Biochemical Pharmacology, Vol. 32, No. 11, pp. 1745–1751, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + .00

EFFECT OF HEXACHLOROBENZENE ON THE ACTIVITIES OF HEPATIC ALCOHOL METABOLIZING ENZYMES

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(*Received* 20 *September* 1982; *accepted* 13 *December* 1982)

Abstract—To study the effect of experimental hepatic porphyria on the activities of hepatic alcohol metabolizing enzymes, female rats received a chow diet containing 0.05% hexachlorobenzene (HCB). After long-term HCB treatment for 60 days hepatic porphyria developed as evidenced by increased hepatic δ-aminolevulinic acid synthase activity and enhanced urinary excretion of δ-aminolevulinic acid, porphobilinogen and total porphyrins. Concomitantly, the activities of the hepatic microsomal ethanol oxidizing system (MEOS) were strikingly augmented by 213% ($P < 0.05$) and 177% ($P < 0.01$) when expressed per g of liver wet weight or per 100 g of body weight, respectively, whereas hepatic alcohol dehydrogenase activities remained virtually unchanged. Moreover, hepatic catalase showed only a trend for a slightly lower enzymic activity under these experimental conditions. The present data therefore show that experimental hepatic porphyria is associated with alterations of hepatic MEOS activities, which in turn may be a factor for the manifestation of human hepatic porphyrias in the course of alcohol consumption.

The liver is the main organ of alcohol metabolism which is catalyzed by alcohol dehydrogenase (ADH) [1, 2] and the microsomal ethanol oxidizing system (MEOS) [1–6], whereas catalase appears to play no significant role [1]. Alcohol consumption leads to a variety of functional and biochemical changes [7–9] and plays an important role as precipitating factor of hepatic porphyrias [10]. The interactions between porphyria and alcohol are complex, and metabolic consequences of alcohol degradation such as changes of the redox state due to alterations of hepatic alcohol metabolizing enzyme activities rather than alcohol itself may be involved [10–12].

Hexachlorobenzene (HCB) induces a hepatic porphyria both in humans and experimental animals, and the HCB induced porphyria of the rat is the most comparable model for the porphyria cutanea tarda as one form of the hepatic porphyrias [13–19]. In the present investigation the HCB animal model was therefore used to study the effect of hepatic porphyria on the activities of alcohol metabolizing enzymes in the liver.

MATERIALS AND METHODS

Materials. The chemicals were obtained from the following sources: Hexachlorobenzene (HCB), potassium chloride, magnesium chloride, calcium chloride 2-hydrate cryst., 4-dimethyl-aminobenzaldehyde, perchloric acid (70%), sodium dithionate, trichloroacetic acid, tris(hydroxymethyl)aminomethane and saccharose from Merck Corp., Darmstadt, F. R. of Germany; NAD, NADPH (tetrasodium salt, 98%), NADP (disodium salt, 98%), isocitric dehydrogenase (grade II) and 7-ethoxycoumarin cryst. from Boehringer Corp., Mannheim; benzo (*a*)pyrene from Ferak Corp., Berlin; 7-hydroxycoumarin from EGA-Chemie Corp., Steinheim; DL-isocitric acid Na_2-salt and Na_2-EDTA from Serva Corp., Heidelberg.

Animals. Female Wistar rats were purchased from Zentral-Institut für Versuchstierzucht, Hannover (F. R. of Germany) and fed Altromin chow containing 0.05% hexachlorobenzene (HCB) ad libitum over a period of 10 and 60 days. The respective control groups received Altromin chow without HCB for the same period of time. All animals had free access to tap water. The urine was collected over a period of 24 hr preceding sacrifice. The rats were decapitated, and their livers *in situ* perfused with ice-cold 0.15 M KCl through the portal vein and excised.

Biochemical assays. Hepatic catalytic activity of catalase [20], and cytochrome P-450 content [21] were determined in a 20% liver homogenate prepared with 0.15 M KCl and the δ-aminolevulinic acid-synthase (ALA-S) in a 25% liver homogenate prepared with Tris-EDTA-buffer, pH 7.4 [22]. Alcohol dehydrogenase (ADH) activity was assessed in the cytosolic fraction of the hepatocytes [1, 4]. In the microsomal fraction the microsomal cytochrome P-450 content [23] and the activities of the microsomal ethanol oxidizing system (MEOS) [4], NADPH-cytochrome *c* reductase [24], 7-ethoxycoumarin deethylase (7-EOC-D) [25] and arylhydrocarbon-hydroxylase (AHH) [26] were measured. Microsomal losses during the preparative procedures were corrected for as described previously [27]. Protein determination was performed

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according to the method of Lowry *et al.* [28], using crystalline bovine albumin as standard.

