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INACTIVATION OF CONSTITUTIVE HEPATIC CYTOCHROMES P450 BY PHENCYCLIDINE IN THE RAT

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ABSTRACT:

The purpose of this study was to determine whether phencyclidine (PCP) inhibits constitutive hepatic cytochrome P450 (CYP) isozymes when administered to naive adult male Sprague-Dawley rats. Animals were pretreated with PCP (25 mg/kg/day for 2 days), killed 3 and 16 hr after the last dose, and liver microsomes prepared. The washed microsomes were then assayed for benzphetamine, methamphetamine (MA), and methylenedioxymethamphetamine (MDMA) *N*-demethylation together with MDMA demethylenation and MA 4-hydroxylation activities. MDMA demethylenation (low substrate concentration), MA 4-hydroxylation, and metoprolol α-hydroxylation reactions, which are catalyzed by CYP2D isozymes, were reduced >74% 3 hr after the last PCP dose and were only partially restored 13 hr later. Benzphetamine and (−)-MDMA *N*-demethylation activities were restored to control values 16 hr after the last dose. These results indicate that PCP suppresses constitutive isozymes, including CYP2C11 and members of the CYP2D subfamily. The suppression of cytochromes P450 activity by PCP *in vivo* is consistent with its *in vitro* actions found in this and other studies, and demonstrates that alteration of CYP activity is another pharmacological effect of this compound.

PCP[2] (fig. 1) is a psychotomimetic agent with a history of abuse (1). Its action as a noncompetitive inhibitor of the NMDA receptor has resulted in the extensive study of it and its congeners as research tools (2, 3) and potential therapeutic agents (4). In abuse situations and in some research protocols, the drug is administered repeatedly and occasionally with other drugs. One of the reported properties of PCP (5–7) and its congeners (8) is the mechanism-based inhibition of CYP. In these studies, the focus was mostly on *in vitro* experiments with phenobarbital-inducible isozymes in the rabbit (6) and rat (7). Thus, Osawa and Coon (6) have demonstrated that PCP inhibits CYP2B4 isozyme without affecting CYP1A1, 1A2, 2C3, and 2E1 isozymes in liver microsomes from phenobarbital-pretreated rabbits. Crowley and Hollenberg (7) studied the actions of the drug in reconstituted preparations of rat liver CYP2B1 and demonstrated irreversible inhibition and binding of the drug.

PCP also affects the CYP system when administered to intact animals. Ho *et al.* (9) have found that chronic administration of PCP to mice caused an increase in hydroxylase and demethylase activities. In studies with rats, Owens *et al.* (10) report that repeated administration to rats caused a decrease in the ability of microsomes to bind

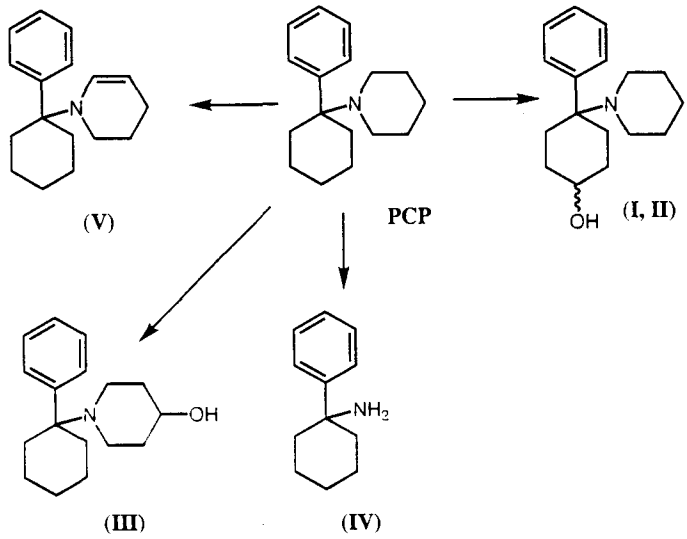

FIG. 1. *Structures of PCP and measured metabolites.*

radiolabeled PCP. We report herein results of a study examining the CYP activities of liver microsomes after systemic administration of PCP to rats. The study focused on the effects of PCP on the metabolic transformations of amphetamine derivatives (fig. 2) that are catalyzed by CYP2C11 and members of the CYP2D subfamily, the major constitutive isozymes associated with the metabolism of these and other abused substances. MDMA and MA are oxidized on the aromatic ring by members of the CYP2D family; MDMA is demethylenated (11) and MA is 4-hydroxylated (unpublished observations). CYP2C11 seems to play an important role in the *N*-demethylation of MA (12) and benzphetamine (13). The effect of PCP on the metabolism of these compounds is of interest because of the potential for drug interaction in poly drug abuse situations and the possible alterations of CYP activity associated with repeated exposure to PCP. This paper describes the results of the study.

