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Enzymatic and Chemical Demethylenation of (Methylenedioxy)amphetamine and (Methylenedioxy)methamphetamine by Rat Brain Microsomes

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The metabolism of (methylenedioxy)amphetamine (MDA) and (methylenedioxy)methamphetamine (MDMA) was examined in microsomal preparations from rat brains. The products generated from MDA and MDMA were identified as dihydroxyamphetamine (DHA) and dihydroxymethamphetamine (DHMA), respectively. The demethylenation reaction required NADPH and was strongly inhibited by CO/O_2 (4:1 v/v), suggesting that the formation of DHA and DHMA is mediated by cytochrome P450. The conversion was inhibited by desipramine, imipramine, and methimazole, whereas SKF-525A and α-naphthoflavone had little effect. Lineweaver–Burk plots of MDA and MDMA demethylenation were biphasic in both cases, indicating that multiple isozymes may participate in the oxidation. The microsomal preparation showed no significant stereoselectivity in the demethylenation of either MDA or MDMA. Catechol formation differed with the incubation buffer and was 2.6 times greater when phosphate rather than HEPES buffer was used. This difference disappeared, however, when desferrioxamine B methanesulfonate (desferal) and hydroxyl radical (•OH) scavenging agents were added to either buffer. The demethylenation was also sensitive to catalase and was stimulated by the addition of ferric ion and EDTA to the microsomal incubation mixture. These results indicate that the demethylenation of MDA and MDMA by rat brain microsomes has a cytochrome P450-mediated component as well as a chemical component involving •OH.

Introduction

Cytochrome P450-dependent oxidase activities are present in many tissues other than liver. The existence of cytochrome P450 in the brain has been known for quite some time (*1–3*), but few studies have been conducted to examine its role in the metabolism of xenobiotics in the central nervous system.

The two amphetamine analogues, (methylenedioxy)amphetamine (MDA,[1] Figure 1) and (methylenedioxy)methamphetamine (MDMA, Figure 1), are serotonergic neurotoxins (*4–6*). The enantioselectivity and the delayed neurotoxicity demonstrated by Schmidt (*7*) and subsequent studies by Gollamudi et al. (*8*) in phenobarbital-treated animals suggest that the neurotoxicity may be due to the formation of an active metabolite. We have previously demonstrated with rat liver microsomes that MDMA and MDA undergo a cytochrome P450-dependent demethylenation and that the catechol metabolites are further oxidized to a quinone or semiquinone, which can react with sulfhydryl functions (*9*). However, this demethylenation is not limited to the action of cytochrome P450; it can be mediated by hydroxyl radical (•OH) as demonstrated with two •OH generating systems: the coupled oxidation of xanthine by xanthine oxidase and the autoxidation of ascorbate in the presence of Fe-EDTA (*10*).

Because of the existence of a tissue-dependent variation in the distribution of cytochrome P450 isozymes, an individual drug may appear to have a metabolic fate that varies from tissue to tissue. This is an important consideration in tissues such as brain, into which there is restricted permeability such that polar metabolites formed in the periphery do not enter the brain. Methylated derivatives of the highly polar catecholamine metabolites of MDMA (*11*) have been found in the brain after peripheral administration, however, suggesting that demethylenation occurs in this tissue. Consistent with this notion, the demethylenation has been reported to take place in brain homogenates (*11*). This report describes results of studies with rat brain microsomes investigating the role of brain P450 in the metabolism of MDMA and MDA. Inasmuch as the levels of cytochrome P450 in the brain are very low, the possibility that the demethylenation may occur through an indirect pathway that generates •OH was also considered. The results demonstrate that the formation of these catechols from MDA and MDMA in brain microsomes is mediated by cytochrome P450 and, depending on incubation conditions, by an •OH-mediated process as well.

