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Metabolic Interactions of Phencyclidine (PCP) and Δ^9-Tetrahydrocannabinol (THC) in the Rat[1]

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LAMÉ, M. W. AND S. HUSAIN. *Metabolic interactions of phencyclidine (PCP) and Δ^9-tetrahydrocannabinol (THC) in the rat.* PHARMACOL BIOCHEM BEHAV **25**(4) 827–833, 1986.—The in vitro effects of THC on the metabolism of PCP by rat liver were determined. Samples containing 1 mM PCP were incubated for 1 hr at 37°C with an NADPH-generating system containing 10,000 × g supernatant or Ca^{++}-precipitated rat liver microsomes. These incubations were carried out in the presence or absence of THC and at the end of 1 hr, PCP metabolites were determined by gas chromatography. In the presence of 0.1, 0.05, 0.025 and 0.0125 mM THC, the production of 1-(1-phenyl-4-hydroxycyclohexyl)piperidine (metabolite I) by the 10,000 × g supernatant was decreased by 46, 29, 23 and 16% respectively. Similarly, production of 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (metabolite II) was reduced significantly by 58, 44, 34 and 23% with the respective concentrations of THC. However, the production of 1-phenylcyclohexylamine (metabolite III) was increased by 18, 32, 30 and 22% with 0.1, 0.05, 0.025 and 0.0125 mM THC. Incubations with Ca^{++}-precipitated liver microsomes revealed similar trends in PCP metabolism in the presence or absence of THC. Metabolites I and II were reduced by 62 and 67% by 0.1 mM THC. Another concentration of THC (0.025 mM) caused a 50 and 62% decrease in I and II. These observations suggest that THC alters the in vitro microsomal metabolism of PCP.

Phencyclidine Δ^9-Tetrahydrocannabinol Metabolic interactions Rat 10,000 × g liver supernatant Ca^{++}-Precipitated liver microsomes

PHENCYCLIDINE (PCP) is often smoked in combination with parsley, tobacco, or marihuana which contains the active component, Δ^9-tetrahydrocannabinol (THC) [28]. The possible interactions that may occur between PCP and THC have not been extensively studied. Freeman and Martin have reported significant behavioral interactions in mice when THC and PCP are coadministered by smoke inhalation [11]. Pryor *et al.* have also described some of the behavioral effects of this combination [26]. When given alone, both drugs impaired avoidance and rotarod performance in rats, and PCP caused a marked increase in photocell activity. When combined, THC enhanced the impairing effect of PCP on avoidance and rotarod performance and antagonized the stimulation of photocell activity caused by PCP. Although this study was unable to demonstrate any metabolic interactions between PCP and THC, subsequent studies have provided some evidence in support of this possibility. The oral administration of THC (10 mg/kg), 90 minutes prior to the intraperitoneal ^{3}H-PCP (7.5 mg, 35 μCi/kg), resulted in the elevation of plasma tritium levels by 1.1, 4.0, 15.9, 20.5, 19.1, 12.1, 16.1, 15.4 and 17.1% over the control rats not receiving THC, for the respective time periods of 0.25, 0.50, 1.0, 2.5, 4.5, 6.5, 9.5, 12.5 and 24.0 hr [16]. These differences were significant at 1.0, 2.5, 4.5, 9.5 and 12.5 hr with $p<0.05$ for a 2-tailed t-test ([16] and unpublished results). These changes in plasma kinetics of PCP on concomitant administration of THC could be due to the alterations in PCP metabolism through THC-derived interactions with the liver microsomes.