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[2] Abbreviations used are: PCP, phencyclidine; CYP, cytochrome P450; MDMA, 3,4-methylenedioxymethamphetamine; MA, methamphetamine; DHMA, 3,4-dihydroxymethamphetamine; α-HM, α-hydroxymetoprolol; TFAA, trifluoroacetic anhydride; BSTFA, *N,O*-bis(trimethylsilyl-trifluoroacetamide; SOD, superoxide dismutase; HPLC-ECD, High-pressure liquid chromatography-electrochemical detector; MDA, 3,4-methylenedioxyamphetamine; TFA, trifluoroacetic acid; TMS, trimethylsilyl.

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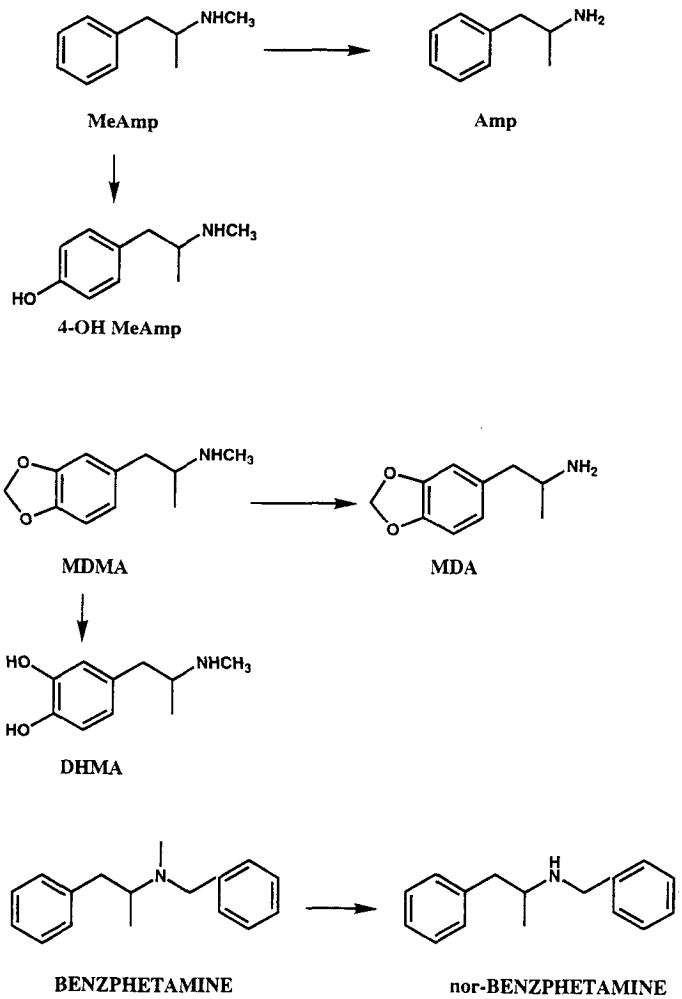

FIG. 2. *Metabolic reactions of amphetamine derivatives.*

Materials and Methods

1-Phenylcyclohexylamine hydrochloride (**IV**) (see fig. 1 for structures), *cis*, and *trans*-4-phenyl-4-piperidinocyclohexanol (**I**) and (**II**), 1-(1-phenylcyclohexyl)4-hydroxy piperidine (**III**), (+)- and (−)-MDMA hydrochloride and (+)-MA hydrochloride were obtained from the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD). PCP HCl (14) and (−)-MA hydrochloride (15) were prepared by previously reported procedures. DHMA hydrochloride, the product of MDMA demethylenation, was prepared by modification of the procedure of Smissman and Borchardt (16). Metoprolol tartarate, α-HM, and guanoxane sulfate were gifts from Dr. M. S. Lennard, University Department of Therapeteutics, Royal Hallamshire Hospital. Benzphetamine hydrochloride was a gift from the Upjohn Co. (Kalamazoo, MI). NADP, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, and HEPES were purchased from Aldrich Chemical Co. (Milwaukee, WI). TFAA and BSTFA were from Pierce Chemical Co. (Rockford, IL). All other compounds used were of reagent grade.