Experimental Procedures

Chemicals. MDA and MDMA were obtained from the Research Technology Branch of The National Institute on Drug Abuse (Rockville, MD). Dihydroxyamphetamine (α-methyldopamine, DHA) was obtained from Merck Sharp and Dohme Laboratories (West Point, PA). Dihydroxymethamphetamine (*N*-methyl-α-methyldopamine, DHMA) was synthesized according to the method of Smissman and Borchardt (*12*). Ferric chloride, EDTA, ascorbic acid, superoxide dismutase (SOD), catalase, benzoic acid, HEPES, potassium, phosphate, and thiourea were obtained from Sigma Chemical Co. (St. Louis, MO). Desferrioxamine B methanesulfonate (desferal) was obtained from CIBA Laboratories (Horsham, Sussex, U.K.). SKF 525-A was a gift from Smith Kline and French Laboratories (Philadelphia, PA). All other chemicals used were of the highest grade available.

Microsomal Preparation. The frozen rat brains were obtained from Pelfreeze (Rogers, AK). The frozen brains used were mixtures of male and female SD rats of unknown age. The rats

[1] Abbreviations: MDA, (methylenedioxy)amphetamine; MDMA, (methylenedioxy)methamphetamine; DHA, dihydroxyamphetamine; DHMA, dihydroxymethamphetamine; desferal, desferrioxamine B methanesulfonate; SOD, superoxide dismutase; HPLC-ECD, high-performance liquid chromatography–electrochemical detection; •OH, hydroxyl radical; EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid.

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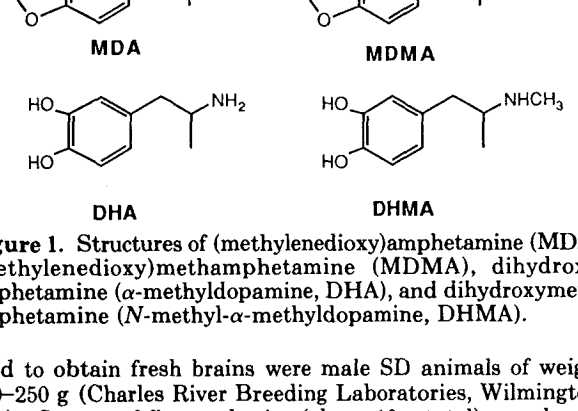


Figure 1. Structures of (methylenedioxy)amphetamine (MDA), (methylenedioxy)methamphetamine (MDMA), dihydroxyamphetamine (α-methyldopamine, DHA), and dihydroxymethamphetamine (*N*-methyl-α-methyldopamine, DHMA).

used to obtain fresh brains were male SD animals of weight 200–250 g (Charles River Breeding Laboratories, Wilmington, MA). Groups of five rat brains (about 10 g total) were homogenized in 30 mL of 1.15% KCl solution containing 1.0 mM EDTA and 100 μM phenylmethanesulfonyl fluoride using a Potter–Elvehjem homogenizer and Teflon pestle (*13*). The homogenates were centrifuged at 9000*g* for 20 min in a refrigerated centrifuge. The supernatants were recentrifuged at 105000*g* for 60 min. The pellets were washed with the solution described above and recentrifuged at 105000*g* for 30 min. The pellets were stored at −80 °C.

Incubation. The incubation mixture consisted of 100 mM HEPES buffer, pH 7.4, 0.5 mM NADPH, microsomal preparation of rat brain (3.0–3.8 mg of protein), 50 units of SOD, and 0.25–10 mM of substrate in a final volume of 1.0 mL. SOD was added to block the conversion of the catechol to the *o*-quinone, a reaction mediated by superoxide generated by cytochrome P450 (*9*). Reactions were initiated at 37 °C by the addition of the enzyme preparation and were terminated by the addition of 0.5 mL of 7.5% perchloric acid.