Quantitative excretion of total urinary porphyrins [29], of the precursors δ-aminolevulinic acid (ALA) and porphobilinogen (PBG) by ion exchange chromatography [30], the separation of total porphyrins and their transformation as methyl-esters [31] as well as the determination of porphyrin-metabolites by high pressure liquid chromatography (HPLC) [32] were performed by standard methods.

Statistical analysis. The results obtained are expressed as means ± S.E.M., and the significances of the differences were assessed by the Student's *t*-test.

RESULTS

Liver weight and protein

Compared to the respective control groups, treatment with HCB for either 10 or 60 days failed to significantly change the liver weight and total protein (Table 1). Moreover, after short-term treatment with HCB for 10 days no significant alterations of liver microsomal protein, liver cytosolic protein and body weight were observed. Long-term treatment with HCB for 60 days resulted in a significant rise of liver microsomal and cytosolic protein and in a slight reduction of body weight (Table 1).

Alcohol dehydrogenase (ADH)

Compared to controls, treatment with HCB for 10 days led to a significant reduction of hepatic ADH activity only when expressed per g of liver, whereas no significant alterations could be demonstrated when the activity was expressed per mg of cytosolic protein or per 100 g of body weight (Table 2). Prolonged application of HCB for 60 days failed to alter hepatic ADH activity.

Microsomal ethanol-oxidizing system (MEOS)

Short-term treatment for 10 days with HCB as well as prolonged administration for 60 days resulted in a significant rise of the hepatic MEOS activity (Table 2). The enhancement was observed whether the activity was expressed per mg of microsomal protein, per g of liver or per 100 g of body weight.

Catalase

In comparison with the controls, treatment with HCB for 10 days resulted in a significant fall of hepatic catalase activity (Table 2). Similarly, after 60 days of treatment with HCB a reduction of hepatic catalase activity was observed, but this decrease was only significant when the activity was expressed per g of liver protein rather than per g of liver wet weight or per 100 g of body weight.

Cytochrome P-450

The hepatic microsomal content of cytochrome P-450 was significantly increased after 10 days of treatment with HCB but not following long-term application for 60 days when expressed per mg of microsomal protein (Table 3). A pronounced enhancement could be demonstrated for the cytochrome P-450 content when calculated per g of liver

Table 1. Effect of HCB treatment on liver weight, liver protein and body weight. Each experimental group consisted of eight animals

	Treatment			
	10 Days		60 Days	
	Controls	HCB	Controls	HCB
Liver weight (g)	8.8 ± 0.4	8.7 ± 0.5	10.5 ± 0.6	9.2 ± 0.9
Liver weight (g/100 g of body weight)	4.2 ± 0.1	4.4 ± 0.2	4.3 ± 0.2	4.2 ± 0.3
Liver total protein (mg/g of liver wet weight)	129.0 ± 12.0	141.0 ± 14.0	132.0 ± 7.0	141.0 ± 4.0
Liver microsomal protein (mg/g of liver wet weight)	38.3 ± 2.9	32.3 ± 3.0	26.5 ± 3.1	81.1 ± 29.3†
Liver cytosolic protein (mg/g of liver wet weight)	31.3 ± 1.0	30.2 ± 1.7	36.0 ± 3.1	43.2 ± 2.3†
Body weight (g)	208.0 ± 7.0	198.0 ± 3.0	242.0 ± 6.0	219.0 ± 8.0*

* $P < 0.025$.
† $P < 0.05$

Table 2. Effect of HCB on the hepatic activities of alcohol metabolizing enzymes. Each experimental group consisted of eight animals