Kammerer *et al.* have found that the in vitro incubation of PCP (1.0 to 0.1 mM), with rabbit liver 9,000 × g supernatant, produces primarily three known metabolites; 1-(1-phenyl-4-hydroxycyclohexyl)piperidine (I), 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (II), 4-(4-hydroxypiperidino)-4-phenylcyclohexanol (VI), an additional metabolite N-(5-hydroxypentyl)-1-phenylcyclohexylamine (IV) and lesser amounts of unidentified metabolites [4, 18, 19]. Other studies have shown that the metabolism of PCP by rabbit microsomes results in the formation of electrophilic iminium ions which covalently bind to macromolecules [13, 29]. An amino acid metabolite, 5-[N-(1-phenylcyclohexyl)amino] pentanoic acid, has been detected in dog and human urine and from in

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vitro incubations containing PCP (1 mM) and mouse microsomes [1]. Metabolism studies utilizing rats have detected metabolites in the urine of animals receiving PCP. These studies have observed the formation of I, II, 1-phenylcyclohexylamine (III) and some dihydroxy metabolites [7,33].

Considering past data on the rat and the availability of pure standards from the National Institute on Drug Abuse (NIDA), this study has attempted to determine the in vitro effect of THC on the production of I, II and III. Alterations in the formation of these metabolites by THC have led us to postulate the potential for metabolic interactions between these two compounds.

METHOD

Animals

Male Sprague-Dawley rats, mean weight of 230 g, were used in all experiments. Animals had food and water ad lib; however, 24 hours prior to experiments they were fasted to facilitate a consistent recovery of microsomes [8].

Drugs

PCP and THC were kindly provided by NIDA. The metabolites, I, II and III, were also obtained from NIDA. All other chemicals were standard analytical grade and are described below.

Tissue Preparation for the In Vitro Liver Metabolism of Phencyclidine (PCP)

Following decapitation, livers were rapidly removed, weighed and placed in ice cold 0.9% NaCl. The tissue was then minced and transferred to a precooled homogenizing tube. The temperature was maintained between 0 and 2°C for all subsequent steps. Livers were homogenized in 3 volumes of 0.066 M phosphate buffer and 1.15% KCl (pH 7.4). The homogenate was centrifuged at 10,000 × g for 10 minutes. The supernatant was diluted with 1.15% KCl in phosphate buffer to give 0.33 g of original wet weight of liver per 3 ml of solution.

Liver microsomes were prepared by Ca^{++} precipitation [5]. After mincing, tissue was homogenized in 0.25 M sucrose (Grade I, crystalline, Sigma) in the same phosphate buffer described above. The resulting homogenate was centrifuged at 12,000 × g for 10 minutes. One ml of 40 mM $CaCl_2$ solution was added to 4 ml of the supernatant to give a final concentration of 8.0 mM Ca^{++}. This supension was vortexed and centrifuged at 27,000 × g for 15 minutes. The supernatant was discarded, and the pellet containing the microsomes was resuspended in 3 ml of 1.15% KCl in phosphate buffer. This was again centrifuged (27,000 × g, 15 minutes) and the supernatant discarded. The pellet was resuspended in KCl phosphate buffer to give the equivalent of 0.33 g of original wet weight of liver per 3 ml of solution.

Tissue preparations were used immediately to prevent loss of activity. The amount of protein in each preparation was determined by the Lowry method [22].

Metabolism of Phencyclidine (PCP) in the Presence and Absence of THC with 10,000 × g Liver Supernatant or Ca^{++}-Precipitated Microsomes