The chemical demethylenation by $^{\bullet}OH$ was examined in a reaction mixture consisting of MDA or MDMA (1 or 5 mM), 10 μM ferric chloride, 20 μM EDTA, 1 mM ascorbate, and 100 mM phosphate or HEPES buffer, pH 7.4, in a final volume of 1.0 mL. The reaction was initiated by the addition of ascorbate. The reaction was carried out at 37 °C for 10 min and terminated by the addition of 0.5 mL of 7.5% perchloric acid containing 30 mM of thiourea. The reaction mixtures were centrifuged at 13500*g* for 5 min, and the supernatants were analyzed by high-performance liquid chromatography–electrochemical detection (HPLC-ECD).

Determination of Metabolites. A 20-μL aliquot of the supernatant was injected into a Biophase ODS 5-μM column (Bioanalytical System, Inc., IN). The catecholamines were separated from the other components of the reaction mixture by a mobile phase consisting of 0.1 M citrate buffer, pH 3.5, containing 1 mM octyl sodium sulfate/acetonitrile/methanol (8:1:1 v/v) at a flow rate of 0.7 mL/min. The catecholamines were detected by an electrochemical detector (ECD) set at +0.7 V. Signals were recorded with a Hewlett Packard 3390A recording integrator, and the peak height of each compound was compared with that of standard samples. Under these conditions, retention times for DHA and DHMA were about 9.8 and 11.3 min, respectively. The data were corrected for recoveries from the amount of catecholamines contained in control samples and normalized with respect to protein determined by the Bio-Rad protein assay using IgG as the standard.

Results

Demethylenation of MDA and MDMA by Rat Brain Microsomes. The demethylenation activity of frozen rat brain microsomes was compared to that of fresh female and male tissues (Table I). The results exhibited sex differences; female microsomal activity was slightly lower (78%) than that of the male. Frozen rat brain microsomes exhibited about 64% and 82%, respectively, of the MDA demethylenation activity of fresh male and female tissue. Since the frozen brains used were a mixture of male and female tissues of unknown age, the reduced activity found in comparison with fresh tissue could reflect sex and age differences as well as a loss in enzyme activity. Frozen brains were used because of the large quantity of tissue needed to perform these studies. All subsequently described experiments used frozen rat brains.[2]

Table I. Comparison of Enzyme Activities of MDA and MDMA Demethylenation by Native and Frozen Microsomes of Rat Brains[a]

source	demethylenation activity [pmol/(min·mg of protein)] MDA	MDMA
native		
male	1.93, 1.80	2.52, 2.78
female	1.44, 1.47	2.02, 2.12
frozen[b]	1.24, 1.16	1.53, 1.54

[a] Each substrate (1 mM) was incubated under the conditions described under Experimental Procedures. The results of two experiments are shown. [b] Obtained as mixtures of male and female brains; the ages are not known.

Table II. Effects of Various Compounds on the Demethylenation of MDA and MDMA[a]

conditions	demethylenation activity (% of control) MDA	MDMA
complete	100	100
+CO/O_2 (4:1 v/v)	39 ± 11[c]	22, 56*
+SKF-525A (0.1 mM)	108 ± 6	105 ± 8
+α-naphthoflavone (0.01 mM)	99 ± 6	95 ± 9
+iprindole (0.1 mM)	83 ± 9[b]	88 ± 8
+methimazole (0.25 mM)	74 ± 1[c]	66 ± 4[c]
+imipramine (0.1 mM)	79 ± 6[c]	81 ± 3[b]
+desipramine (0.1 mM)	79, 81*	79 ± 4[b]

[a] MDA or MDMA (1 mM) was incubated with microsomes (3.3–3.5 mg of protein) from frozen rat brain in the presence of 0.5 mM NADPH for 20 min. Under these conditions, the activities of MDA and MDMA demethylenation were 1.44 and 1.70 pmol/(min·mg of protein), respectively. The values represent the mean ± SD of three determinations except for the entry marked with an asterisk (*). [b] $p < 0.01$. [c] $p < 0.05$ vs control.

