Critical Reviews in . . .

Toxicology

Halogenated hydrocarbon

Solvents

File: ZK-2511-(1100) 1993 Chloroform ρ

K-2516- 1993 Carbon tetrachloride

DR-0060-0011- ρ 1,1,1,2-Tetrafluoroethane

K-622- ρ 1,2-Dibromomethane

K-1715- ρ Ethylene dibromide

K-1711- 1993 Vinyl chloride

K-739- 1993 Vinyl bromide

K-2526- 1993 Trichloroethylene

Anesthetics

K-24793- Halothane

DR-0169-9108- Enflurane

DR-0181-3424- Sevoflurane

K-30950- Methoxyflurane

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Bioactivation of Halogenated Hydrocarbons by Cytochrome P4502E1

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ABSTRACT: Numerous halogenated hydrocarbons of the alkane, alkene, and alkyne classes are metabolized by P450 enzymes to products that elicit cytotoxic and/or carcinogenic effects. Such halogenated hydrocarbons include anesthetics (e.g., halothane and enflurane) and industrial solvents (e.g., carbon tetrachloride, chloroform, and vinylidine chloride). Formation of reaction intermediates from these compounds occurs via P450-promoted dehalogenation, reduction, or reductive oxygenation, with certain hydrocarbons undergoing all three reaction types. Of the multiple forms of P450 present in liver microsomes, P4502E1 has been identified as the primary catalyst of hydrocarbon bioactivation in animals and, most likely, in humans as well. As hepatic concentrations of this P450 enzyme are highly inducible by ethanol and similar agents, prior exposure to 2E1-inducing compounds can play a pivotal role in halogenated hydrocarbon toxicity. Considering that metabolism governs the cytotoxicity and carcinogenicity of halogenated hydrocarbons, an understanding of the mechanism(s) underlying 2E1 induction in man becomes all the more important.

KEY WORDS: P4502E1, P450, bioactivation, halogenated hydrocarbons.

I. INTRODUCTION

Halogenated hydrocarbons are a structurally diverse class of chemicals that have been widely used as solvents, pesticides, plasticizers, fuel additives, flame retardants, anesthetics, and as precursors, intermediates, or byproducts of industrial organic syntheses.[1] Several of these halogenated compounds have been identified as environmental pollutants, and have been detected in air, food, water, soil, and in tissues of livestock. The general utility of halogenated hydrocarbons leads to the manufacture and use of billions of pounds of these compounds each year, resulting in environmental and occupational exposure. Because of this exposure, it is important to understand their potential human toxicity.

The toxic effects of halocarbons can be both general and specific. The effects observed in experimental animals or in occupational settings depend on many factors, including solvent structure, level and frequency of exposure, concom-

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itant exposure to other agents, and individual sensitivity. General effects include those on the central nervous or cardiovascular systems, such as depression, paralysis, convulsion, and death from respiratory or cardiovascular collapse. Specific effects include selective organ toxicities, such as nephro- and hepatotoxicity.

Specific organ toxicities caused by halogenated hydrocarbons are related directly to metabolism of these agents. The phenomenon of toxic metabolite generation is termed bioactivation, and it is largely mediated by P450 enzymes.[2] However, not all metabolism of a given compound results in bioactivation. Usually, multiple P450s convert the compound to one of several metabolites, with only one enzyme promoting formation of a reactive intermediate. The other P450s involved convert a large percentage of the dose to nontoxic metabolites, a process termed detoxication. In the absence of metabolism by P450 enzymes, halogenated hydrocarbons generally do not exhibit specific organ toxicity.

Many halogenated hydrocarbons are low molecular weight compounds and, as such, fit the substrate model for metabolism by one particular P450 enzyme, 2E1.[3] This P450 is present both in animals and man, and is found not only in liver, but also in several extrahepatic tissues. Regulation of 2E1 enzyme levels involves both endogenous and exogenous factors, and the extent to which the enzyme is expressed may play an important role in the outcome of human exposure to halogenated hydrocarbons. Endogenous regulation of 2E1 expression entails physiological conditions such as fasting, diabetes, and obesity.[4] Exogenous factors include exposure to xenobiotics such as alcohol, which can result in the marked induction of 2E1 enzyme levels.[4]

This review focuses on three different areas of investigation. The first area discussed is the research on the metabolism of halogenated hydrocarbons, including those that require biotransformation to exhibit toxicity. The emphasis is on haloalkanes and haloalkenes; aromatic halogenated compounds are not considered here. Evidence also is provided for the involvement of specific P450 enzymes in the bioactivation process. Another area focused on is the functional characteristics of 2E1, the P450 enzyme that produces reactive intermediates from many halogenated hydrocarbons. Finally, current theories on the mechanisms through which cellular levels of 2E1 are regulated are described.

II. METABOLISM

Biotransformation of halogenated hydrocarbons has been known to occur for quite some time. Metabolism is promoted primarily by P450 enzymes, which often catalyze the initial steps in both activation and detoxification of xenobiotics and comprise a superfamily of multifunctional forms encoded by distinct genes that are independently influenced by factors such as heredity, sex, tissue, and age.[5] Factors that affect the relative balance between activation and detoxification as catalyzed by the P450 enzymes can affect the outcome of exposure to toxins. Among these factors is the alteration in enzyme expression that occurs as a result of either genetic polymorphisms or exposure to compounds that induce or repress individual enzymes. The fact that several P450 enzymes with differing substrate selectivity and modes of regulatory control existed led to the concept of variability in relative rates of substrate metabolism to both toxic and nontoxic products. Therefore, from a quantitative standpoint, the formation of toxic metabolites depends on the concentrations of specific P450s present at the time of exposure to halogenated hydrocarbons.

Several human P450 enzymes have been identified whose expression can be influenced by either polymorphism or induction.[6] These include members of the 1A, 2A, 2C, 2D, 2E, and 3A subfamilies. With regard to toxicity, 2E gene subfamily proteins are among the most important due to their extensive capacity to metabolize drugs, solvents, and environmental procarcinogens to cytotoxic and/or carcinogenic metabolites.[3,7] Studies to date indicate that structural features of the substrate play a critical role in determining whether catalysis by 2E1 will result in toxication or detoxication.[8]

The initial step in the bioactivation of halocarbons occurs with the breakage of the C–H bond, which is usually the rate-limiting step in metabolism.[2] Hepatotoxicity of these agents has been associated with the ease with which a hal-

ogen can be removed to produce a reactive metabolite. The toxicity of halogenated hydrocarbons is known to increase as the numbers of halogens, size, and ease of homolytic cleavage of the molecule increase.[9] Toxicity from halogenated hydrocarbons may arise as either cytotoxicity or carcinogenesis, and generally occurs in the liver and/or kidney. Of the many halogenated hydrocarbons, evidence for carcinogenicity has been found in the case of chloroform, carbon tetrachloride, vinyl chloride, vinyl bromide, vinylidene chloride, and Tris (2,3-dibromopropyl)phosphate.[1] Methylene chloride, 1,1,2,2-tetrachloroethane, tetrachloroethylene, and trichloroethylene are strongly suspected to be carcinogenic.[1] The known hepatotoxins among these agents include carbon tetrachloride, chloroform, 1,2-dibromoethane, trichloroethylene, vinylidene chloride, tetrachloroethane, and 1,2-dichloroethane. These same compounds also are nephrotoxic, as is ethylene dibromide.[2]

For the many halogenated hydrocarbons that undergo bioactivation, our discussions are divided into two areas, solvents and anesthetics, with specific examples of each provided.