Three ml of diluted 10,000 × g supernatant or Ca^{++}-precipitated microsomes were added to 25 ml Erlenmeyer flasks and placed on ice. Flasks were previously treated with 5% dichlorodimethylsilane in benzene. All glassware used for the metabolism studies was treated in this manner to reduce the binding and loss of drugs and metabolites to glass surfaces. One ml of phosphate buffer was added to the 3 ml of liver preparations. This buffer contained 30 μmol of monosodium salt of D-glucose-6-phosphate (G-6-P), 2.6 μmol of the sodium salt of β-nicotinamide adenine dinucleotide phosphate (β-$NADP^+$), and 150 μmol $MgCl_2$. Eight units of glucose-6-phosphate dehydrogenase (G-6-PDH) (Bakers yeast type XV, 1.1.1.59, Sigma) was added next in 96 μl of phosphate buffer followed by 704 μl of phosphate buffer, pH 7.4. Control flasks received 3.1 μl of 95% ethanol while test flasks received appropriate amounts of THC dissolved in 3.1 μl of ethanol to yield the following concentrations: 0.1, 0.05, 0.025 and 0.0125 mM in 5 ml of incubation medium. PCP·HCl in 200 μl of phosphate buffer was the last component to be added to give a 1 mM concentration in the reaction mixture. A control was run for each test flask. Differences between control and test flasks were analyzed for significance by a paired *t*-test [27]. Two blank flasks containing all components except PCP were incubated in the presence and absence of THC. The blank flasks were initially run to determine if THC, its metabolites, or other endogenous components of the incubation medium, would interfere with the detection of PCP and its metabolites during gas chromatography. Results from these blank flasks indicated the absence of components which would co-elute with PCP or its metabolites during gas chromatography. All flasks were incubated at 37°C for 1 hour in a water bath under an atmosphere of 100% oxygen [20]. At the end of 1 hour the incubation was terminated by placing the flasks on ice.

Extraction and Chromatography of PCP and Metabolites

Aliquots (2 ml) of the incubation mixture were taken from the ice bath and added to 15-ml, conical centrifuge tubes containing 5 ml of ice cold chloroform and 10 or 15 μg of benzphetamine. Concentrated ammonium hydroxide was then added to bring the aqueous layer to a pH of 9 to 10. The tubes were shaken on an Eberbach shaker for 25 minutes and centrifuged at 1,300 × g for 30 minutes. After centrifugation, the $CHCl_3$ layer containing PCP and its metabolites was transferred to 15-ml conical centrifuge tubes and 5 ml of 0.2 N sulfuric acid was added. The tubes were vortexed for 2 minutes. The H_2SO_4 layer containing PCP and metabolites was adjusted to pH 9 to 10. Chloroform was added and the mixture vortexed for 2 minutes. The aqueous layer was removed and the $CHCl_3$ layer was evaporated to approximately 10 μl in tapered reaction vials (Regis). The contents of the vials were derivatized by adding 50 μl of tetrahydrofuran (THF) and 50 μl of bis(trimethylsilyl)-trifluroacetamide (BSTFA), followed by heating for 1 hour at 55°C.

Gas chromatography (GC) was employed for the detection, separation and quantitation of PCP and its metabolites. A Hewlett Packard gas chromatograph, model 5830A, equipped with a flame ionization detector and a 18850 GC terminal, was used throughout. A glass column 2 mm × 1.8 meters, packed with 80 to 100 mesh Gas-Chrom Q, loaded with Methylphenyl silicone (OV-17) 5% (Applied Science Laboratories), was used to obtain separation of PCP and metabolites. Samples (2.1 μl) were run under the following conditions: N_2=25 ml/minute, air=240 ml/minute, H_2=30 ml/minute, column temperature=187°C, flame ionization detector temperature=250°C and injector temperature=200°C.

TABLE 1

EFFECTS OF Δ^9-TETRAHYDROCANNABINOL (THC) ON THE PRODUCTION OF 1-(1-PHENYL-4-HYDROXYCYCLOHEXYL) PIPERIDINE (METABOLITE I) FROM PHENCYCLIDINE (PCP) WITH 10,000×g RAT LIVER SUPERNATANT FRACTION

THC[a]	Control[b]	Test[b]	% Change of test in relation to control	Significance[c]
0.1	12.8 ± 0.3[d] (7)	6.9 ± 0.1[d] (7)	−46	0.0001
0.05	12.9 ± 0.3 (9)	9.2 ± 0.2 (9)	−29	0.0001
0.025	14.8 ± 0.1 (8)	11.4 ± 0.1 (8)	−23	0.0001
0.0125	16.2 ± 0.7 (8)	13.6 ± 0.7 (8)	−16	0.0001

[a]mM concentrations of THC in the test flasks.
[b]Control and test flasks contained 1 mM PCP as the substrate.
[c]Significance determined by a pair *t*-test.
[d]Data expressed as the mean value of metabolite formed in μg ± S.E. per 50 mg protein for a 1 hour incubation. Data in parentheses represents the number of experiments.