Previous studies with rat liver microsomes showed that DHMA formed from MDMA was oxidized rapidly to the *o*-quinone by superoxide (*9*). Superoxide anion radicals can be formed by microsomes through the uncoupling reaction sequence of cytochrome P450 (*16*), and for this reason, all experiments were carried out in the presence of SOD.

Demethylenation was dependent on the presence of microsomes, substrate, and NADPH. (In the absence of NADPH, there is an 82 ± 1% reduction of MDMA demethylenation and a 69 ± 17% reduction in MDA demethylenation.) The reaction was also shown to be markedly reduced in an incubation atmosphere with CO/O_2 (4:1) (Table II), suggesting that demethylenation of MDMA and MDA is mediated by the cytochrome P450 monooxygenase system.

Catechol formation from MDMA and MDA in incubates of rat brain microsomes was proportional to protein content over a range of 2–8 mg (Figure 2A). The rates of the catechol formation are linear with time for at least 20 min and approached a plateau after 60 min of incubation

[2] Determination of cytochrome P450 content in frozen brain by CO- and dithionite-difference spectra by the method of Omura and Sato (*14*) and Estabrook et al. (*15*), respectively, was not possible, apparently due to the presence of hemoglobin or cytochrome oxidase from mitochondrial fraction as contaminants which interfere with the spectral properties of P450.

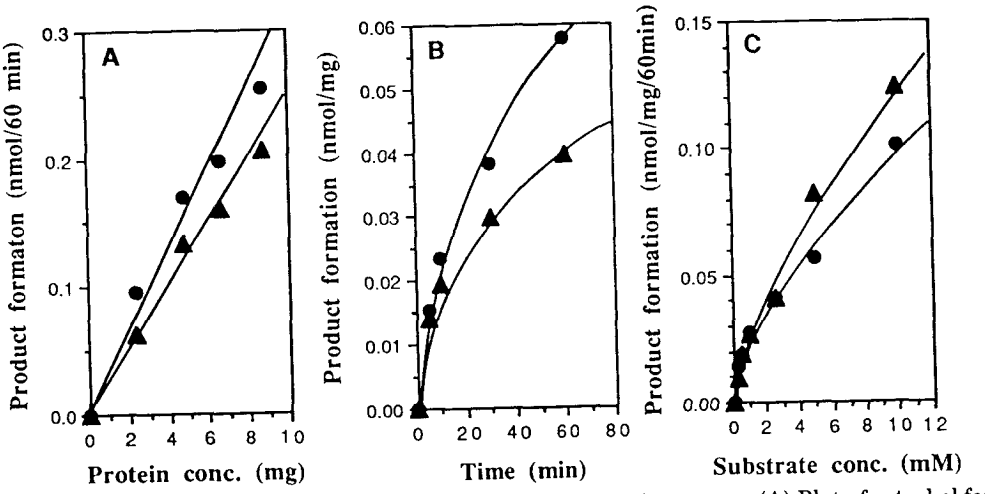


Figure 2. Characterization of MDMA and MDA demethylenation in rat brain microsomes. (A) Plot of catechol formed from 1 mM MDMA or MDA at 60 min vs different microsomal protein concentrations. (B) Time course of catechol formation from 1 mM MDMA or MDA incubated with rat brain microsomes. (C) Plot of catechol formed at 60 min vs substrate concentration. MDA = ●; MDMA = ▲. Each data point is the mean of three determinations.