	Treatment			
	10 Days		60 Days	
	Controls	HCB	Controls	HCB
Alcohol dehydrogenase (ADH)				
(nmoles NADH/min/mg cytosolic protein)	11.5 ± 1.1	9.4 ± 1.6	13.0 ± 2.7	11.5 ± 1.5
(nmoles NADH/min/g of liver wet weight)	360.0 ± 33.0	274.0 ± 35.0‡	486.0 ± 104.0	479.0 ± 41.0
(nmoles NADH/min/100 g of body weight)	1509.0 ± 158.0	1299.0 ± 143.0	2186.0 ± 493.0	2031.0 ± 289.0
Microsomal ethanol oxidizing system (MEOS)				
(nmoles acetaldehyde/min/mg of microsomal protein)	6.8 ± 0.7	11.0 ± 0.7‡	8.6 ± 0.7	10.4 ± 0.7†
(nmoles acetaldehyde/min/g of liver wet weight)	259.0 ± 31.0	361.0 ± 44.0‡	228.0 ± 32.0	711.0 ± 198.0‡
(nmoles acetaldehyde/min/100 g of body weight)	1104.0 ± 136.0	1566.0 ± 196.0‡	960.0 ± 105.0	2661.0 ± 567.0†
Catalase				
($U \times 10^3$/g of liver protein)	29.5 ± 2.5	19.7 ± 2.2†	24.5 ± 1.6	17.7 ± 2.8‡
($U \times 10^3$/g of liver wet weight)	3.7 ± 0.4	2.7 ± 0.3‡	3.2 ± 0.3	2.5 ± 0.4
($U \times 10^3$/100 g of body weight)	15.8 ± 1.7	12.0 ± 1.6‡	13.2 ± 1.3	10.6 ± 2.3

* $P < 0.001$.
† $P < 0.01$.
‡ $P < 0.05$.

or per 100 g of body weight after both short-term and prolonged HCB treatment.

NADPH-cytochrome c reductase

Hepatic NADPH-cytochrome-c reductase activity remained virtually unchanged after HCB treatment for 10 days (Table 3). A significant rise could only be demonstrated after prolonged administration for 60 days when compared to the controls.

7-Ethoxycoumarin deethylase (7-EOC-D)

In comparison with the respective controls, both short-term administration of HCB for 10 days and prolonged treatment for 60 days resulted in a striking rise of hepatic microsomal 7-EOD activities (Table 3).

Arylhydrocarbonhydroxylase (AHH)

Hepatic microsomal AHH activity was significantly increased after treatment with HCB for 10 as well as 60 days when compared to the respective controls (Table 3).

δ-Aminolevulinic acid synthase (ALA-S)

Treatment with HCB for 10 days led to a slight enhancement of hepatic ALA-S activity with significant differences when expressed per g of liver wet weight or per 100 g of body weight but not when the data are given per g of liver protein (Table 4). Compared to controls, the 60 days lasting treatment with HCB resulted in a pronounced increase of hepatic ALA-S activity.

Porphyrin excretion

Compared to controls, treatment with HCB for 10 days had no or only little influence on the urinary excretion of porphyrin precursors such as δ-aminolevulinic acid (ALA) or porphobilinogen (PBG) as well as of total porphyrins and the percentage distribution of porphyrin metabolites (Table 4). After 60 days of HCB treatment, however, a striking increase of urinary excretion of ALA, PBG and total porphyrins was measured compared to the respective untreated controls (Table 4). HCB treatment for 60 days enhanced urinary excretion of porphyrin metabolites such as uro-, hepta- and hexa-porphyrins whereas the one of copro- and proto-porphyrins was significantly reduced when calculated on a percentage basis (Table 4).

DISCUSSION

The present study shows that the hepatic activity of the microsomal ethanol oxidizing system (MEOS) is strikingly enhanced after both short-term treatment for 10 days with HCB as well as after prolonged administration for 60 days (Table 2). Conversely, hepatic alcohol dehydrogenase (ADH) activity remained virtually unchanged under these experimental conditions. Hepatic catalase activities were significantly reduced after 10 days of treatment with HCB and showed only a trend for a slightly lower activity following 60 days of administration (Table 2). HCB, therefore, exhibits a striking enzyme inducing property with respect to MEOS activity (Table 2) comparable to other substances such as

Table 3. Effect of HCB on hepatic microsomal parameters. Each experimental group consisted of eight animals