Pretreatment of Animals and Preparation of Hepatic Microsomes. Adult male Sprague-Dawley rats (weighing 250–300 g) were used. PCP HCl was dissolved in 0.9% NaCl. Rats were injected with PCP HCl (25 mg/kg ip) once daily for 2 days and killed 3 or 16 hr after the last injection. This relatively high dose is in the range used to protect animals against the toxic effects of MA (17) and in chronic studies (18). Control animals were given 0.9% NaCl (1.0 ml/kg) alone. Rats were decapitated and the livers (10 g) were homogenized in 30 ml of 1.15% KCl using a Potter-Elvehjem homogenizer and a Teflon pestle. Homogenates were centrifuged at 9,000*g* for 20 min and the supernatants recentrifuged at 105,000*g* for 60 min. The pellets were washed twice by resuspension in fresh 1.15% KCl and 0.1 M pyrophosphate containing 0.1 mM EDTA (pH 7.4) and recentrifugation at 105,000*g* for 30 min. The washed pellet was stored under 0.5 ml of 1.15% KCl at −80°C. For incubation, the thawed pellet was resuspended in 1.15% KCl and diluted to an appropriate concentration. The protein concentration was determined by the Bio-Rad (Hercules, CA) protein assay using bovine serum albumin as the standard. Total CYP content in the suspension was determined from the sodium dithionite-reduced carbon monoxide difference spectrum using a molar extinction coefficient of 91 cm^{-1} mM^{-1} between 450 and 490 nm (19).

***In Vitro* Inactivation.** Experiments on the inactivation of microsomal CYP were conducted in two stages. The first stage reaction mixture consisted of 100 mM HEPES buffer (pH 7.6), microsomal preparation (1–3 mg of protein), 1 mM EGTA, the desired concentration of PCP and an NADPH-generating system composed of 0.5 mM NADP, 4 mM $MgCl_2$, 8 mM glucose 6-phosphate, and 1 unit of glucose 6-phosphate dehydrogenase/ml (final concentrations) in a volume of 3.0 ml. Controls were incubated for the same length of time, but did not contain PCP. In the second stage, aliquots of 100 μl were taken from the first-stage mixtures and examined for metabolic activity by dilution to 1 ml. The second-stage reaction mixture for the benzphetamine *N*-demethylase assay (8) contained 1 mM benzphetamine, the microsomal fraction (0.1 ml) to be analyzed, and the same NADPH-generating system and buffer as described in a total volume of 1.0 ml. The incubation mixture for MDMA demethylenation used the same buffer and cofactors described herein, but contained 100 units of SOD and 1 mM MDMA.

MDMA Demethylenation. The demethylenation of MDMA was determined by a HPLC-ECD method based on that described by Kumagai *et al.* (20). The incubation mixture consisted of 100 mM HEPES buffer (pH 7.4), microsomal preparation (0.1–1.0 mg of protein), and an NADPH-generating system composed of 0.5 mM NADP, 8 mM glucose 6-phosphate, 5 mM $MgCl_2$, 1 unit of glucose 6-phosphate dehydrogenase, (+)- or (−)-MDMA (10 μM or 10 mM), and 100 units of SOD in a final volume of 1.0 ml. After preincubation for 5 min, the reactions were initiated by the addition of the enzyme preparation and were terminated by the addition of 0.5 ml of 7.5% (w/v) perchloric acid. Incubations were conducted for 5 min at 37°C. The quenched reaction mixture was centrifuged at 13,500*g* for 5 min, and a portion of the supernatant (10 μl) was analyzed by an HPLC-ECD system.

DHMA Analysis by HPLC-ECD. MDMA metabolites were separated by reversed-phase HPLC using Biophase ODS column (4.6 × 250 mm, 5 μM, Bioanalytical System, Inc., West Lafayette, IN) and a mobile phase consisting of 0.1 M citrate buffer (pH 3.5), containing 1 mM sodium octylsulfate: acetonitrile:methanol (10:1:1, v/v) at a flow rate of 0.7 ml/min. DHMA was detected by an electrochemical detector set at + 0.7 V. Signals were recorded with a Hewlett-Packard 3390A recording integrator, and the peak height of metabolite was compared with that of standard samples. Retention time for DHMA was ~11.3 min.

MDMA *N*-demethylation. (+)- and (−)-MDMA (5 mM) were incubated under the same conditions as described, and the MDA produced was determined by GC/MS (20). Reactions were terminated by the addition of 0.5 ml of 7.5% (w/v) $HClO_4$ containing internal standard, [$^2H_2$]MDA (2.5 nmol) and the incubation tubes were kept on ice for 30 min. After centrifugation (13,500*g* for 5 min), 0.3 ml of supernatant was transferred to a 50-ml extraction tube containing 2 ml of 1.5 M $NaHCO_3$:Na_2CO_3 buffer (pH 9.5) and 10 ml of CH_2Cl_2. The resulting mixture was shaken for 10 min. After centrifugation (2900 rpm for 10 min), the organic layer was collected and evaporated down to 50 μl under N_2. Then 20 μl of CH_3CN was added, followed by 100 μl of TFAA, mixed (vortex mixer), and allowed to stand at room temperature for 5 min. Excess TFAA was then allowed to evaporate upon standing, and the sample was reconstituted in 120 μl CH_3CN. A volume of 1 μl was then injected into the GC/MS system.