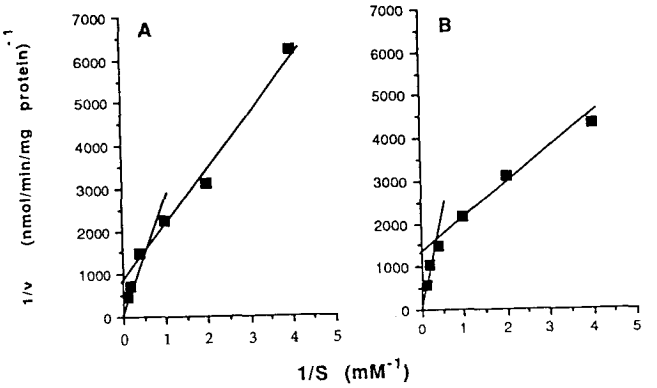


Figure 3. Lineweaver–Burk plots of the conversion of MDMA to DHMA (A) and MDA to DHA (B) by rat brain microsomes.

Table III. Stereochemistry of MDA and MDMA Demethylenation by Rat Brain Microsomes[a]

substrate	demethylenation activity [pmol/(min·mg of protein)] with (+)	with (−)	(+)/(−)
MDA	1.40 ± 0.10	1.34 ± 0.05	1.04
MDMA	1.39 ± 0.06	1.48 ± 0.06	0.94

[a] Each substrate (1 mM) was incubated under the conditions described under Experimental Procedures. Each value is the mean ± SD of three determinations.

(Figure 2B). Lineweaver–Burk plots of demethylenation were nonlinear over the range 0.25–10 mM, suggesting that a multiplicity of isozymes may be responsible for the reaction (Figure 3).

Inhibition of Catechol Formation from MDA and MDMA. Various compounds were added to the reaction mixture to characterize the demethylenation catalyzed by microsomal enzymes. The results, shown in Table II, indicate that desipramine, imipramine, and methimazole inhibited the demethylenation of MDMA and MDA whereas iprindole inhibited only that for MDA. In contrast, SKF-525A and α-naphthoflavone, a selective inhibitor of the cytochrome P450 isozyme IA family (*17*), did not show any inhibitory effect on demethylenation. Table III compares the demethylenation activities of (+) and (−) isomers of MDA and MDMA, which were almost equal.

Role of ·OH in the Demethylenation by Rat Brain Microsomes. The suppression of microsomal demethylenation by ·OH scavengers such as thiourea and benzoate (Table IV) reflected participation of ·OH in the demethylenation reaction. The reaction was also found to be suppressed by addition of desferal (Table IV), an iron

Table IV. Effects of Buffer Components on the Enzymatic and Chemical Demethylenation of MDMA

addition	concentration (mM)	buffer system: phosphate (A)	HEPES (B)	A/B
		Enzymatic[c]		
control		4.81, 5.11	1.76, 2.02	2.6
desferal	0.1	1.60, 1.16	0.99, 0.95	1.4
thiourea	1	3.15, 3.06	1.12, 1.20	2.7
	10	1.45, 1.52	0.89, 0.93	1.6
benzoate	5	3.50, 3.56	1.67, 1.39	2.3
	50	1.40, 1.29	1.51, 0.66	1.3
		Chemical[c]		
control		2.60, 2.77	1.26, 1.29	2.1
desferal	0.1	0.53, 0.43	0.91, 0.90	0.5
thiourea	1	1.93, 2.08	1.23, 1.16	1.7
	10	0.65, 0.70	0.68, 0.63	1.0
benzoate	5	1.02, 1.09	0.79, 0.71	1.4
	50	0.33, 0.38	0.22, 0.22	1.6

[a] Enzymatic demethylenation and chemically promoted demethylenation of MDMA were carried out in the presence of substrate (5 mM) with microsomes of rat brain and the ascorbate system, respectively, under the conditions described in Experimental Procedures. The results of two experiments are shown. [b] Values for enzymatic demethylenation are given in units of pmol/(min·mg of protein). [c] Values for chemical demethylenation are given in units of nmol/min.

