Sexual Dimorphism in Phencyclidine In Vitro Metabolism and Pharmacokinetics in Rats

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ABSTRACT

Studies were conducted to determine the differences in phencyclidine (PCP) in vitro metabolism and pharmacokinetics in female and male Sprague-Dawley (SD) rats. Formation rates of five major PCP metabolites in liver microsomes were significantly higher (p < .05) in males compared with females in three different rat strains (SD, Fischer 344, and Dark Agouti). In addition, the formation rate for an irreversibly bound PCP metabolite in males was the second highest of the six metabolites measured in these studies. However, the liver microsomes from the females produced essentially no metabolite binding in any strain. To determine the in vivo consequences of these in vitro metabolism results, we determined PCP's pharmacokinetic profile in female SD rats after a pharmacologically active i.v. dose of PCP (1 mg/kg) and then compared these data with the pharmacokinetic profile in male SD rats. The value for PCP systemic (and nonrenal) clearance was more than 45% lower (p < .05) in female rats. In addition, the terminal elimination T1/2 was significantly longer (p < .05) in the female rats (5.5 versus 3.4 h, respectively). Because the initial serum concentration, volume of distribution at steady state, and renal clearance were not significantly different between the sexes, the longer half-life was attributed directly to a decreased ability of females to metabolize the drug. Consequently, these pharmacokinetic data suggest pharmacological differences in PCP effects between female and male rats are due primarily to a decreased ability of female rats to metabolize the drug.

Sex-related differences in mammalian metabolism and pharmacokinetics have been known for the last few decades (Zaphiropoulos et al., 1989; Mugford and Kedderis, 1998). In particular, there are major differences in the metabolic potential in rats, which often result from differences in either the amount of enzyme(s) and/or the level of expression of individual members of the cytochrome P-450 (CYP) family of enzymes. For example, male rats express CYP2C11, CYP2C13, and CYP3A2 whereas females express CYP2C12 (Zaphiropoulos et al., 1989). These enzymes are important to physiological function and homeostasis in the rat because they catalyze the oxidation of many steroids, xenobiotics, and drugs (Waxman, 1988). Because male and female rats have different levels of expression of these key enzymes, the net result is a significant difference in both the qualitative and quantitative processing of endogenous and xenobiotic agents.

These sex-specific isozyme profiles can result in pharmacokinetic differences for some drugs, which likely leads to profound differences in the pharmacological effects produced in males versus females. Nabeshima et al. (1984) suggest that decreased liver metabolism in female Sprague-Dawley (SD) rats results in a longer plasma and brain half-life after an acute i.p. dose of phencyclidine (PCP). They postulate this is a reason for the increased pharmacological effects of PCP in females. Although the differences in behavioral effects between male and female rats in this acute study are clearly significant, the pharmacokinetic analysis and in vitro metabolism studies are very incomplete. For instance, suggested differences in PCP half-life (83 min for males and 108 min for females) are the sole basis for concluding there are differences in PCP pharmacokinetics between the sexes. These reported differences in PCP half-life after an i.p. dose were determined from a 2-h blood collection period, which included the drug distribution, absorption, and elimination phases. More recent studies show the true terminal elimination T1/2 in males is 3.4 h (Valentine and Owens, 1996). Thus, the pharmacokinetic analysis was not determined from a complete data set, and the half-life was a hybrid value that failed to reflect the drug distribution phase.
to adequately describe a distribution, absorption, or terminal elimination phase. This makes it impossible to determine whether differences in metabolism, in the volume of distribution, or in renal elimination or a combination of these factors contributes to the sexual dimorphism in the pharmacological effects of PCP.

Differences in pharmacological effects also are found after chronic PCP dosing. Wessinger (1995) found that the operant behavior of female rats is profoundly affected for the first 7 days of a 10-day s.c. infusion of PCP at 10 mg/kg/day. Male rats show little effects at this dose, and, based on results of a previous study (Wessinger and Owens, 1991), a dose of about 18 mg/kg/day would be required to produce similar effects in the males. These sex-dependent differences in operant behavior during chronic dosing could not be explained by differences in PCP receptor binding between males and females, because the $K_d$ and $B_{max}$ values for [$^3$H]dizocilpine binding (MK-801; (+)-methyl-10,11-dihydro-5H-dibenzo(a,d)cy clohepten-5,10-imine) were not statistically different. This radioligand measures receptor binding to the N-methyl-D-aspartate-associated PCP-binding site (Vincent et al., 1979; Zukin and Zukin, 1979; Lodge et al., 1983). This PCP receptor has been implicated in PCP-induced neurotoxicity. Indeed, Olney et al. (1991) found that adult female SD rats are twice as sensitive to PCP-induced neuropathological changes (vacuolization of neuronal cytoplasm) in cingulate and retrosplenial neurons.

In spite of these profound differences in PCP sensitivity and neurotoxicity between sexes, the possible role of PCP metabolism and pharmacokinetics in producing these differences is still unclear. Consequently, the purpose of these studies was to determine PCP in vitro liver metabolism in female and male rats and in vivo pharmacokinetics in female rats (after i.v. administration) as an aid to understanding the mechanisms underlying the sex differences in PCP behavioral and neurotoxic effects.