MDA Analysis by GC/MS. MDMA metabolites were separated by GLC using a capillary column (12.5 m × 0.2 mm) with cross-linked methylsilicone, 0.33 μm film thickness, operated with a temperature program from 65°C to 250°C at rate of 30°C/min. Under these conditions, the retention time for MDA-TFA was 5.5 min. A Hewlett-Packard 5971A GC/MS system was used in the selected-ion monitoring mode with [$^2H_2$]MDA as internal standard. The mass spectrometer was operated in its specific ion detection mode focused on *m/z* 162. Quantitation was achieved by comparing the mass spectrometer responses at *m/z* = 162 and 164 (internal standard). The internal standard used in the assay was [$^2H_2$]MDA of 99.9% isotopic purity.

MA *N*-demethylation. The incubation mixture for MA demethylation used

TABLE 1

Effects of PCP on the metabolism of metoprolol, MDMA, and MA

Treatment	Metoprolol α-HM	MDMA (10 μM) DHMA (+)	MDMA (10 μM) DHMA (−)	MDMA (10 mM) DHMA (+)	MDMA (10 mM) DHMA (−)	MA (20 μM) 4-OHMA (+)	MA (20 μM) 4-OHMA (−)
Saline	0.52 (100)	0.59 (100)	0.48 (100)	2.24 (100)	2.40 (100)	137.87 ± 46.46 (100)	166.58 ± 45.46 (100)
PCP (3 hr)	0.12 (24)	0.15 (25)	0.12 (26)	1.61 (72)	1.66 (69)	27.10 ± 5.35 (20)	50.31 ± 8.59 (30)
PCP (16 hr)	0.21 (40)	0.24 (59)	0.18 (38)	1.76 (79)	2.04 (85)	49.18 ± 11.17 (29)	83.35 ± 16.72 (50)

Groups of five rats were given PCP HCl (25 mg/kg, ip, daily) for 2 days and killed 3 or 16 hr after the final dose. Control animals were given saline (1.0 ml/kg) in the same regimen. Liver microsomes were prepared from each group of animals and the activities evaluated by procedures described in *Materials and Methods*. Metoprolol and MDMA analyses were performed in duplicate. MA conversion to 4-hydroxy-MA (4-OHMA) was monitored in triplicate, and the results shown are the mean ± SD of two separate experiments. Values are expressed as nmol/min/mg protein, with the percentage of activity of the saline control in parentheses.

the same concentration of buffer and cofactors as described, but without SOD. The final concentration of (+)- and (−)-MA was 20 μM, and the amphetamine produced was determined by a GC/MS method described earlier (21). Reactions were initiated at 37°C by the addition of the enzyme preparation, terminated by the addition of 0.5 ml of 7.5% (w/v) $HClO_4$ containing internal standard, [$^2H_3$]amphetamine (2.5 nmol), and the immersion of the incubation tubes in an ice bath. After centrifugation (13,500*g* for 5 min), 0.4 ml of supernatant was transferred to a 20-ml extraction tube containing 1 ml of 10% Na_2CO_3, 0.5 g NaCl, and 6 ml of isopropanol:CH_2Cl_2 (1:4). The resulting mixture was shaken for 10 min. After centrifugation (2000 rpm for 10 min), the organic layer was collected and evaporated to dryness under N_2. Then, 50 μl of CH_3CN was added, followed by derivatization with 100 μl of TFAA and heating at 60°C for 15 min. TFAA was allowed to evaporate under a stream of nitrogen, and the residue was reconstituted in 100 μl CH_3CN. A volume of 1 μl was then injected into the GC/MS system.

Amphetamine Analysis by GC/MS. MA metabolites were separated by GC using a capillary column (12.5 m × 0.2 mm) with cross-linked methylsilicone, 0.33 μm film thickness, operated with a temperature program from 65°C to 190°C at a rate of 30°C/min. Under these conditions, the retention time for amphetamine-TFA was 4.0 min. A Hewlett-Packard 5971A GC/MS system was used in the selected-ion monitoring mode with [$^2H_3$]amphetamine as internal standard. The mass spectrometer was operated in its specific ion detection mode focused on *m/z* 140. Quantitation was achieved by comparing the mass spectrometer responses at *m/z* = 140 and 143 (internal standard). The internal standard used in the assay was [$^2H_3$]amphetamine of 99.9% isotopic purity.