TABLE 2

EFFECTS OF Δ^9-TETRAHYDROCANNABINOL (THC) ON THE PRODUCTION OF 1-(1-PHENYLCYCLOHEXYL)-4-HYDROXYPIPERIDINE (METABOLITE II) FROM PHENCYCLIDINE (PCP) WITH 10,000×g RAT LIVER SUPERNATANT FRACTION

THC[a]	Control[b]	Test[b]	% Change of test in relation to control	Significance[c]
0.1	64.5 ± 1.3[d] (7)	26.9 ± 0.4[d] (7)	−58	0.0001
0.05	59.7 ± 0.9 (9)	33.6 ± 0.4 (9)	−44	0.0001
0.025	71.5 ± 0.9 (8)	47.3 ± 0.5 (8)	−34	0.0001
0.0125	76.9 ± 5.0 (8)	59.0 ± 4.7 (8)	−23	0.0001

[a]mM concentrations of THC in the test flasks.
[b]Control and test flasks contained 1 mM PCP as the substrate.
[c]Significance determined by a pair *t*-test.
[d]Data expressed as the mean value of metabolite formed in μg ± S.E. per 50 mg protein for a 1 hour incubation. Data in parentheses represents the number of experiments.

Using benzphetamine (15 μg) as an internal standard, standard curves were constructed to determine the amounts of remaining PCP and metabolites formed during in vitro liver metabolic studies. Standard curves were constructed by spiking 2 ml of 10,000 × g incubation medium retained on ice with the following: PCP (20 to 280 μg), III (0.8 to 5.6 μg), I (0.4 to 5.6 μg) and II (2.0 to 28 μg). The extraction protocol was identical to what was previously described. The resulting curves were linear within these ranges with correlation coefficients of 0.999 for all compounds except III (0.993). Mean extraction efficiencies±S.E. for 4 determinations from mediums containing 10,000 × g supernatant were 60.7±4.0, 72.2±4.9, 74.8±3.0, 70.6±2.2 and 70.3±1.8 for III, benzphetamine, I, II and PCP, respectively. Standard curves were also constructed by extracting metabolites and PCP from mediums containing Ca^{++}-precipitated microsomes. These curves were constructed using similar concentrations described above except that the amount of benzphetamine used was reduced to 10 μg. Mean extraction efficiencies±S.E. for four determinations were 85.5±2.4, 88.3±1.9, 89.7±2.8 and 89.9±2.1 for PCP, II, benzphetamine and I.

RESULTS AND DISCUSSION

Under in vitro conditions, THC affected the metabolism of PCP as shown by the altered production of metabolites I, II and III. The 10,000 × g liver supernatant production of I and II was inhibited by all concentrations of THC ranging from 0.0125 to 0.1 mM (Tables 1 and 2). There are several methods by with THC could alter the liver microsomal metabolism of different drugs including PCP. For example, prior subchronic treatment of rats with THC (10 mg/kg, IP, for 21 days) was able to competitively inhibit the in vitro activity of liver microsomal dimethylamine-N-demethylase and p-nitroanisole-o-demethylase enzymes [25]. Similarly, in vitro exposure of microsomes to THC (2, 4 and 8 μg/mg protein) inhibited both demethylase activities in a competitive manner, while aniline hydroxylase activity showed