A. Solvents

1. Trihalomethanes

The trihalomethanes cause damage to both the liver and kidney, and have been found to promote cancer in those organs.[10] The most widely studied of this class of compounds is chloroform.[11] Chloroform-promoted liver damage has been observed in humans as well as in experimental animals.[12] The mechanism by which chloroform produces toxicity involves metabolism to a reactive metabolite(s) that covalently binds to hepatic proteins and depletes glutathione. Bioactivation of chloroform proceeds via P450 oxidation to trichloromethanol, which undergoes spontaneous elimination of HCl to form phosgene.[1] Phosgene has been incriminated as the reactive intermediate that acylates proteins, giving rise to the hepatic centrilobular necrosis and renal proximal tubular necrosis observed with chloroform poisoning. Carcinogenesis occurring in these tissues may stem from recurrent cytotoxicity with chronic tissue regeneration rather than from the formation of chloroform-derived DNA adducts.[1]

Evidence for the involvement of a specific P450 in the bioactivation of chloroform was provided by Brady et al.,[13] who demonstrated that purified 2E1 from rat liver microsomes metabolized chloroform to trichloromethanol more efficiently than other purified P450 enzymes (Table 1). Chloroform metabolism by liver microsomes also was potentiated (threefold) when animals were pretreated with 2E1 inducers, such as the secondary ketones acetone, 2-butanone, and 2-hexanone.[13] Moreover, antibodies against this P450 inhibited 81% of the chloroform metabolism in microsomes obtained from acetone-treated rats[13] and also were effective inhibitors of trichloromethanol formation by human liver microsomes.[8]

It has been proposed that the hepatotoxic and nephrotoxic effects of chloroform occur independently as a result of the differential metabolism of chloroform in the two tissues.[14] Furthermore, gender-specific chloroform metabolism appears to promote toxicity in mice. Male mice display both liver and kidney toxicity, whereas only the former is observed in female mice. The underlying mechanism probably involves conversion of chloroform to a reactive metabolite by renal 2E1, which is under regulation by testosterone. Hong et al.[15] reported that 2E1 was found in kidney from male mice but not from female mice; however, treatment of females with testosterone resulted in renal expression of the enzyme. In contrast, the corresponding liver enzyme does not appear to be regulated in a sex-specific manner. Strain differences in the susceptibility to chloroform kidney toxicity also have been shown to exist.[16] DBA/2J mice are more sensitive to nephrotoxicty than are C57BL/6J mice, which may stem from quantitative differences in renal 2E1 expression among the two strains.

2. Tetrahalogenated Methanes

Carbon tetrachloride (CCl_4), an exemplary tetrahalogenated methane, is metabolized by reductive pathways to reactive intermediates that

TABLE 1
P450 Enzymes Involved in the Bioactivation of Halogenated Hydrocarbons

Halogenated hydrocarbon	Major P450 involved in bioactivation	Ref.
Solvents		
Chloroform	2E1	8, 13
Carbon tetrachlonde	2E1	8, 25–28, 30, 31, 105
	2B4	23
1,1,1,2-Tetrafluoroethane	2E1, 1A1	38
1,2-Dibromomethane	2E1	8
Ethylene dibromide	2E1	8
Vinyl chloride	2E1, 2B1/2	8, 41, 42
Vinyl bromide	2E1, 2B1/2	8, 41, 42
Trichloroethylene	2E1, 2B1/2, 2C11/6	46–51, 85
Anesthetics		
Halothane	2E1, 2B4, 2B1/2, 2A2	67, 68
Enflurane	2E1	71, 72
Sevoflurane	2E1	71
Methoxyflurane	2E1, 2B1/2, 2B4	71, 73

can cause hepatic as well as renal damage.[2] Centrilobular necrosis and fat accumulation occur in the liver after CCl_4 exposure and, although the mechanism of toxicity has yet to be defined, both lipid peroxidation and covalent binding of CCl_4-derived reactive metabolites have been implicated in the process.[17] The reductive pathway initially involves a P450-mediated one electron reduction leading to the cleavage of a C–Cl bond to yield a trichloromethyl radical (CCl_3·) and a chloride radical (Cl·).[18] The trichloromethyl radical may dismutate to give chloroform, bind covalently to critical cellular macromolecules, or attack enoic fatty acids within the membranes of the endoplasmic reticulum, leading to the production of secondary lipid free radicals.[19] In addition, the trichloromethyl radical can react with molecular oxygen to form an even more reactive species, the trichloromethylperoxyl free radical, which also exhibits covalent binding and/or elicits lipid peroxidation.[20] Alternatively, under anaerobic conditions, another reductive pathway of CCl_4 metabolism can result in carbene-type products that bind to proteins and nucleic acids.[21] Thus, it appears that aerobiosis promotes covalent binding and lipid peroxidation by CCl_4 via P450-catalyzed formation of the trichloromethyl (or trichloromethylperoxyl) free radical, whereas carbene metabolites may play an important role in CCl_4 toxicity when oxygen tension is low.[21,22]

Experimental evidence suggests that 2E1 and, possibly, phenobarbital-inducible P450 enzymes are involved in the metabolism of CCl_4 (Table 1). In early studies, it was shown that animals treated with phenobarbital exhibited greater hepatotoxicity upon CCl_4 exposure than untreated animals,[23] an effect most likely due to the generalized inductive properties of phenobarbital on drug-metabolizing enzymes. A majority of recent studies, however, implicate 2E1 as the major catalyst of both reductive and oxidative CCl_4 bioactivation. Pretreatment of rats with acetone or isopropanol results in enhanced lipid peroxidation[24] and covalent binding of CCl_4 metabolites to protein.[25] This enhanced bioactivation occurred with a concomitant increase in metabolism of two specific 2E1 substrates, N-nitrosodimethylamine (NDMA)[25] and *p*-nitrophenol.[24] Ethanol pretreatment also results in an increase in the rate of CCl_4 metabolism by rat liver microsomes. This increase in CCl_4 metabolism was accompanied by a parallel increase in immunoreactive 2E1 content in liver microsomes.[26] Administration of pyrazole, acetone, or other aliphatic alcohols to rats

causes a 15- to 30-fold potentiation of CCl_4-dependent hepatotoxicity.[27,28] All of these agents are known to be potent 2E1 inducers.[3] A role for this enzyme in the reduction of CCl_4 also was provided by Johansson and Ingelman-Sundberg,[24] who showed that purified rabbit 2E1, upon reconstitution, was 100-fold more active in metabolizing CCl_4 to chloroform and in initiating lipid peroxidation compared to P4501A2 or P4502B4. Inhibition studies with selective inhibitors or antibodies also have demonstrated the involvement of 2E1 in the bioactivation of CCl_4. Disulfiram, a chemical inhibitor of the enzyme, was shown to block CCl_4 hepatoxicicity in rats.[29] Furthermore, anti-2E1 IgG produced marked inhibition (80%) of CCl_4 reduction mediated by human liver microsomes,[8,30] indicating that 2E1 participates in CCl_4 metabolism in man as well.