Metoprolol α-Hydroxylation. The α-hydroxylation of metoprolol was determined by an HPLC method based on that described by Lennard *et al.* (22). The incubation mixture consisted of 0.2 M phosphate buffer (pH 7.4) microsomal preparation (0.1–1.0 mg of protein), 0.1 mM metoprolol dissolved in 1.15% (w/v) KCl solution, and an NADPH-generating system composed of 1 mM NADP, 5 mM $MgCl_2$, 10 mM glucose 6-phosphate, and 1 unit of glucose 6-phosphate dehydrogenase/ml, in a final volume of 1.0 ml. Reactions were initiated at 37°C by addition of the microsomal preparation and were terminated by transferring 0.45 ml of the incubation mixture to capped plastic vials containing 6% (v/v) perchloric acid solution (50 μl) and guanoxane sulfate (30 μl of a 2 μg/ml solution) as the internal standard. After centrifugation and precipitation of microsomal protein, 0.45 ml of the supernatant was transferred to glass tubes containing 0.5 ml of 4 M NaOH and 5 ml of methyl *tert*-butyl ether. The tubes were vortexed for 2 min and then centrifuged at 1,500 rpm for 10 min. The organic layer was transferred to a glass test tube and evaporated to dryness under a stream of nitrogen at room temperature. The residue was dissolved in 200 μl of mobile phase, which had been adjusted to pH 3, and 100 μl was injected into the HPLC column.

α-HM Analysis by HPLC. Metoprolol metabolites were separated by reversed-phase chromatography using Biophase ODS column (4.6 × 250 mm, 5 μm, Bioanalytical System, Inc., West Lafayette, IN) and a mobile phase consisting of water:acetonitrile (88:12, v/v) and 1% (w/v) triethylamine adjusted to pH 3 with orthophosphoric acid at a flow rate of 1.0 ml/min. α-HM was detected by Hewlett-Packard 1064 fluorescence detector with an excitation wavelength of 193 nm and an emission wavelength of 280 nm. The concentration of α-HM was estimated from a standard curve based on peak area ratios. Retention times for α-HM and internal standard, guanoxane, were 8.2 and 16.5 min, respectively.

PCP Metabolism. Incubation of PCP with microsomes and subsequent GLC analysis of its metabolites were conducted as previously reported (23). The incubation mixture consisted of 0.1 M potassium phosphate buffer (pH 7.4), microsomal preparation (1–3 mg of protein), and an NADPH-generating system composed of 0.5 mM NADP, 8 mM glucose 6-phosphate, 5 mM $MgCl_2$, 1 unit of glucose 6-phosphate dehydrogenase, and 0.5 mM PCP HCl in a final volume of 5.0 ml. After incubation, the mixture was transferred to glass tubes containing 10 ml of ice-cold CH_2Cl_2 containing internal standard, extracted by shaking for 20 min, and then centrifuged at 3,000 rpm for 10 min. The organic layer was collected and evaporated under a stream of N_2 to ~200 μl. Samples of the underivatized mixture were injected into the GC for analysis of PCP, 1-phenylcyclohexylamine, and 1-(1-phenylcyclohexyl)-1,2,3,4-tetrahydropyridine. BSTFA (100 μl) was then added and the mixture heated at 50°C for 1 hr for analysis of the hydroxylated compounds. An aliquot of 2 μl was then injected into the GC.

Analysis of PCP Metabolites by GC. A Hewlett-Packard 5890 gas chromatograph fitted with an HP-5 capillary column with 0.32 μm thickness, 0.2 mm i.d., and 25 m length was used. The conditions of chromatography were: carrier gas flow (He), 0.7 ml/min; injection port temperature, 210°C; flame ionization detector temperature, 220°C; and oven temperature, 200°C. The retention time (min) of the measured compounds were: PCP (6.3), 1-(1-phenylcyclohexyl)-4-hydroxypiperidine TMS (**III**, 14.94), 1-phenylcyclohexylamine (**IV**, 2.3), *cis* (**I**, 14.14), and *trans*-4-phenyl-4-piperidinocyclohexanol TMS (**II**, 13.79), and 1-(1-phenylcyclohexyl)-1,2,3,4-tetrahydropiperidine (**V**, 7.46). The area ratio of the compounds analyzed to the internal standard (500 nmol of benzphetamine) was calculated and compared with a standard curve that was conducted through each experiment. Compounds **IV**, **V**, and PCP were analyzed separately from the others, because the sample was not derivatized.