TABLE 3

EFFECTS OF Δ^9-TETRAHYDROCANNABINOL (THC) ON THE METABOLISM OF PHENCYCLIDINE (PCP) WITH Ca-++-PRECIPITATED MICROSOMES

	THC[a]	Control[b]	Test[b]	% Change of test in relation to control	Significance[c]
I	0.1	14.9 ± 0.2[d] (6)	5.7 ± 0.3[d] (6)	−62	0.0001
	0.025	14.1 ± 0.2 (6)	7.0 ± 0.1 (6)	−50	0.0001
II	0.1	56.8 ± 0.9 (6)	18.8 ± 1.3 (6)	−67	0.0001
	0.025	56.5 ± 0.8 (6)	21.6 ± 0.5 (6)	−62	0.0001

[a]mM concentrations of THC in the test flasks.
[b]Control and test flasks contained 1 mM PCP as the substrate.
[c]Significance determined by a pair *t*-test.
[d]Mean ± S.E. of μg of metabolite produced per 10 mg of microsomal protein. Value in parentheses represents the number of experiments conducted.
I=1 (1-Phenyl-4-hydroxycyclohexyl) piperidine.
II=1 (1-Phenylcyclohexyl)-4-hydroxypiperidine.

TABLE 4

EFFECTS OF Δ^9-TETRAHYDROCANNABINOL (THC) ON THE PRODUCTION OF 1-PHENYLCYCLOHEXYLAMINE (METABOLITE III) FROM PHENCYCLIDINE (PCP) WITH 10,000×g RAT LIVER SUPERNATANT FRACTION

THC[a]	Control[b]	Test[b]	% Change of test in relation to control	Significance[c]
0.1	3.8 ± 0.1[d] (7)	4.5 ± 0.1[d] (7)	+18	0.003
0.05	3.7 ± 0.05 (9)	4.9 ± 0.05 (9)	+32	0.0001
0.025	5.3 ± 0.2 (8)	6.9 ± 0.3 (8)	+30	0.0001
0.0125	5.8 ± 0.8 (8)	7.1 ± 1.0 (8)	+22	0.0003

[a]mM concentrations of THC in the test flasks.
[b]Control and test flasks contained 1 mM PCP as the substrate.
[c]Significance determined by a pair *t*-test.
[d]Data expressed as the mean value of metabolite formed in μg ± S.E. per 50 mg protein for a 1 hour incubation. Data in parentheses represents the number of experiments.

mixed-type inhibition at the 8 μg level. In contrast, the acute in vivo administration of THC (50 mg/kg, IP), resulted in a mixed-type inhibition for both demethylases and a noncompetitive inhibition for the enzyme aniline hydroxylase. From these studies, Mitra *et al.* [25] concluded that the mixed-type inhibition observed at the high dose of THC (50 mg/kg) was due to both specific and nonspecific interactions of THC with the active site of cytochrome P-450 or lipids of the microsomal membrane. Similarly, inhibition of the noncompetitive type could be the result of membrane perturbations by THC which results in conformational changes of membrane components. Gill and Lawrence [12] have also conducted studies which support the conclusions of Mitra *et al.* and indicate that THC can alter the structure of biomolecular membranes. Additional studies have shown that THC can inhibit the microsomal metabolism of aminopyrine and hexobarbital as well as the conjugation of estradiol and p-nitrophenol [9]. THC can also combine with microsomes to give a typical type I difference spectrum [6]. Similarly, microsomal metabolism of compounds like ethylmorphine which also exhibit type I difference spectra, is inhibited by THC [6].

On the basis of these studies it is reasonable to speculate that THC might have similar effects in altering the in vitro metabolism of PCP and the production of metabolites I and II. This could occur by direct interactions with cytochrome P-450 or through alterations in microsomal membrane structure which may be primary to conformational alterations in membrane components.

