-450s by BNF, TAO or DEX treatment was not impaired in the selenium-deficient rats, it would appear that the mechanism by which PB induction of the P-450s occurs is uniquely affected by the selenium status of the animals.

Previous studies have also demonstrated that heme synthesis is similar in the selenium-deficient and control rats [11]. Thus, the PB induction of MHO in the selenium-deficient rats does not appear to be the result of a defect in the synthesis of heme. The activity of MHO has been demonstrated to be induced by the accumulation of heme in the cell [12]. Thus, our results suggest that in PB-treated selenium-deficient rats the induction of MHO may be the result of an increase in the cellular heme pool due to a decrease in the accumulation of the PB-inducible P-450s. The increase in cellular heme would appear to result from an increase in the rate of degradation of the P-450s since the synthesis of these proteins has been shown to be unaffected by the selenium status of the rats [7, 8]. In support of this is the observation that the accumulation of the mRNAs encoding the PB-inducible P-450s was not decreased in the PB-treated selenium-deficient rats. Therefore, the lack of accumulation of the PB-inducible P-450s would appear to be the result of either decreased translation of the mRNAs encoding these P-450s or increased degradation of the P-450s as a result of selenium-deficiency. The effects of selenium-deficiency on the turnover of the PB-inducible isozymes is the subject of future research.

Acknowledgements—The authors wish to thank Dr Milton Adesnik for supplying the cDNA clone for P-450b + e, Dr M. Almira Correia for reviewing this manuscript, Drs Phil Guzelian and Erin Schuetz for discussions concerning this work, and Tamatha Steward for secretarial assistance.

REFERENCES

1. Adesnik M and Atchison M, Genes for cytochrome P-450 and their regulation. *CRC Crit Rev Biochem* **19**: 247–305, 1985.
2. Nebert DW and Gonzalez FJ, P-450 Genes: structure, evolution and regulation. *Annu Rev Biochem* **56**: 945–993, 1987.
3. Whitlock JP Jr, The regulation of cytochrome P-450 gene expression. *Annu Rev Pharmacol Toxicol* **26**: 333–369, 1986.
4. Cresteil T, Beaune P, Celier C, Leroux JP and Guengerich FP, Cytochrome P-450 isoenzyme content and monooxygenase activities in rat liver: effect of ontogenesis and pretreatment by phenobarbital and 3-methylcholanthrene. *J Pharmacol Exp Ther* **236**: 269–276, 1985.
5. Burk RF, Mackinnon AM and Simon FR, Selenium and hepatic microsomal hemoproteins. *Biochem Biophys Res Commun* **56**: 431–436, 1974.
6. Burk RF and Masters BSS, Some effects of selenium deficiency on the hepatic microsomal cytochrome P-

450 system in the rat. *Arch Biochem Biophys* **170**: 124–131, 1975.
7. Mackinnon AM and Simon FR, Impaired hepatic heme synthesis in the phenobarbital-stimulated selenium-deficient rat. *Proc Soc Exp Biol Med* **152**: 568–572, 1976.
8. Burk RF and Correia MA, Accelerated hepatic haem catabolism in the selenium-deficient rat. *Biochem J* **168**: 105–111, 1977.
9. Correia MA and Burk RF, Defective utilization of haem in selenium-deficient rat liver. *Biochem J* **214**: 53–58, 1983.
10. Burk RF, Biological activity of selenium. *Annu Rev Nutr* **3**: 53–70, 1983.
11. Correia MA and Burk RF, Hepatic heme metabolism in selenium-deficient rats: effect of phenobarbital. *Arch Biochem Biophys* **177**: 642–644, 1976.
12. Correia MA and Burk RF, Rapid stimulation of hepatic microsomal heme oxygenase in selenium-deficient rats. *J Biol Chem* **253**: 6203–6210, 1978.
13. Newman S and Guzelian PS, Stimulation of *de novo* synthesis of cytochrome P-450 by phenobarbital in primary nonproliferating cultures of adult rat hepatocytes. *Proc Natl Acad Sci USA* **79**: 2922–2926, 1982.
14. van der Hoeven TA and Coon MJ, Preparation and properties of partially purified cytochrome P-450 and reduced nicotinamide adenine dinucleotide phosphate–cytochrome P-450 reductase from rabbit liver microsomes. *J Biol Chem* **249**: 6302–6310, 1974.
15. Lawrence RA and Burk RF, Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* **71**: 952–958, 1976.
16. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. *J Biol Chem* **239**: 2379–2385, 1964.
17. Schacterele GR and Pollack RL, A simplified method for the quantitative assay of small amounts of protein in biologic material. *Anal Biochem* **51**: 654–655, 1973.
18. Wrighton SA, Maurel P, Schuetz EG, Watkins PB, Whitley B and Guzelian PS, Identification of the cytochrome P-450 induced by macrolide antibiotics in rat liver as the glucocorticoid responsive cytochrome P-450p. *Biochemistry* **24**: 2171–2178, 1985.
19. Hostetler KA, Wrighton SA and Guzelian PS, Immunochemical evidence for multiple steroid-inducible hepatic cytochromes P-450 in the rat. *Biochem J* **245**: 27–33, 1987.
20. West SB, Huang M-T, Miwa GT and Lu AYH, A simple and rapid procedure for the purification of phenobarbital-inducible cytochrome P-450 from rat liver microsomes. *Arch Biochem Biophys* **193**: 42–50, 1979.
21. Chirgwin JM, Przybyla AE, MacDonald RJ and Rutter WI, Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**: 5294-5299, 1979.
22. Wrighton SA, Schuetz EG, Watkins PB, Maurel P, Barwick J, Bailey BS, Hartle HT, Young B and Guzelian PS, Demonstration in multiple species of inducible hepatic cytochromes P-450 and their mRNAs related to the glucocorticoid-inducible cytochrome P-450 of the rat. *Mol Pharmacol* **28**: 312–321, 1985.
23. Kumar A, Raphael C and Adesnik M, Cloned cytochrome P-450 cDNA. Nucleotide sequence and homology to multiple phenobarbital-induced mRNA species. *J Biol Chem* **258**: 11280–11284, 1983.
24. Simmons DL, McQuiddy P and Kasper CB, Induction of the hepatic mixed-function oxidase system by synthetic glucocorticoids. *J Biol Chem* **262**: 326–332, 1987.