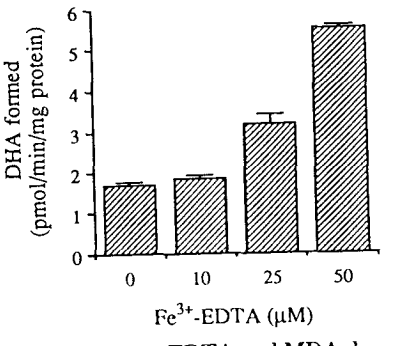


Figure 4. The effect of iron–EDTA and MDA demethylenation by rat brain microsomes. Substrate (1 mM) was incubated under the conditions described under Experimental Procedures. Mixtures of iron–EDTA were prepared by the addition of a 2-fold molar excess of EDTA to ferric chloride. Each value for the mixture is expressed as the concentration of iron.

chelator which blocks the production of •OH by microsomes (*18*), and was augmented by the addition of ferric ion and EDTA (Figure 4). The latter effect is indicative of the ability of a microsomal component to convert ferric iron to ferrous iron, which then generates •OH via a Fenton process.

The possibility that impurities in the buffer used may contribute to •OH generation was considered as phosphate buffer is known to contain small amounts of iron (*19*). A comparison of the effects of phosphate and the HEPES buffers on the demethylenation of MDMA is shown in Table IV. DHMA formation with phosphate buffer was 2.6 times greater than that with the HEPES buffer. Desferal and •OH scavenging agents reduced DHA and DHMA formation in both buffers. The reaction was inhibited in a concentration-dependent manner, and the ratio of DHMA formed in phosphate buffer to DHMA formed in HEPES buffer decreased to almost unity as desferal concentration was increased. Similar results were also obtained by chemical cleavage of the methylenedioxy group of MDMA by the •OH generating system.

Addition of desferal (0.1 mM) to the reaction mixture of fresh male and female and frozen rat brain microsomes in HEPES buffer gave results identical to those without desferal (Table I), indicating that the sex differences in activity were due to cytochrome P450 and not •OH, differentially generated from the microsomes.

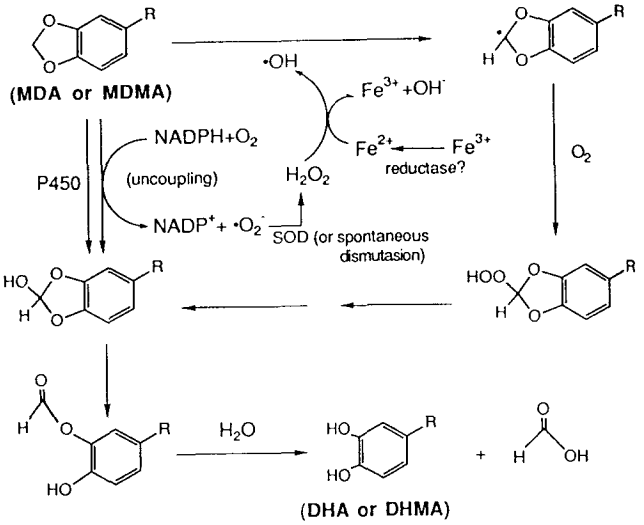
Discussion

Rat brain microsomal preparations are capable of demethylenating MDA and MDMA by a cytochrome P450-mediated process. The reaction requires NADPH and is strongly inhibited when the atmosphere was replaced by a CO/O_2 mixture (4:1 v/v), consistent with involvement of the cytochrome P450 monooxygenase. DHA and DHMA formation by rat and rabbit liver were markedly suppressed by SKF-525A (*9*, *20*), but the compound had no effect on the brain enzyme. This differential inhibition indicates that the cytochrome P450 isoenzyme effecting the reaction in brain is different from that in the liver. As P450 isozymes IA1 and IA2 had been shown to be present in the brain (*21*) by immunological procedures, their involvement was assessed by sensitivity of demethylenation to the selective inhibitor, α-naphthoflavone. This compound also showed no inhibitory effect, indicating that these isozymes were unlikely catalysts. Iprindole, which blocks amphetamine oxidation by cytochrome P450 (*22*, *23*) in rats, inhibits the demethylenation of MDA only.