The results with Ca^{++}-precipitated microsomes were similar to those obtained using the 10,000 × g liver supernatant fraction (Table 3). The extent of inhibition produced in the metabolism of I and II was larger than that seen at comparable doses for the 10,000 × g studies. This may be due to a greater effective concentration of THC in microsomes as compared to the 10,000 × g liver supernatant. For instance,

there was an average of 30.2 mg protein per 3 ml 10,000 × g liver supernatant, while in the Ca^{++} preparation there was 2.74 mg protein per 3 ml. With the 10,000 × g fraction, much of the THC may have bound to non-microsomal components, proteins and lipoproteins [31,32].

Unlike metabolites I and II, THC caused an increase in the formation of III from PCP (Table 4). This effect gradually increased from the 0.0125 mM to 0.05 mM THC. The stimulatory effect began to fall at concentrations of 0.1 mM. For Ca^{++}-precipitated microsomes the level of metabolite III was too low for reliable quantification. This reduced production of metabolite III by microsomes, as compared to 10,000 × g supernatant, has also been reported previously by Cho *et al.* [3].

In our study, THC has been shown to both stimulate and inhibit the metabolism of PCP depending on the metabolite being monitored. Dingell *et al.* [9] have also shown that THC can exert both an inhibitory and a stimulatory effect on microsomal metabolism of a variety of substrates. In their study, THC at concentrations less than 0.1 mM was able to reduce the microsomal metabolism of aminopyrine and hexobarbital while it stimulated the reduction of p-nitrobenzoic acid.

Other previous studies have shown that PCP is metabolized by multiple forms of cytochrome P-450. Holsztynska *et al.* [15] pretreated several different strains of mice with sodium phenobarbital or 3-methylcholanthrene once daily for five days. Phenobarbital induction significantly increased the formation of I and II from PCP, however, no change was noted for the production of 1-(1-phenyl-3-hydroxycyclohexyl) piperidine. Exposure to 3-methylcholanthrene was only slightly effective in altering the ratios of formation of the above metabolites in relation to controls pretreated with saline. These results were interpreted as evidence for the involvement of different forms of cytochrome P-450 in the biotransformation of PCP. Other studies with different compounds have arrived at similar conclusions. For example, the antipyrine metabolites, 4-hydroxy-antipyrine, 3-hydroxymethyl-1-antipyrine and norantipyrine, are produced by separate monooxygenases [2]. Similarly, work using purified cytochrome P-450 isozymes have indicated that they possess remarkable regional and stereoselectivity. Rat cytochrome P-450a, P-450b and P-450c preferentially hyroxylated testosterone at the 7α, 16α and 16β positions, respectively [23]. Therefore, it is conceivable that the formation of metabolites I, II and III from PCP is accomplished by different cytochrome P-450 isozymes. Such isozymes may be expected to respond differently to THC depending on their geometric configuration in relation to the microsomal membrane. As a result, THC could stimulate the production of metabolite III while inhibiting the formation of I and II. A previous report has shown that one particular microsomal enzyme, glucuronyltransferase, can be activated by an isomer of THC, Δ^8-THC [30]. In this study, it was postulated that this particular enzyme, which is tightly bound to the microsomal membrane, could be activated by a modification of the surrounding membrane environment by Δ^8-THC. Stimulation in the production of metabolite III by a particular cytochrome P-450 isozyme(s) may follow a similar mechanism.

In our study, the remaining unmetabolized quantity of PCP was also measured for each reaction vessel. For the 10,000 × g supernatant incubations, containing 0.33 g equivalent of liver and 5 μmoles of PCP per 5 ml of incubation medium, the following amounts of PCP were recovered. Control incubations contained 1.35 μmoles of PCP while incubations containing 0.1 mM THC retained 1.53 μmoles of unmetabolized PCP. Therefore, in controls, 73% of the original PCP was metabolized in which 0.014 μmoles, 0.0317 μmoles and 0.159 μmoles were recovered as III, I and II, respectively. When Ca^{++}-precipitated microsomes were used, 2.05 μmoles and 1.99 μmoles of PCP remained for control and test incubations containing 0.1 mM THC. No significant differences could be detected between control and test incubation with respect to the total amount of PCP remaining after 1 hour incubations. The metabolite concentrations presented here are similar to those reported by Krammerer *et al.* [17]. The major metabolite reported in their study was found to be II, with 0.17±0.03 μmoles being produced for a 60-minute incubation containing 0.33 g of liver and 1 mM PCP per 5 ml of medium. Therefore, the production of I and II in their study is similar to our findings with one exception. We found that 73% of the initial PCP concentration was lost during a one-hour incubation as compared to 23% reported by Kammerer *et al.* This may be a reflection of our use of a 100% O_2 atmosphere instead of room air [20].