	Treatment			
	10 Days		60 Days	
	Controls	HCB	Controls	HCB
Cytochrome P-450				
(nmoles/mg of microsomal protein)	0.7 ± 0.03	1.4 ± 0.1*	0.7 ± 0.04	0.8 ± 0.1
(nmoles/g of liver wet weight)	23.5 ± 1.4	48.4 ± 4.5*	24.4 ± 2.7	68.9 ± 18.5‡
(nmoles/100 g of body weight)	95.8 ± 9.1	210.0 ± 19.0*	97.7 ± 5.0	278.0 ± 64.0†
NADPH-cytochrome *c* reductase				
(nmoles/min/mg of microsomal protein)	136.0 ± 7.0	137.0 ± 8.0	144.0 ± 11.0	167.0 ± 7.0‡
(nmoles/min/g of liver wet weight)	5199.0 ± 439.0	4528.0 ± 586.0	3573.0 ± 306.0	13,603.0 ± 4802.0‡
(nmoles/min/100 g of body weight)	22,073.0 ± 2023.0	19,717.0 ± 2728.0	15,419.0 ± 1404.0	49,247.0 ± 14,251.0‡
7-Ethoxycoumarin deethylase (7-EOC-D)				
(nmoles/min/mg of microsomal protein)	0.6 ± 0.04	5.5 ± 0.3*	0.4 ± 0.02	2.3 ± 0.2*
(nmoles/min/g of liver wet weight)	22.1 ± 2.4	188.0 ± 17.0*	16.2 ± 2.9	216.0 ± 64.0†
(nmoles/min/100 g of body weight)	93.2 ± 10.8	820.0 ± 77.0*	62.9 ± 6.9	869.0 ± 220.0†
Arylhydrocarbon hydroxylase (AHH)				
(nmoles/20 min/mg of microsomal protein)	3.8 ± 0.7	47.8 ± 4.1*	4.6 ± 0.4	30.5 ± 3.1*
(nmoles/20 min/g of liver wet weight)	139.0 ± 28.0	1639.0 ± 179.0*	162.0 ± 16.0	3821.0 ± 1231†
(nmoles/20 min/100 g of body weight)	579.0 ± 115.0	7109.0 ± 760.0*	655.0 ± 50.0	15,280.0 ± 4422.0†

* $P < 0.001$.
† $P < 0.01$.
‡ $P < 0.05$.

Table 4. Effect of HCB on hepatic δ-aminolevulinic acid synthase activity and urinary excretion of porphyrins. Each experimental group consisted of eight animals

	Treatment			
	10 Days		60 Days	
	Controls	HCB	Controls	HCB
Hepatic δ-aminolevulinic acid synthase				
(nmoles/hr/g of liver protein)	758.0 ± 81.0	893.0 ± 90.0	648.0 ± 98.0	2960.0 ± 508.0*
(nmoles/hr/g of liver wet weight)	93.0 ± 6.0	118.0 ± 4.0†	82.0 ± 10.0	419.0 ± 72.0*
(nmoles/hr/100 g of body weight)	391.0 ± 27.0	518.0 ± 34.0†	333.0 ± 36.0	1706.0 ± 250.0*
Urinary δ-aminolevulinic acid (nmoles/24 hr)	141.0 ± 12.0	204.0 ± 22.0‡	153.0 ± 8.0	1040.0 ± 56.0*
Urinary porphobilinogen (nmoles/24 hr)	58.3 ± 4.8	65.4 ± 4.3	56.1 ± 5.9	890.0 ± 86.0*
Urinary total porphyrins (nmoles/24 hr)	4.6 ± 0.6	4.7 ± 0.6	5.0 ± 0.7	106.0 ± 5.0*
Uroporphyrin (%)	10.0 ± 1.0	3.0 ± 1.0	12.0 ± 1.0	53.0 ± 3.0*
Heptaporphyrin (%)	4.0 ± 1.0	5.0 ± 1.0	3.0 ± 1.0	8.0 ± 2.0*
Hexaporphyrin (%)	1.0 ± 1.0	4.0 ± 1.0	0	4.0 ± 1.0*
Pentaporphyrin (%)	2.0 ± 1.0	5.0 ± 2.0	1.0 ± 1.0	0
Koproporphyrin (%)	48.0 ± 3.0	45.0 ± 3.0	51.0 ± 5.0	28.0 ± 4.0*
Triporphyrin (%)	4.0 ± 1.0	0	3.0 ± 2.0	0
Protoporphyrin (%)	31.0 ± 3.0	38.0 ± 2.0	30.0 ± 3.0	7.0 ± 1.0*

* $P < 0.001$.
† $P < 0.01$.
‡ $P < 0.05$.

alcohol [3, 6, 27, 33], testosterone [34, 35], thyroid hormones [36] and propylthiouracil [35].