CCl_4 metabolism has been shown to be closely linked with the concomitant destruction of P450. Indeed, metabolic reduction of CCl_4 was accompanied by a rapid loss in immunoreactive 2E1 with no changes in two other P450 enzymes, P4502C and P4501A.[31] Moreover, the suicide destruction of P450 by CCl_4 was greater in liver microsomes from pyrazole-treated rats than in microsomes from untreated animals. A selective decrease in both low K_m NDMA demethylase and the conversion of CCl_4 to chloroform has been reported in rats treated with CCl_4,[32] again suggesting that 2E1 was the target of destruction.

3. Vicinal Halogenated Alkanes

Certain vicinal dihalogenated ethanes are both genotoxic and acutely cytotoxic.[33] The most widely studied is the pesticide 1,2-dibromomethane and the lead scavenger ethylene dibromide (EDB). A major route of EDB metabolism is oxidative dehalogenation by P450 yielding 2-bromoacetaldehyde, which preferentially binds to protein.[34] EDB-mediated hepatic, renal, and cardiac necrosis have been observed after exposure of humans to fatal doses of EDB.[35] Recently, it has been shown that 2E1 mediates the hepatic metabolism of both 1,2-dibromomethane and EDB in man.[8]

Vicinal polyhalogenated ethanes, such as 1,1,2,2-tetrachloroethane and 1,1,12-tetrafluoroethane, also are hepatoxic.[3] Tetrachloroethane is oxidatively dechlorinated by P450 to 2,2-dichloroacetyl chloride, which is then hydrolyzed to dichloroacetic acid and acylates proteins.[36,37] Administration of the 2E1-inducing agent pyridine to rats results in an eightfold elevation in the rate of tetrafluoroethane metabolism by liver microsomes. Moreover, in reconstituted systems, purified 2E1 catalyzes reductive defluorination of 1,1,1,2-tetrafluoroethane at high rates compared to other P450s including P4501A1, which is somewhat active toward the substrate, and P4502B1 and P4501A2, which display negligible rates of defluorination (Table 1).[38]

4. Halogenated Alkenes

Monosubstituted haloethylenes, such as vinyl chloride and vinylidene chloride, are metabolized to compounds that are mutagenic and carcinogenic.[2] A common mechanism for haloalkene oxidation may be through formation of an oxygenated intermediate that directly forms haloaldehyde and haloacylhalide derivatives. The nature of the intermediate has been postulated as an oxo-iron complex that can either collapse to a haloaldehyde (in the case of vinylidene chloride) or an epoxide (in the case of vinyl chloride), or can result in heme alkylation and P450 destruction.[1,39] Evidence demonstrates that of the halogenated ethylenes, vinyl chloride, vinyl bromide, and 1,1,2-trichloroethylene (trichloroethylene) are oxidized primarily by 2E1 to cytotoxins (Table 1).

The human carcinogenicity of vinyl chloride was established in 1974, when 50 cases of hepatic angiosarcoma were found within 1 year after workers were exposed to this compound.[1] Animal studies then confirmed the tumorigenic activity of vinyl chloride as well as that of vinyl bromide. Oxidative metabolism of vinyl chloride by P450 was found to result in the production of a reactive epoxide, 2-chloroethylene oxide, and vicinal haloaldehydes,[40] with evidence favoring the epoxide as the ultimate carcinogenic metabolite.[41] The haloaldehyde, 2-chloroacetaldehyde, also is an important alkylating agent derived from vinyl chloride.[41] At least two P450 enzymes may be involved in the activation of vinyl chloride

and vinyl bromide. Microsomes from rats pretreated with phenobarbital exhibited high conversion rates of these compounds to their corresponding epoxides compared to microsomes from naïve animals. Purified rat P4502B1, the major P450 enzyme induced by phenobarbital treatment, was highly active in the conversion of vinyl chloride to its corresponding epoxide.[41,42] More recently, inhibitors of 2E1, e.g., disulfiram, were shown to prevent epoxidation of vinyl chloride and vinyl bromide by human liver microsomes,[8] implying that 2E1 also is involved in the activation of halogenated alkenes in man.

Trichloroethylene (TCE) has been widely used as an organic solvent and general anesthetic. It is weakly hepatocarcinogenic in B6C3F mice and hepatotoxic in rats and humans.[2,43] TCE oxide has been detected as an oxidative metabolite, and may be responsible for the covalent binding to liver proteins and nucleic acids noted with TCE.[44,45] The only other TCE metabolite formed by P450 in detectable amounts is chloral hydrate (CH). Several P450 enzymes may be involved in the oxidation of TCE, with substrate concentrations being a major determinant of which P450 predominates (Table 1). Fasted rats and those treated with phenobarbital or ethanol exhibit enhanced rates of TCE metabolism;[46–48] the latter treatments also potentiate TCE hepatoxicity. However, this enhancement of TCE toxicity by ethanol is noted when the animals are exposed to low TCE concentrations. In contrast, exposure to markedly higher TCE concentrations are required to observe potentiation of TCE toxicity by phenobarbital.[46,47] Nakajima et al.[49,50] showed that 2E1 antibodies were potent inhibitors of TCE metabolism by ethanol-induced liver microsomes at low TCE concentrations, whereas P4502C11 and P4502B1/2B2 antibodies were more inhibitory with phenobarbital-induced microsomes at higher substrate concentrations. Such results indicate that 2E1 is the major TCE-metabolizing enzyme at physiologically relevant concentrations of this halogenated alkene; phenobarbital-inducible P450s are apparently involved in metabolism only at much higher substrate levels. 2E1-catalyzed metabolism of TCE also has been shown to occur in human liver microsomes.[8]

Factors other than exposure to inducing agents appear to affect metabolism of TCE. Age and pregnancy, which are factors that influence 2E1 expression, both alter the extent of TCE oxidation in rats.[51] Three-week-old animals converted TCE to CH at faster rates than did 18-week-old rats (an approximate twofold difference). With regard to pregnancy, TCE metabolism in animals at day 21 of gestation was decreased 50% compared to nonpregnant rats or to those at day 10 of gestation.[51] These changes in microsomal TCE metabolism paralleled those of microsomal 2E1 content, suggesting that TCE metabolism is influenced by the same physiological factors that regulate 2E1 levels.