Results

Liver microsomes, collected from rats pretreated with PCP (25 mg/kg) for 2 days and killed 3 hr after the final dose, exhibited altered metabolic activities. Thus, α-hydroxylation of the CYP2D substrate, metoprolol (22), was suppressed to approximate 24% of control (table 1). MDMA demethylenation activity was determined at two concentrations, because prior studies (11) had shown that different CYP isozymes predominate in the catalysis of the reaction at these two concentrations. Demethylenation at low substrate concentrations (10 μM) was decreased to approximately the same level as metoprolol [25% for (+)-MDMA and 26% for (−)-MDMA], but high substrate concentration (10 mM) activity was affected less and decreased to ~72% [for (+)-MDMA] and 69% [for (−)-MDMA] of control (table 1). The 4-hydroxylation of MA is also catalyzed by CYP2D (unpublished observations), and the 3-hr data are consistent with that notion. Sixteen hr after the last PCP dose, activity had recovered somewhat

TABLE 2

Effects of PCP on microsomal N-demethylation activity of benzphetamine, MDMA, and MA

Treatment	Benzphetamine (1.0 mM) HCHO Formed	MDMA (5 mM) MDA Formed		MA (20 μM) Amphetamine Formed	
		(+)	(−)	(+)	(−)
	nmol/mg protein/min	*nmol/mg protein/min*		*pmol/mg protein/min*	
Saline (3 hr)	11.3 (100)	0.17 ± 0.27 (100)	0.18 ± 0.005 (100)	39.7 ± 15.1 (100)	70.0 ± 3.8 (100)
PCP (3 hr)	7.9 (70)	0.10 ± 0.004 (59)	0.12 ± 0.005 (67)	24.6 ± 1.7 (62)	29.4 ± 3.3 (42)
PCP (16 hr)	11.4 (101)	0.11 ± 0.011 (63)	0.17 ± 0.022 (94)	26.1 ± 9.3 (66)	40.7 ± 2.6 (58)

Rats were pretreated with PCP HCl in the same manner as described in table 1. Microsomes from five rats were pooled and evaluated for metabolic activities. Benzphetamine, MDMA, and MA were incubated with liver microsomes in the presence of NADPH-generating system at 37°C for 10, 5, and 5, min respectively. Except for benzphetamine-*N*-demethylation, which was assayed in duplicates, data represent the mean value of three incubations ± SD, with the percentage of control activity indicated in parentheses.

and microsomal metoprolol α-hydroxylation and (+)- and (−)-MDMA demethylenation at low substrate concentration had returned to 40%, 59%, and 38%, respectively, of control. (+)- and (−)-MDMA demethylenation at high concentration was restored to ~79% and 85% of control, respectively (table 1). Other CYP reactions were also examined. Three hr after the last PCP dose, benzphetamine *N*-demethylation activity decreased to ~70% of control and (+)- and (−)-MDMA and (+)- and (−)-MA *N*-demethylation activities were suppressed to approximate 59% [for (+)-MDMA], 67% [for (−)-MDMA], 62% [for (+)-MA], and 42% [for (−)-MA] of control (table 2). The recovery of activity varied with the substrate and, in the case of MA, the configuration of its α-carbon. Sixteen hr after the last dose of PCP, *N*-demethylation activities of benzphetamine and (−)-MDMA were restored to control values, whereas those of (+)-MDMA and MA were not.

Metabolism of PCP by PCP-Pretreated Rat Liver Microsomes. The metabolism of PCP was investigated in microsomes from both naive and PCP-pretreated rats. Concentrations of PCP, *cis*-, and *trans*-4-phenyl-4-piperidinocyclohexanol (**I** and **II**), 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (**III**), 1-phenycyclohexylamine (**IV**), as well as the relative levels of the tentatively identified *N*-(1-phenylcyclohexyl)1,2,3,4-tetrahydropyridine (**V**), were measured by GC-flame ionization detector. The metabolites generated and the substrate consumed at a PCP concentration of 0.5 mM with fortified liver microsomes from PCP-treated rats are shown in table 3. Microsomal PCP consumption was slightly increased (129%) in the 3-hr preparations and increased to 240% of control in the 16-hr preparation. However, the metabolic formation of **I**, **II**, **III**, **IV**, and **V** were decreased to 74, 71, 82, 87, and 60% of control, respectively, in the 3-hr posttreatment microsomes. On the other hand, in the 16-hr posttreatment micromes, the formation of **I**, **II**, and **III** were increased to 123%, 113%, and 123% respectively, whereas the formation of **IV** and **V** was slightly decreased.