Previous studies have shown that a variety of porphyrogenic compounds may alter the hepatic content of hemoproteins [15, 38–42]. Among these are allylisopropylacetamide (AIA) [40, 41] and cobaltous chloride [39] which decrease both the hepatic catalase activity and the hepatic content of cytochrome P-450 [38–41]. Moreover, dicarbethoxydihydrocollidine (DDC), an inhibitor of the ferrochelatase activity, reduces hepatic catalase activity [42] without a consistent effect on the hepatic content of cytochrome P-450 [39, 42]. The present study using HCB as a porphyrogenic agent shows a significant fall of hepatic catalase activity after short-term administration of HCB (Table 2). In contrast to DDC [39, 41], HCB leads to a pronounced increase of the hepatic content of cytochrome P-450 (Table 3), confirming previous reports [15, 38]. HCB causes similar changes in the pattern of cytochrome P-450 content (Table 3) and MEOS activity (Table 2). In the short-term experiments with HCB a pronounced increase of the two parameters was observed when the data are expressed per mg of microsomal protein (Tables 2 and 3). However, after prolonged treatment with HCB for 60 days the increase of cytochrome P-450 content and MEOS activity on the basis of microsomal protein was only marginal. Since HCB causes an extensive proliferation of the smooth endoplasmic reticulum of the hepatocyte [43], these microsomal parameters are elevated when the data are given per g of liver or per 100 g of body weight (Tables 2 and 3). The participation of cytochrome P-450 and/or P-448 has been shown for MEOS [44–46], 7-EOC-D [47] and AHH [47–49], which are inducible in activity by HCB administration (Tables 2 and 3). It is evident from the present study that the degree of microsomal enzyme induction is dependent on the substrate used (Tables 2 and 3), a finding compatible with the existence of various forms of cytochrome P-450 exhibiting striking differences in substrate specificity [44–49]. Indeed, HCB causes a so-called mixed type induction of cytochrome P-450 since a simultaneous increase of both the phenobarbital and benzo[*a*]pyrene type can be observed [38]. The present experiments show that the activity of NADPH-cytochrome *c* reductase as another component of the microsomal mixed-function oxidase [47, 49] remained unaffected by the short-term administration of HCB (Table 3) and substantiate that the degree of microsomal metabolism of exogenous compounds depends on the cytochrome P-450 and/or P-448 rather than on the reductase.

Biochemical changes suggestive for experimental hepatic porphyria are observed after long-term application of HCB for 60 days but not following treatment for only 10 days (Table 4), confirming thereby previous reports [15–17]. Short-term administration of HCB for 10 days led to a pronounced enhancement of microsomal parameters such as MEOS (Table 2), 7-EOC-D (Table 3), AHH (Table 3) and cytochrome P-450 (Table 3) without changing urinary porphyrin excretion (Table 4). It has, therefore, been concluded [15] that the induction of the cytochrome P-450 system acts only indirectly on the manifestation of the porphyria by forming a porphyrogenic HCB metabolite after reaching a certain degree of microsomal enzyme induction.

A close association exists between alcohol consumption and human hepatic porphyria, since porphyric attacks may be precipitated by alcohol ingestion [10, 11]. Alcohol has been shown to decrease the activity of uroporphyrinogen decarboxylase in the liver [50], and similar changes can be observed in human hepatic porphyria as well as in the HCB animal model for experimental hepatic porphyria [19]. Alcohol itself appears to have little if any effect on porphyrin metabolism [12], whereas metabolic consequences of alcohol degradation such as redox changes of NADH/NAD seem to be more important [12–14]. Redox changes after alcohol are dependent in part upon the activities of both ADH requiring NAD as cofactor and producing NADH and MEOS consuming reducing equivalents in form of NADPH [8]. It is, therefore, reasonable to assume that after alcohol intake alterations of the redox change due to changes of hepatic alcohol metabolizing enzyme activities may be of some importance in hepatic porphyria in man. Other metabolic alterations associated with prolonged alcohol consumption include increased production of the hepatotoxic acetaldehyde due to an enhancement of MEOS activity [3, 6–8, 27], raising the question of acetaldehyde as one of the precipitating factors for hepatic porphyria, but further studies are necessary to firmly establish the biochemical basis for the relationship between alcohol and porphyria.

Acknowledgements—The authors are grateful to Mr. N. Crijns for his valuable technical assistance.

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