B. Anesthetics

1. Halogenated Anesthetics

The most widely used inhalation anesthetics are polyhaloalkanes or alkyl ethers containing fluorine. Most of these anesthetics have been shown to be oxidized and/or reduced by P450 enzymes to reactive metabolites that can bind to proteins and lipids.[52,53] This biotransformation of volatile anesthetics is of concern because it has been linked in a number of instances to toxic side effects, including hepatotoxicity and nephrotoxicity.[54] The best studied of the inhalation anesthetics is halothane (1-bromo-1-chloro-2,2,2-trifluoroethane), an agent that causes hepatotoxicity in man and animals.[55–57] Evidence supports P450-dependent formation of the 2-chloro-1,1,1-trifluoroethyl radical as the major reactive and toxic metabolite produced under conditions of low oxygen tension.[58–61] This reductive metabolite also acts as a suicide inhibitor of P450 and, under anaerobic conditions, there is a significant inactivation of P450 and a parallel loss of the prosthetic heme group.[62] P450 destruction by halothane was found to be irreversible, was inhibitable by carbon monoxide, and showed biphasic, pseudo-first-order kinetics, thus fulfilling all the conditions of a typical "suicide" inactivation reaction.[62]

Oxidative biotransformation of halothane is predominant in humans and results in lesions of a far more extensive nature than those produced by reductive metabolism.[54] Oxidation of halothane proceeds through a halohydrin to produce

either trifluoroacetyl chloride or bromide, which subsequently hydrolyzes to trifluoroacetic acid and acylates proteins.[63,64] A trifluoroacetyl-P450 adduct has been detected on the outer cell surface of rat hepatocyte membranes after treatment with halothane. This acylated P450 (Mr = 54,000) may be immunogenic and may promote the development of halothane hepatitis.[56,57,65,66]

The metabolism of halothane involves at least two different P450 enzymes (Table 1). In rabbits, both phenobarbital and imidazole induce metabolism of halothane, although the extent of induction is much greater with imidazole.[67] Moreover, purified rabbit P4502B4 and 2E1 catalyze the oxidation of halothane to trifluoracetic acid. Immunoinhibition studies have revealed that 2E1 is the major catalyst of halothane oxidation from imidazole-treated rabbits, whereas the reductive metabolism of halothane was shown to be catalyzed mainly by phenobarbital-inducible enzymes.[67] Utilizing purified rat liver P450 enzymes, VanDyke et al.[68] demonstrated that P4502A2, P4502B1, and P4502B2 catalyzed the reduction of halothane, with all three enzymes producing 2-chloro-1,1,1-trifluoroethane. However, P4502B2 was the only enzyme that formed 2-chloro-1,1-difluoroethylene.[68]

Human liver microsomes also metabolize halothane, in fact at rates comparable to microsomes from imidazole-treated rabbits.[67] Furthermore, metabolism of halothane was found to be elevated in obese individuals, as serum levels of inorganic fluoride, trifluoroacetic acid, and bromide ion were higher in these subjects than in non-obese subjects following halothane administration.[69] Because obesity is among those pathophysiological conditions that results in 2E1 induction,[70] the elevated metabolism of halothane noted in obese patients implicates 2E1 involvement.

Enflurane and isoflurane are chemical isomers that are metabolized to a lesser extent than another related halogenated anesthetic, methoxyflurane.[54] Fluoride is released during the oxidative metabolism of these compounds and, during prolonged enflurane anesthesia, enough fluoride ion is generated to produce subclinical nephrotoxicity. Oxidative biotransformation of both enflurane and isoflurane results in the production of reactive intermediates that bind covalently to liver proteins in a manner analogous to that of halothane.[54]

Several studies have revealed that 2E1 is the predominant catalyst of enflurane metabolism (Table 1).[71,72] Hoffman et al.[71] demonstrated that treatment of rabbits with imidazole resulted in a 250% increase in the microsomal oxidation of enflurane, methoxyflurane, and sevoflurane, the latter being a structurally related halogenated anesthetic. Antibodies to 2E1 were found to inhibit microsomal enflurane defluorination by over 90% but inhibited methoxyflurane dehalogenation to a much lesser extent (40%). In the case of methoxyflurane, such a finding was not unexpected inasmuch as other P450 enzymes (e.g., P4502B1) are known to be involved in metabolism of this compound (Table 1).[73] However, reconstitution studies showed that 2E1 was capable of oxidizing not only enflurane, but also methoxyflurane and sevoflurane.[71] A role for 2E1 as the major enflurane-oxidizing enzyme in liver microsomes also was provided by Tsutsumi et al.,[72] who found that liver microsomes from ethanol-treated rats defluorinated enflurane at rates eightfold higher than microsomes from control animals; this increase in hepatic enflurane metabolism by ethanol was attendant with a potentiation of the compound's hepatotoxicity. Antibodies to 2E1 also were used by Tsutsumi and co-workers[72] to demonstrate involvement of the ethanol-inducible P450 in dehalogenation of the anesthetic. In clinical studies, elevated serum fluoride ion levels were found in subjects who received isoniazid prior to enflurane administration.[71] Obese patients also were found to exhibit enhanced rates of enflurane metabolism.[74] These alterations in enflurane disposition observed in both types of subjects presumably stemmed from the isoniazid and obesity-mediated induction of 2E1.

III. P4502E1

As discussed in detail previously, 2E1 plays an integral role in the metabolism of numerous halogenated hydrocarbons. Furthermore, in many instances, induction of this P450 enzyme causes enhanced susceptibility to the toxicity of such compounds. In light of the toxicological impact of 2E1, the remainder of this review focuses on

specific metabolic properties of the enzyme itself as well as on mechanisms involved in its cellular regulation.