The nature of the inhibitory activity elicited by PCP after administration was examined *in vitro* to assess its similarity with observations made earlier in rabbit preparations (5, 8, 6). Incubation of untreated rat liver microsomes with PCP resulted in NADPH-dependent loss of benzphetamine *N*-demethylation and MDMA demethylenation activities. The decrease in these activities by PCP was concentration-dependent between 0 and 1.0 mM. Under these conditions, maximal inhibition occurred when PCP concentration was 1.0 mM, and the remaining activities were 55% for benzphetamine and 35% for MDMA (fig. 3). However, no suppression of activity was observed when either PCP or NADP was omitted from these incubation mixtures (data not shown). Exposure of the microsomes to PCP (1 mM) in the presence of NADPH-generating system for 60 min did not inhibit activity completely. Benzphetamine and MDMA activities were 50% and 30% of control, respectively (results not shown).

Effect of the Cyanide Ion. The presence of cyanide ion in the incubation mixture protected microsomal P450 against the inactivation by PCP (table 4). This protective effect of cyanide ions was concentration-dependent, ranging from no observable effect at 10 μM NaCN to maximal protection at 100 μM NaCN. This effect is attributed to the formation of the α-iminonitrile (5), suggesting that oxidation of the α-carbon of the piperidine ring of PCP is an essential step in the inhibition process.

Discussion

PCP administration to naive rats caused a decrease in metoprolol α-hydroxylation and (+)- and (−)-MDMA demethylenation (low K_M) activities of liver microsomes (table 1). Both of these reactions have been shown to be catalyzed by CYP2D isozymes (11, 22). Benzphetamine, (+)-, (−)-MA *N*-demethylation, and (+)- and (−)-MDMA demethylenation (high K_M) and *N*-demethylation were also decreased but less effectively (tables 1 and 2). The inability to reverse the PCP inhibition by washing the microsomes indicates the effect is irreversible, possibly due to turnover-based conversion of PCP to an irreversible inhibitor (5, 6, 8).

These results are consistent with the changes in PCP binding to microsomes reported by Owens *et al.* (10). These workers found that continued administration of PCP to rats for 1–4 days caused a reduction in the binding of radiolabeled PCP to microsomal protein. When

TABLE 3

Effect of PCP administration on PCP metabolism by liver microsomes

Treatment	PCP Consumed	Metabolites Formed				
		I	II	III	IV	V
Saline (3 hr)	21.02 ± 6.73 (100)	0.95 ± 0.08 (100)	1.20 ± 0.16 (100)	12.15 ± 0.82 (100)	3.99 ± 0.25 (100)	0.015 ± 0.003 (100)
PCP (3 hr)	27.08 ± 12.7 (129)	0.70 ± 0.09 (74)	0.85 ± 0.12 (71)	0.98 ± 0.89 (82)	3.48 ± 0.34 (87)	0.009 ± 0.001 (60)
PCP (16 hr)	50.50 ± 2.25 (240)	1.17 ± 0.04 (123)	1.36 ± 0.10 (113)	14.91 ± 0.60 (123)	3.84 ± 0.37 (96)	0.013 ± 0.005 (87)

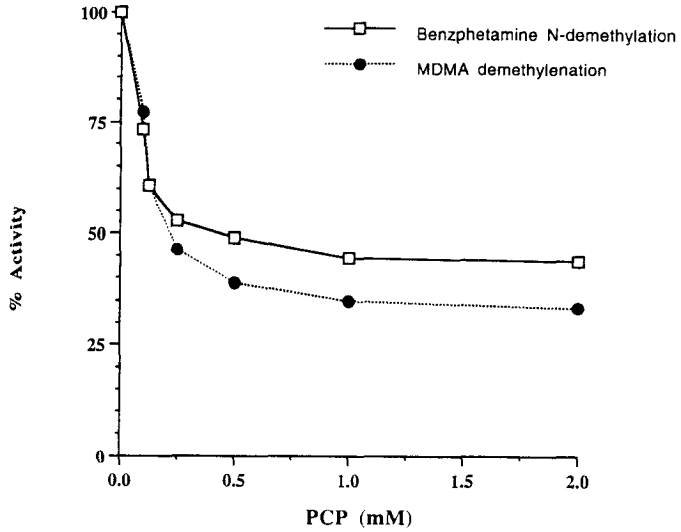

FIG. 3. *In vitro effects of PCP on benzphetamine N-demethylation and MDMA demethylenation.*

Microsomes from livers of untreated rats were exposed to PCP at the indicated concentrations for 30 min under conditions described in *Materials and Methods*. Treated microsomes were then assayed for *N*-demethylation (*open squares*) and demethylenation activity (*closed circles*) as described. Results are expressed as the percentage of activities of microsomes preincubated in the absence of PCP.