In summary, under in vitro conditions, THC was able to stimulate the production of metabolite III while inhibiting the formation of I and II from PCP. If this process also occurs with respect to other metabolites, the total balance of unmetabolized PCP will remain the same in the absence or presence of THC. Therefore, the elevated plasma radioactivity, observed in earlier studies is not due to an increase in the levels of ^{3}H-PCP by THC-induced inhibition of its metabolism. These findings, however, do not rule out the possibility that THC may be stimulating the production of, or reducing the further metabolism of, a metabolite(s) which is retained in plasma longer than other readily excreted metabolites. It should be noted that previous studies do not show similar alterations in the plasma kinetics of PCP following the administration of THC [11,26]. Pryor *et al.* [26] were unable to find significant elevations in ^{3}H-PCP equivalents following the same protocol as employed in our acute studies with THC ([16] and unpublished results). However, their study used lower doses of PCP (5.0 mg/kg, IP) and a different strain of rats (Fischer). The importance of strain differences has previously been shown to be a factor in PCP metabolism. For instance, Holsztynska and Domino [15] have found that different strains of mice hydroxylate PCP at different rates. ICR, C57BL/6J mice have been shown to be fast hydroxylators while A/J and DBA/2J strains are slow hydroxylators of PCP.

Therefore, to explain the behavior interactions and plasma kinetic changes derived from the coadministration of THC and PCP, this in vitro study indicates that the amount and possibly the types of metabolites formed from PCP are more important than the quantity of unmetabolized parent compound. In this regard, the increased production of metabolite III from incubations containing THC is of particular interest. One of the postulated mechanisms for the production of III is through the hydroxylation of the piperidine ring at the two position to give 1-(1-phenylcyclohexyl)-2-hydroxypiperidine followed by hydroxylation at the six position to yield (1-phenylcyclohexyl)-2,6-dihydroxypiperidine. This latter compound, due to its instability, decomposes to III [14]. Metabolite III could also be formed from the conversion of 1-(1-phenylcyclohexyl)-2-hydroxypiperidine to PCP aldehyde with further degradation by cystoplasmic enzymes [3].

The common intermediate in both schemes, 1-(1-phenylcyclohexyl)-2-hydroxypiperidine, has also been postulated to be the precursor of an iminium ion [29]. The electrophilic iminium ion has the potential for binding to endogenous nucleophiles, such as glutathione and cysteine in addition to nucleophilic groups present on proteins and other macromolecules. Such binding could result in cytotoxic alterations and irreversible lesions in essential brain macromolecules [14]. Other potential reactive metabolites of PCP are hydroxylamines. These compounds are known to mediate the toxic effects of amino and nitro compounds [24]. Primary (III) and secondary amines formed during PCP biotransformation are a potential source of hydroxylamines [14]. Such reactive intermediates have previously been hypothesized to be responsible for the long term effects of PCP reported in human subjects following a single dose [14,21]. Symptoms resembling schizophrenia persist for several weeks to several months following a single dose of PCP [10]. Such symptoms occur even when plasma and urine assays for PCP are negative. Therefore, reactive intermediates such as iminium ions and hydroxylamines could possibly explain the behavior interactions associated with the use of THC and PCP, especially when it is considered that the data presented here show that THC can stimulate the production of metabolite III.

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