The initial observation in humans that chronic ethanol consumption resulted in proliferation of liver smooth endoplasmic reticulum[75,76] led to the discovery of a unique pathway of ethanol oxidation, which was termed the microsomal ethanol oxidizing system or MEOS. MEOS was dependent on P450 and NADPH, and displayed increased activity following chronic ethanol exposure in rats.[77] The P450 enzyme responsible for this ethanol-oxidizing activity was initially characterized upon purification from ethanol-treated rabbits and was termed P450LM-3a.[78] The purified enzyme displayed an enhanced ability to oxidize ethanol and other primary aliphatic alcohols to their corresponding aldehydes when compared to other purified rabbit P450s.[79] An homologous P450 enzyme, initially termed P450j, was then purified from isoniazid-treated rats.[80,81] Rabbit LM-3a and rat P450j, now collectively designated 2E1,[82] not only metabolized alcohols, but also converted environmental procarcinogens to ultimate carcinogenic metabolites. In fact, 2E1 is now known as the sole microsomal low K_m NDMA demethylase in most animal species.[83] This P450 enzyme also plays an important role in the generation of cytotoxic metabolites from acetaminophen, a widely used therapeutic agent.[84] Other compounds whose metabolism is catalyzed primarily by 2E1 include aniline, pyridine, 4-methylpyrazole, benzene, chlorzoxazone, carbon disulfide, p-nitrophenol, acetone, glycerol, diethyl ether, and the halogenated hydrocarbons discussed earlier in this review.[3]

Experimental studies have revealed that xenobiotics other than ethanol also increase 2E1 levels in liver microsomes.[85] Such compounds include acetone, benzene, dimethylsulfoxide, imidazole (in rabbits only), isoniazid, isopropanol, pyrazole, 4-methylpyrazole, and pyridine.[85–88] In fact, one of the polyhalogenated hydrocarbons, TCE, also is a 2E1-inducing agent.[85] As previously noted, most of the same compounds that induce 2E1 also are substrates for the enzyme. The ability of 2E1 to metabolize acetone has led to the proposal that ketone disposition may represent one of the physiological functions of the enzyme.[89] Under conditions where blood acetone levels are elevated (e.g., starvation, diabetic ketoacidosis, and obesity), hepatic 2E1 levels are usually induced and, hence, increased conversion of acetone to acetol and methoxyglycol, proposed gluconeogenic intermediates, may occur. This pathway could constitute an important source of glucose during starvation or diabetic ketoacidosis.

Multiple P450s also are present in human liver, and 2E1 constitutes one of the enzymes found in most, if not all, individuals. Immunochemical studies using antibodies to rat or rabbit 2E1 first revealed the presence in human liver of an enzyme (Mr = 54,000) that promoted aniline hydroxylation and low K_m NDMA demethylation.[90,91] 2E1 was subsequently purified in a catalytically active state from human liver microsomes and, upon its reconstitution, was shown to effectively oxidize ethanol, aniline, and NDMA, the latter at concentrations similar to those found *in vivo*.[92] Other metabolic properties later attributed to human 2E1 include activation of acetaminophen to the reactive metabolite *N*-acetyl-*p*-benzoquinone imine,[93] chlorzoxazone 6-hydroxylation,[94] benzene hydroxylation, and styrene epoxidation;[8] the role of this human P450 enzyme in halocarbon bioactivation was already discussed. Furthermore, the amino acid sequence of human 2E1, which was derived from the complementary DNA, was found to exhibit an extensive degree of homology with both the rabbit and rat enzymes.[96,97] Southern blot analysis revealed that the human *CYP*2E gene subfamily consists of only a single gene without closely related members.[96] In contrast, rabbits possess two *CYP*2E gene subfamily members, 2E1 and 2E2, which exhibit different modes of regulation.[98] The human *CYP*2E1 gene was mapped to chromosome 10.[99]

More importantly, 2E1 is among the P450 enzymes that are inducible in man. Investigations by Wrighton et al.[91] and Tsutsumi et al.[95] revealed a marked increase in hepatic 2E1 levels among subjects who had recently consumed alcohol or were administered isoniazid. In fact, microsomes from these subjects were found to exhibit enhanced rates of NDMA metabolism.[91] Furthermore, 2E1 induction was found to occur primarily among perivenular hepatocytes,[95] the same cells that exhibit most of the damage after

exposure to ethanol, halogenated hydrocarbons, and other 2E1-activated hepatotoxins.[7]

A. Xenobiotic Regulation

Because 2E1-mediated metabolism is believed to play such an important role in the cytotoxicity and carcinogenesis of halocarbons, the mechanisms involved in regulation of this P450 also are of importance. Indeed, the enhanced bioactivation of halocarbons noted after exposure to certain xenobiotics stems from the elevation in 2E1 levels elicited by such agents. However, the mechanisms underlying induction of this enzyme remain somewhat controversial. In this final section of the review, we consider the various modes of regulation proposed for 2E1. The toxicological significance of 2E1 regulation as it relates to halocarbon bioactivation also is discussed.

The modulation of cellular 2E1 levels by xenobiotics has been demonstrated in several mammalian species, including man. However, controversy surrounds the mechanism(s) via which xenobiotics induce 2E1. Proposed mechanisms include increases in 2E1 mRNA stemming from transcriptional activation or mRNA stabilization, enhanced protein synthesis, and decreased enzyme degradation. The three mechanisms may act individually or in concert with each other to increase 2E1 content in the liver. Species differences, the particular inducing agent, and the duration of inducer treatment (acute or chronic) appear to govern which of the mechanisms predominates.

Of the various treatments that increase hepatic 2E1 in rodents, ethanol and similar agents (acetone, pyrazole, 4-methylpyrazole, and imidazole) have little effect on 2E1 mRNA[4,96,98,100] but reportedly induce the enzyme by stabilizing it against proteolytic degradation. Numerous experimental studies have failed to document substantial increases in hepatic 2E1 transcripts following acute and/or chronic administration of ethanol or ethanol like agents.[4] In fact, 2E1 mRNA levels in rabbit liver actually decrease as treatment with ethanol progresses.[98] Although treatment of rabbits with imidazole results in a transient, twofold increase in 2E1 mRNA, it was concluded that this transient increase was not sufficient to account for the marked induction of the enzyme by imidazole."[7] Wrighton et al.[91] could find no correlation between 2E1 mRNA and 2E1 protein levels in human liver, and concluded that ethanol-mediated 2E1 in man, as is the case in rodents, does not require the accumulation of 2E1 transcripts. Such observations led to the consensus that the induction of 2E1 caused by xenobiotics occurs primarily via post-translational mechanisms.

A decrease in 2E1 protein degradation as a result of inducer-mediated enzyme stabilization has been proposed by a number of investigators.[101-105] The rapid decrease in 2E1 protein levels noted in primary cultures of rat hepatocytes was shown by Eliasson et al.[101] to be prevented by including isopropanol, dimethylsulfoxide, or imidazole in the culture media. Song et al.[106] measured the turnover of 2E1 *in vivo* in untreated and acetone-treated rats and found that the former animals exhibited biphasic 2E1 degradation kinetics with respective estimated half-lives of 7 and 37 h. In animals treated with acetone, however, only the slow phase component (37 h) was observed. The mechanism by which inducers block 2E1 enzyme degradation was examined by Tierney et al.,[107] who found that mice treated with 4-methylpyrazole possessed much lower levels of ubiquitin-conjugated microsomal protein than did mice treated with CCl_4. These workers proposed that ubiquitination is an intracellular signal for 2E1 proteolysis, and that the inhibition of 2E1 degradation by 4-methylpyrazole resulted from inhibition of the formation of microsomal ubiquitin conjugates by this compound.[107] However, because specific ubiquination of 2E1 was not measured in this study, the proposal that inducers of the enzyme prevent its ubiquination, and thus its degradation, may be somewhat premature.