TABLE 4

Effects of cyanide on the inhibition of microsomal benzphetamine N-demethylase activity by PCP

NaCN	HCHO Formed		% Inhibition
	With PCP	Without PCP	
μM	*nmol/mg protein/min*		
0	6.02 ± 0.20	10.13 ± 0.17	40.6
10	6.72 ± 0.15	10.93 ± 0.20	38.5
25	7.98 ± 0.16	11.02 ± 0.13	27.6
50	8.12 ± 0.12	10.21 ± 0.14	20.5
100	8.44 ± 0.27	9.63 ± 0.08	12.4

Microsomes were incubated with or without PCP (1.0 mM) at 30°C for 30 min in HEPES buffer + NaCN as described in *Materials and Methods*. After this incubation, aliquots were taken from the mixtures and examined for benzphetamine *N*-demethylation activity. Each value represents the mean ± SD of three determinations.

infused for 20 days, however, the binding had returned to predosing levels. The binding was proposed to be CYP2D-dependent on the basis of its inhibition by quinine and quinidine. Thus, both their results and those reported herein indicate that PCP treatment for up to 4 days causes a reduction in CYP2D activities in liver microsomes. However, this inhibitory effect is not observed after continued dosage at 20 days, suggesting that either induction has occurred or the liver has somehow compensated for the initial loss in CYP2D, for example, by increasing activity of an alternate metabolic pathway. This recovery may also account for the results of Ho *et al.* (9), because their dosage regimen was over a 7-day period. The temporal changes in CYP are likely to be different in rats and mice.

The *in vitro* experiments with microsomes from untreated animals were consistent with metabolic activation of PCP, because the inhibition in that paradigm was dependent on NADPH. The reaction occurring in microsomes from untreated animals seems to be similar if not identical to that occurring in phenobarbital-induced preparations, whereas coincubation with cyanide provides partial protection in the same manner found earlier by Hoag *et al.* (5). Recovery from such an irreversible process could require protein or prosthetic group turnover, and the partial restoration of activity observed between 3 and 16 hr may reflect that process, with the differences in restoration reflecting differences in isozyme turnover. Alternatively, the concentration of another CYP isozyme capable of effecting these reactions could be sufficiently increased by induction to contribute to observed reactions.

Low concentration MDMA demethylenation and metoprolol α-hydroxylation had similar suppression and restoration patterns, in keeping with their common catalysis by CYP2D (11, 22). The participation of this isozyme in the 4-hydroxylation of MA has been demonstrated in unpublished experiments with anti-CYP2D IgG (inhibition) and experiments with microsomes from female Dark Agouti rats (<10% of the activity compared to that of female Sprague–Dawley rats). Female Dark Agouti rats have been shown to be deficient in CYP2D isozymes (24–26). Benzphetamine *N*-demethylation have been ascribed to CYP2C11 and CYP2B1 isozymes, among others (13), and high concentration MDMA demethylenation seems to be mediated by isozymes in the CYP2B subfamily (11). MA *N*-demethylation was shown by Baba *et al.* (12), in studies with purified proteins, to be mediated by CYP-SD **IV** and **V**, which seem to be identical to CYP2C13 and CYP2C11, respectively. However, our more recent studies of MA *N*-demethylation suggest a stereoselective participation of other enzymes as well; (+)-MA is a better substrate for the flavin-containing monooxygenase and (−)-MA *N*-demethylation, at μM concentrations, seems to be catalyzed by CYP2D subfamily members (unpublished observations). These observations may account for the rather large difference in suppression of MA *N*-demethylation, in which (−)-MA seems to be more sensitive 3 hr after PCP exposure. CYP2C11 is a major (13, 27) and CYP2D1 is a minor (24) constitutive isozyme in the uninduced adult male rat, whereas CYP2B1 is one of the isozymes inducible by phenobarbitol (11, 27, 28). These isozymes are important in the metabolism of the basic structures commonly found in drugs in general and abused substances in particular. Thus, after PCP exposure under the condition used herein, drug metabolic activities in liver catalyzed by certain P450 isozymes are substantially suppressed so that the pharmacokinetics of all other substrates of the affected isozymes would be altered.

In addition to this general effect, PCP pretreatment affects its own metabolism by reducing or enhancing it at 3 and 16 hr after dosage, respectively. Although the Owens *et al.* study (10) observed only a decrease in metabolic activity after 3 days of PCP pretreatment, the metabolites monitored were, for the most part, different with only *trans*-4-phenyl-4-piperidinocyclohexanol as the only common metabolite. The results reported herein suggest that the other pathways of PCP metabolism are increased after exposure. Therefore, in addition to the central actions of PCP (3), its action on CYP isozymes must also be considered in evaluating *in vivo* experiments.

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