Methimazole (*N*-methyl-2-mercaptoimidazole), generally considered an inhibitor of the flavin-containing monooxygenase (*24*), also inhibited the reaction. One possible explanation for the observation is that the thiol function of methimazole serves as an •OH scavenger and inhibits the chemical reaction that is participating in the demethylenation. In fact, a dose-dependent inhibition (up to 96%) of •OH-mediated demethylenation by methimazole was observed (data not shown). Other studies have demonstrated that the flavin-containing monooxygenase or cytochrome P450 catalyzes the conversion of methimazole to an active metabolite that decreases microsomal cytochrome P450 content (*25*, *26*) and reactions (*26*, *27*). Although the presence of flavin-containing monooxygenase in the brain has been reported (*28*), its unstable nature suggests that its activity in the frozen brain preparations would be low, and these results would argue against its participation in the reaction.

On the basis of data showing that the inhibition of the uptake system can protect serotonergic neurons from the

Scheme I. Proposed Direct and Indirect Role of Cytochrome P450 in the Demethylenation of (Methylenedioxy)amphetamines



toxic effects of (+)-MDMA, Schmidt hypothesized that the (+)-MDMA-derived toxin is a substrate for the 5-HT uptake carrier (*7*). However, as uptake inhibitors such as desipramine and imipramine also inhibit metabolism, their protective actions may reflect changes in metabolism as well. Demethylenation by brain microsomes did not exhibit stereoselectivity. However, the nonlinear Lineweaver–Burk plot (Figure 3) indicates a multiplicity of isozymes, and as there is a differential isozyme distribution in brain regions (*29*), it is possible that some regions may exhibit stereoselectivity. The toxicity of the catechol metabolite of MDA has been demonstrated in studies with NG108-15 cells, which have properties similar to those of a 5-HT nerve terminal (*30*). If MDA and MDMA toxicities were associated with the production of the catechols, a stereoselective difference in the demethylenation would be expected. The selective toxicity toward the serotonergic neurons could be related to localization of the cytochrome P450 isozymes that are involved in the oxidation of these compounds.

The low demethylenation activity by whole-brain microsomes is noteworthy as it is about one-thousandth that of the liver (cf. ref 9). This low activity introduces an artifact due to the chemical demethylenation reaction that can proceed simultaneously unless appropriate measures are taken to eliminate iron. As shown in Scheme I, superoxide is released during NADPH–microsomal electron transfer (*16*). The superoxide is converted to hydrogen peroxide by spontaneous dismutation or by the action of SOD, which is added in these incubations to prevent further oxidation of DHA and DHMA. The stimulation of the demethylenation by external ferric–EDTA and the inhibition by desferal, which nearly completely abolished the production of •OH by microsomes, indicate that the brain microsomes have the potential to oxidize MDA or MDMA by two pathways, the cytochrome P450-dependent system and the •OH-dependent system proposed by Cederbaum and Dicker (*18*). Addition of ferric–EDTA increased catechol formation in a concentration-dependent manner, demonstrating the capability of brain microsomal components to reduce ferric iron to ferrous ion, which reacts with hydrogen peroxide to produce •OH via the Fenton-type reaction (*31*). Winston and Cederbaum (*32*) have pointed out that NADPH–cytochrome P450 reductase primarily contributes to the •OH-dependent oxidation. The relatively high amount of this flavoprotein present in

the brain (*33*) also argues for its role as a reductant in the generation of •OH. The addition of 100 units of catalase, the scavenger of hydrogen peroxide, resulted in a 13 ± 2% and 16 ± 1% decrease in the DHA formation from MDA and DHMA formation from MDMA, respectively. The weak inhibition by catalase may indicate that the hydrogen peroxide is generated at the active site of the enzyme and reacts directly, thereby limiting access to catalase (*34*, *35*).