Another mechanism of 2E1 induction involves inducer-promoted increases in both 2E1 mRNA and protein synthesis. In contrast to previous investigations, more recent studies have shown that elevations in liver 2E1 mRNA content after the administration of xenobiotics do indeed occur in several species. In cultured rabbit hepatocytes, an increase in 2E1 transcripts was noted in cells that had been treated with acetone for

short periods of time (6 to 24 h), which paralleled the increase in newly synthesized 2E1 protein. The concomitant exposure of rabbit hepatocytes to acetone and α-amanitin, an inhibitor of gene transcription, blocked the increase of both 2E1 mRNA and protein produced by the former compound,[108] suggesting that the enhanced rate of 2E1 protein synthesis found in acetone-treated hepatocytes may stem from increased transcription of the *CYP*2E1 gene, at least in rabbits. Treatment of acetone appears to stimulate rates of 2E1 protein synthesis in rats as well.[87,109] A single injection of acetone was found to increase aniline hydroxylation by liver microsomes both 30 min and 48 h after administration. The more rapid increase in this drug-metabolizing activity (69%), which was maximal at 0.5 h, returned to control levels after 4 h while the slower increase in activity (168%) peaked after 48 h. Because treatment with cycloheximide, an inhibitor of protein synthesis, blocked the slower increase in aniline hydroxylation by acetone, it was concluded that enhanced rates of protein synthesis were involved at least in the slower phase of induction.[87] Elevations of 2E1 mRNA and protein have been reported in rats fed ethanol in liquid diets[110] as well as in rats receiving continuous ethanol infusion.[111] An interesting phenomenon was noted with the continuous-infusion model of ethanol treatment. Despite the fact that ethanol was infused intragastrically at a constant rate, blood ethanol levels were cyclical in nature, and ranged from 10 to 500 mg/dl over a 7-day period.[112] At blood ethanol levels <250 mg/dl, a 6-fold increase in hepatic 2E1 content was found, whereas at levels >250 mg/dl, the enzyme was elevated 12-fold and a marked increase in 2E1 mRNA content also was observed.[113,114] In addition, nuclear run-on experiments showed that when blood alcohol concentrations were high, the elevation of 2E1 mRNA occurred via transcription of the *CYP*2E1 gene.[115]

Other species in which 2E1 protein induction involves increases in 2E1 mRNA synthesis include hamsters and, more importantly, humans. Ethanol and pyrazole given chronically to hamsters resulted in an enhancement of hamster 2E1 protein and translatable mRNA.[116] In ethanol-abusing patients, Takahashi et al.[117] recently demonstrated that the induction of 2E1 protein occurring within the hepatic perivenular zone is accompanied by an increase in 2E1 transcripts within the same acinar zone. Furthermore, it was found that levels of 2E1 protein and mRNA in perivenular, pericentral, and periportal hepatocytes were significantly correlated among all patients studied.[117]

Translational control of protein synthesis also may play a role in the 2E1 induction process. For instance, Kim and Novak[118] found that pyridine (and acetone) increased the hepatic 2E1 content, not by enhancing levels of 2E1 transcripts but by increasing the efficiency with which preexisting transcripts were translated. The two- to fourfold increase in 2E1 content noted 6 to 24 h following a single pyridine injection could be completely blocked by prior administration of cycloheximide, whereas actinomycin D, an inhibitor of transcription, had no effect. The stimulation of mRNA translational efficiency by pyridine was attributed to a shift in 2E1 transcripts from light to heavy polyribosomes, which are more effective at protein synthesis.

B. Regulation by Pathophysiological States and Diet

1. Fasting/Diet/Obesity

Fasting markedly enhances microsomal drug-metabolizing activities, including those mediated by 2E1. Twenty-four- and 48-h fasts were found to cause 59 and 116% increases, respectively, in microsomal NDMA demethylation in rats. These increases in enzyme activity were accompanied by parallel increases in 2E1 protein and its corresponding mRNA;[119] induction of 2E1 protein and mRNA was noted mainly in the hepatic perivenular zone.[120] Elevated 2E1 protein and mRNA in liver and kidney also have been observed in fasted rabbits, although renal 2E1 protein levels were enhanced more than hepatic levels of the enzyme.[98] The molecular events underlying the elevation in 2E1 mRNA and protein that occurs during fasting are not clear. However, a number of studies have implied that 2E1 levels during fasting are under pretranslational control that involves either *CYP*2E1 gene transcription or stabilization of 2E1 mRNA.[98,119,120] Initially, in-

creased circulating ketone levels were proposed as the cause for induction inasmuch as the administration of ketogenic compounds, such as n-hexane, 2-hexanone, and acetonyl acetone, increased 2E1 concentrations in the liver.[121] However, even prolonged fasting does not produce blood ketone levels high enough to account for the extent of 2E1 induction observed. Therefore, physiological factors other than increased circulating ketones are apparently responsible for the elevation of 2E1 that occurs when animals are starved.

Dietary alterations also may result in the induction of 2E1 protein. Ketogenic diets, including those deficient in carbohydrate or high in fat, are known to enhance metabolism of chloroform, CCl_4, 1,2-dichloroethane, 1,1,-dichloroethylene, trichloroethylene, and enflurane in rats.[122,123] The increased rates of metabolism observed with these halogenated hydrocarbons most likely stem from the increase in 2E1 elicited by the high levels of circulating ketones attendant with the diets. Compared to animals fed fat-free diets, animals fed diets enriched in fat (20%) exhibited higher serum levels of acetone, β-hydroxybutyrate, and acetoacetate, which were found to correlate with the increased content of 2E1 and its corresponding activity, NDMA demethylation, in liver microsomes.[124] Both hepatic and renal 2E1 were induced by high fat diets, and the increase in enzyme was accompanied by a threefold increase in 2E1 mRNA.[125] Such results indicate that high fat diets can influence expression of 2E1, possibly in a manner similar to that observed with fasting. In addition, the long-term (6 months) feeding of high fat diets to render rats obese also resulted in induction of 2E1 protein and its corresponding catalytic activities.[70] Because obese animals exhibit an elevation in serum ketones, either the ketogenic diet or obesity itself may be the cause of the increase in 2E1 enzyme concentrations. The ability of high fat diets, as well as the obesity produced by these diets, to elevate hepatic 2E1 content may have significant implications for man, considering that these diets reflect, to a certain degree, the human diet. This could place the obese individual at greater risk to halogenated hydrocarbon-promoted toxicity. Moreover, it has been shown that high fat diets potentiate the 2E1 induction mediated by xenobiotics, again demonstrating the interaction between xenobiotics and physiological factors in terms of 2E1 regulation. Administration of ethanol to rats in liquid diets containing 35% fat resulted in a greater induction of 2E1 compared to animals fed ethanol in liquid diets containing 5% fat.[126] Finally, feeding rats a diet deficient in thiamine resulted in an increase in 2E1 content and NDMA demethylase activity in liver microsomes.[127] As thiamine deficiency does not affect ketone levels, the mechanism of 2E1 induction remains obscure.

2. Diabetes

Chemically induced and spontaneous diabetes are conditions that may influence the outcome of exposure to halogenated hydrocarbons bioactivated by 2E1. Uncontrolled diabetes results in a dramatic elevation of serum ketone levels. In fact, it has been shown in spontaneously diabetic (BB) rats that plasma β-hydroxybutyrate levels (but not those of glucose or insulin) correlated with microsomal 2E1 content and associated activities, i.e., aniline hydroxylation and NDMA demethylation.[128] Moreover, in rats made diabetic by the administration of streptozotocin or alloxan, both the 2E1 protein and 2E1 mRNA were elevated to levels found in fasted rats.[129,130] Interestingly, insulin treatment was found to reverse the induction of 2E1 caused by diabetes[131,132] via a mechanism that most likely involves reversal of the ketosis rather than through a direct effect on the *CYP*2E1 gene. Indeed, insulin has no effect on 2E1 expression when added to cultured hepatocytes.[133] With regard to diabetes itself, the elevations noted in 2E1 protein and 2E1 transcripts are reportedly due to mRNA stabilization.[134] However, as ketones have been shown to increase 2E1 mRNA levels by activating *CYP*2E1 gene transcription in fasted animals, it seems likely that a similar mechanism is at work in the diabetic animal.

3. Hormonal Regulation

At least two hormones, growth hormone and testosterone, have been implicated in the regu-

lation of 2E1 expression. Testosterone appears to be important in regulating 2E1 levels in the kidney and is discussed in the next section. Investigations into the effects of growth hormone on 2E1 have been performed mainly with hypophysectomized rats, which exhibit multiple hormonal deficiencies. Hypophysectomy results in an increase in 2E1 and, in a parallel fashion, its corresponding mRNA.[135-137] Replacement therapy with growth hormone reverses the hypophysectomy-mediated increase in enzyme content. These results imply that of the various hormones altered by removal of the pituitary, growth hormone is the most important in regulating hepatic 2E1 expression. That 2E1 protein and mRNA levels are both coordinately induced by hypophysectomy indicates that growth hormone acts directly on the *CYP*2E1 gene and suppresses its expression by inhibiting gene transcription.[136] It also has been suggested that growth hormone suppresses 2E1 concentrations through a somatogenic receptor-mediated process.[136] Although 2E1 protein content is markedly increased by hypophysectomy, catalytic activities associated with the enzyme (p-nitrophenol hydroxylation, NDMA demethylation, and aniline hydroxylation) were elevated only slightly. This dissociation of 2E1 content and activity is most likely the result of a decrease in P450 reductase concentrations caused by hypophysectomy; P450 reductase content fails to return to control levels after growth hormone replacement therapy.[138,139] Thus, the depression of P450 reductase, which is the rate-limiting enzyme in many P450-dependent reactions, may be responsible for the lower 2E1-mediated drug metabolizing activities noted in hypophysectomized rats.

C. Tissue Distribution/Localization

Similar to most other P450 enzymes, the highest concentrations of 2E1 are found in the liver. Both prior to and after induction, 2E1 expression predominates among hepatocytes comprising the perivenular zone of the liver acinus.[95,120,140,141] This regiospecific pattern of 2E1 expression in liver has been invoked to explain the enhanced susceptibility of perivenular cells to damage from hepatotoxins, including halogenated hydrocarbons. Because it is now realized that 2E1 is not only expressed but also is induced in extrahepatic tissues, the halocarbon-promoted toxicity observed in these tissues also may be due to 2E1-mediated bioactivation. Most often, immunochemical procedures have been used to localize the enzyme in extrahepatic tissues. For instance, Shimizu et al.[142] used protein blotting to show that 2E1 was present and inducible by ethanol treatment in rat lung, kidney, and nasal epithelial microsomes. A more sensitive technique, immunohistochemistry, was used by these investigators to reveal expression and ethanol-mediated induction of the enzyme in rat duodenal and jejunal villous cells, and in epithelial cells derived from the cheek mucosa, tongue, esophagus, forestomach, and proximal colon.[142] In rabbits, 2E1 has been found in kidney, nasal mucosa, and, interestingly, in bone marrow.[143-145] Treatment with ethanol or acetone induced the enzyme nearly 5-fold in kidney and over 12-fold in bone marrow but was without effect in nasal mucosa. The elevation of enzyme content in kidney and bone marrow was accompanied by a similar enhancement of 2E1-catalyzed activities (e.g., aniline and benzene hydroxylases) in these tissues.[144,145] 2E1 is present mainly within the proximal tubules of mouse kidney. In contrast to the rat and rabbit homologs, however, mouse renal 2E1 is regulated in a sex-dependent manner. Kidney microsomes obtained from adult male mice exhibit substantial levels of immunoreactive 2E1 and also demethylate NDMA, whereas those obtained from female and immature male mice do not.[15,146] The role of testosterone in regulation of mouse renal 2E1 was shown by the appearance of a new microsomal low K_m NDMA demethylase as well as 2E1 mRNA in kidneys from female animals treated with the hormone. With regard to halocarbon toxicity, this gender-specific expression of renal 2E1 in mice most likely explains the kidney damage observed in male but not in female mice after exposure to chloroform.[16]

Besides the endoplasmic reticulum (i.e., microsomes), 2E1 also is found in the plasma membrane of rat hepatocytes.[147] In fact, purified preparations of plasma membranes demethylate NDMA at rates nearly one third of those obtained with microsomes, reflecting the difference in 2E1

content between the two organelles, 2E1 levels in the hepatocyte plasma membrane have proved inducible,[147] an observation that may have toxicological significance with regard to the production of acylated protein adducts on the cell surface. The formation of acylated 2E1 adducts in plasma membranes as a result of halocarbon bioactivation may, in turn, elicit the immunogenic (and toxic) response observed in certain types of drug-promoted hepatitis, such as that described for halothane.[56,57,65,66]