Recently, Steele et al. (*36*) reported a high enzyme activity for MDMA demethylenation by brain microsomes, almost equal to the activity observed in liver microsomes. The discrepancy between their results and ours is likely due to the use of phosphate buffer. As shown above, the iron contained in the phosphate buffer can act as a source of catalytic iron available for the microsomes to generate •OH and, depending on its concentration, could exceed the activity of the cytochrome P450 reaction. However, other metal ions such as copper could also effect the reaction. This possibility was confirmed by the studies conducted with HEPES buffer, which itself has the ability to scavenge •OH (*37*). Similar interpretations must be applied to the work of Lim and Foltz (*11*), as they used phosphate buffer as well. Thus, although cytochrome P450 in the rat brain catalyzes the demethylenation of both MDA and MDMA, artificially high levels of activity might be observed unless precautions are taken to block the iron-mediated •OH reaction.

In summary, these studies demonstrate that brain cytochrome P450 can effect the demethylenation of MDA and MDMA, but at very low levels of overall activity. The reaction must, however, be conducted with appropriate care to avoid contributions from a •OH-mediated reaction.

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The Calcium-Binding Protein Calreticulin Is Covalently Modified in Rat Liver by a Reactive Metabolite of the Inhalation Anesthetic Halothane

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A general procedure is presented for the isolation of several liver microsomal target proteins of the reactive trifluoroacetyl halide metabolite of halothane. It was found that most of these proteins could be selectively extracted from microsomes with 0.1% sodium deoxycholate and separated into partially purified fractions by DEAE-Sepharose anion-exchange chromatography. Using this method, we describe the isolation and identification of a 63-kDa target protein of halothane in rat liver. Amino acid sequences of the N-terminal and of several internal peptides of the protein, as well as the deduced amino acid sequence of a nearly full-length rat liver cDNA clone of the protein, showed 98% identity with a reported murine cDNA that encodes for calreticulin, a major calcium-binding protein of the lumen of endoplasmic reticulum. Although it remains to be determined what role calreticulin has in the development of halothane hepatitis, this study has shown that calreticulin can be a target of reactive metabolites of xenobiotics.

Introduction

It has been estimated that more than 600 drugs cause hepatic injury (*1*, *2*). These compounds can be classified as being either intrinsic or idiosyncratic hepatotoxins (*3*). In both cases, however, reactive metabolites have been implicated in producing the toxicity (*4*). For example, reactive metabolites of intrinsic hepatotoxic agents are thought to cause toxicity by covalently altering cellular macromolecules either directly or indirectly. It is believed that they indirectly cause these modifications by causing lipid peroxidation and protein cross-linking and S-thiolation (*4-6*). These reactions may lead to the inactivation of enzymes, to the disruption of intracellular calcium homeostasis, or to a general loss of cellular membrane integrity (*4-7*).

Idiosyncratic drug-induced hepatic damage also might be attributed to similar processes that for some reason are very host dependent. It could be due to the presence of abnormally high levels of enzymes that convert the drug into a reactive metabolite, a mutant form of the enzyme that is catalytically more active than the normal enzyme, or abnormally low levels or activities of enzymes that detoxify either the reactive metabolite or its precursor. Alternatively, a reactive metabolite may produce an idiosyncratic drug-induced hepatotoxicity by an allergic or hypersensitivity reaction that is directed against a covalently altered tissue macromolecule (neoantigen) (*3*).

Lack of knowledge about the target macromolecules in the liver of reactive metabolites of drugs has slowed the elucidation of the mechanism of hepatotoxicity (*4*). In this regard, we have started to characterize liver neoantigens associated with the idiosyncratic hepatotoxicity produced by the inhalation anesthetic, halothane. Patients with this toxicity have been shown by immunoblotting to have serum antibodies that react with at least five distinct rat liver microsomal polypeptide fractions (100, 76, 59, 57, and 54 kDa), which are covalently altered by the trifluoroacetyl

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