D. Polymorphism in Human 2E1 Expression

Various genetically linked polymorphisms in oxidative drug metabolism have been described in man. Such polymorphisms play an important role in the differences observed among individuals with regard to drug response and drug toxicity. One of these polymorphisms, namely that involving the *CYP*2D6 debrisoquine hydroxylase locus, stems from a single base change in a *CYP*2D6 intronic splice site. This point mutation causes aberrant splicing of 2D6 pre-mRNA and, hence, the production of a very unstable 2D6 protein.[148,149] Inasmuch as the mechanisms underlying other inheritable defects in human drug metabolism are far less understood, these polymorphisms also may involve base changes as well as deletions in the corresponding P450 gene. Such mutations may result not only in the absence of P450 proteins, but also in the production of functionally deficient enzymes. For instance, the S-mephenytoin 4-hydroxylase polymorphism appears to stem from a mutation in a *CYP*2C gene subfamily member (e.g., 2C9, 2C10, 2C18) with altered catalytic properties.[150,151]

One approach for identifying structural changes in P450 genes is restriction fragment length polymorphism (RFLP) analysis. Mutations of functional significance in the *CYP*2D6 gene have been detected using this type of analysis.[152] With regard to the human *CYP*2E1 gene, *Taq*I and *Dra*I RFLPs have been discovered,[153,154] the former within intron 7 and the latter within intron 2. Both are two allele polymorphisms that occur with a frequency of 0.1 (*Taq*I) and 0.24 (*Dra*I). Whether either RFLP has ramifications in terms of 2E1 protein expression or function is not known, although Uematsu et al.[154] reported that host susceptibility to lung cancer is associated with the *CYP*2E1 *Dra*I RFLP.

More recently, two other RFLPs in the human *CYP*2E1 gene have been detected. *Pst*I and *Rsa*I polymorphisms were both found in the 5′-flanking (i.e., regulatory) region of the gene, were in complete linkage disequilibrium with each other, and occurred with frequencies of 0.81 (c1 = *Pst*I$^-$, *Rsa*I$^+$) and 0.19 (c2 = *Pst*I$^+$, *Rsa*I$^-$) among 202 unrelated Japanese subjects. The predominant homozygous allele, heterozygous allele, and the homozygous rare allele were designated as type A (c1/c1), type B (c1/c2), and type C (c2/c2), respectively.[155] Comparison of type A and type C gene structure revealed several point mutations within the *CYP*2E1 distal promoter region (bases −1259 through −771) but no changes in the proximal promoter region of the gene.[156] Nevertheless, these base substitutions were shown to have a marked effect on gene transcription. Hayashi et al.[156] subcloned the 5′ regions from types A and C *CYP*2E1 DNA into pSV40CAT (chloramphenicol acetyltransferase) plasmids, and then transfected the different plasmid constructs into the HepG2 human hepatoma cell line. Whereas cells transfected with either the type A or type C DNA pCAT plasmid constructs expressed CAT activity, the level of CAT expression was tenfold higher in cells with the type C construct, indicating not only that *CYP*2E1 bases −1259 through −771 contain a *cis*-acting element capable of activating CAT gene transcription in HepG2 cells, but also that the point mutations found in this *CYP*2E1 DNA region have a profound influence on the extent of CAT gene transcription.[156] If these polymorphisms are found to actually influence expression of the *CYP*2E1 structural gene itself, then individuals of the type C genotype will express more hepatic 2E1 protein than individuals of the type A genotype. Moreover, the type C genotype could give rise to a phenotype that, in all likelihood, exhibits more extensive metabolism of 2E1 substrates, including halogenated hydrocarbons. The identification of such genotypes would be of clinical importance as it could aid in predicting those subjects at risk to exposure to halocarbons.

IV. CONCLUDING REMARKS

In this review, we emphasized the importance of one particular P450 enzyme, ethanol-inducible 2E1, in the bioactivation of halogenated hydrocarbons. The presence of the enzyme in man can predict a variety of pathological sequelae that stem from 2E1-mediated metabolism of these ubiquitous environmental pollutants. Of the many factors that affect halocarbon bioactivation and its resultant toxicity, one of the most prominent concerns variations in 2E1 enzyme concentrations. If conversion of a particular halocarbon to its toxic metabolite is limited by the amount of enzyme present, then enzyme induction may markedly enhance toxicity of the compound. Thus, the induction of 2E1 that results upon ketosis or upon exposure to any of several xenobiotics, including alcoholic beverages, may be especially detrimental to individuals simultaneously exposed to halogenated hydrocarbons. From the experimental and the clinical studies, it has become apparent that the regulation of 2E1 expression by both endogenous and exogenous compounds is rather complex, and involves at least three distinct mechanisms operating either singularly or in concert at the level of transcription, translation, or subsequent to translation. In fact, because of the opposing effects of the many factors that influence 2E1 expression, it is somewhat difficult to predict the outcome of halogenated hydrocarbon exposure. Age, diet, hormonal status, and physiological status are included among the endogenous factors affecting 2E1 enzyme levels. The ketosis that results from starvation, uncontrolled diabetes, or high fat intake can, by increasing 2E1 levels, potentiate liver damage caused by chloroform and CCl_4.[8,13,24,25] Of the exogenous factors, alcohol abuse is the most important due to its prevalence. Chronic ethanol intake is known to enhance the hepatotoxicity of numerous halocarbons. In addition, many halogenated hydrocarbons can induce their own bioactivation, again resulting in increased organ toxicity.

Because several halogenated hydrocarbons, including CCl_4 and halothane, can act as suicide inhibitors of 2E1, halocarbon-mediated destruction of the enzyme could cause a marked decrease in the metabolism of other substrates. This could result in either the reduction or potentiation of xenobiotic toxicity. In the case of environmental agents (e.g., benzene) requiring activation by 2E1, toxicity would be reduced, whereas drugs (e.g., chlorzoxazone) normally converted by the enzyme to an inactive product would exhibit an exaggerated therapeutic response. Inasmuch as 2E1 also may play a role in ketone body homeostasis, suicide inactivation stemming from halocarbon metabolism could have serious consequences in terms of decreased acetone conversion to acetol and methoxyglycol, the latter being a putative glucose precursor. Halogenated hydrocarbons also could influence the metabolism of other 2E1 substrates in a different manner, i.e., through competitive inhibition at the substrate binding site(s) of the enzyme.

Genetic variability in 2E1 expression and/or its induction is another important factor that could influence the outcome of halocarbon exposure. As discussed herein, inheritable polymorphisms within the human *CYP*2E1 structural gene and upstream promoter regions have been discovered. However, it is not known yet whether such polymorphisms ultimately affect the expression or the function of the encoded protein, as has been found to be the case for other human P450 enzymes (e.g., P4502D6). If indeed this is the case, then individuals expressing high levels of 2E1 would exhibit enhanced metabolism of halogenated hydrocarbons to reactive intermediates and, as a result, enhanced susceptibility to their cytotoxic effects.

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