**Experimental Procedures**

**Materials.** Phencyclidine hydrochloride, [$^3$H]1-[1-(phenyl-3-$^3$H)]cyclohexyl)piperidine (PCP, 15.69 Ci/mmol), and the phencyclidine metabolites listed below were obtained from the National Institute on Drug Abuse (Rockville, MD). All concentrations were calculated as the free base. The PCP metabolites were $trans$-4-(4'-hydroxypiperidino)-4-phenylcyclohexanol (t-DIOH), $cis$-1-(1-phenyl-4-hydroxy cyclohexyl)piperidine (c-PPC), $trans$-1-(1-phenyl-4-hydroxy cyclohexyl)piperidine (t-PPC), 1-3-phenylcyclohexyl-4-hydroxypiperidine (PCHP), and 5-[4-N-(1-phenylcyclohexyl)aminol]pentanoic acid (PCAP). Because of the presence of minor impurities in the $[^3H]$PCP, it was purified further by the method of Valentine et al. (1994). Trichloroacetic acid (TCA, 10%) was purchased from Baxter Scientific Products (Grand Prairie, TX). 4-Glucose 6-phosphate, glucose-6-phosphate dehydrogenase, $n$-NADP (NADP$^+$), and EDTA were purchased from Sigma Chemical Company (St. Louis, MO). Trimethylamine was purchased from Aldrich Chemical Company (Milwaukee, WI). Ecoscient A scintillation cocktail was purchased from National Diagnostics Inc. (Manville, NJ). All other chemicals were obtained from Fisher Scientific (Springfield, NJ). The GF/B filters and filtration device (model M24R) for determination of PCP metabolite irreversible binding were purchased from Brandel Laboratories (Gaithersburg, MD). Thin-layer chromatography (TLC) plates were aluminum-backed, silica plates with a fluorescent indicator and a concentrating zone (EM Separations, Gibbstown, NJ).

**Animals.** For preparation of adult rat liver microsomes, female and male SD rats ($n = 4$) were purchased from Charles River Laboratories (Wilmington, MA). Adult Dark Agouti (DA, $n = 4$) and Fischer 344 (F344, $n = 4$) rats were purchased from Bantin and Kingman Laboratories (Fremont, CA). The ages of the animals at the time of liver collection was 13 and 12.5 weeks, 11 and 13.5 weeks, and 8 and 16 weeks for the male and female SD, F344, and DA rats, respectively. For the pharmacokinetic experiments, adult female SD rats (250–300 g) were purchased from Hilltop Lab Animals, Inc. (Scottsdale, PA) with dual venous cannuulas (femoral and jugular) in place. The use and care of the cannuulas are described elsewhere (Valentine et al., 1994). The right femoral and right external jugular vein cannuulas were used for drug administration and blood collection, respectively, with the exception of one animal. The dosing and sampling cannuulas were reversed in that animal because of cather patency problems. Because the results in this animal were not different from the others, we assumed the use of the same cannula for dosing and blood collection did not result in errors. The pharmacokinetic results from these female animals were used for comparison with the pharmacokinetic parameters of five male SD rats that were determined previously in our laboratory (Valentine et al., 1994). All animals were allowed to habituate for at least 1 week before being used in experiments. All animal experiments in these studies were approved by the University of Arkansas for Medical Sciences Animal Use and Care Committee, and all experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

**Preparation of Rat Liver Microsomes.** For preparation of the microsomal enzymes, livers were homogenized in ice-cold 10 mM phosphate, pH 7.4, 1.15% KCl, and 10 mM EDTA buffer (3 ml/g wet tissue weight), and then centrifuged at 10,000g for 30 min to remove mitochondria and other cellular debris. The resultant supernatant was centrifuged at 100,000g for 60 min at 4°C. The supernatant was discarded and the pellet was resuspended in 0.05 M phosphate buffer (pH 7.4 with 1 mM EDTA) and recentrifuged at 100,000g. The final pellet was resuspended in the same buffer (with 20% glycerol) at 0.25% of the original liver weight. Protein concentrations were determined by using the Pierce Coomassie Protein Reagent assay (Rockford, IL) with BSA as the protein standard. CYP content of the microsomes was determined according to the method of Omura and Sato (1964), as modified by Johannesen and DePierre (1978). The microsomes were stored at −80°C until needed.

**In Vitro Metabolic Incubations.** For determination of in vitro PCP metabolite irreversible binding, the microsomal samples were incubated at 37°C for 20 min at 2 mg/ml microsomal protein along with 1 μM unlabeled PCP, a tracer dose of [$^3$H]PCP (approximately 1 × 10$^6$ dpm), and the NADPH-regenerating system consisting of 8 mM glucose 6-phosphate, 1 U/ml glucose-6-phosphate dehydrogenase, 4 mM MgCl$_2$, and the addition or absence (for the controls) of 0.5 mM NADP$^+$'. Immediately after the incubation, the microsomal proteins in duplicate 100-μl aliquots were precipitated by the addition of 1 ml of ice-cold 10% TCA and incubated for 1 h at 4°C with occasional vortexing. The precipitated aliquots were then washed over GF/B filters and washed extensively with additional volumes of 10% TCA and 40% ethanol. The number of TCA and ethanol washes needed to decrease the radioactivity on the filters to a constant, background value was determined carefully before the start of these experiments. The filters were allowed to air-dry, and the concentration of metabolite irreversible binding then was determined by liquid scintillation spectrometry analysis of the filters.

**HPLC Analysis of PCP and Metabolites.** Immediately after the microsomal incubation procedure (see previous section), a 500-μl aliquot was removed from each tube to determine PCP metabolite formation. The metabolic reaction was stopped by passing the metabolic incubation mixture, along with the internal standards of PCP and metabolites, over a solvent-conditioned C$_18$ column (Bakerbond spe, high hydrophobic octadecyl; J. T. Baker Inc., Phillipsburg, NJ). Before adding the metabolic incubation mixture, the column was conditioned with two 2.5-ml washes of 5% CH$_3$CN/H$_2$O containing 0.1% trifluoroacetic acid (TFA) and 1% (CH$_3$)$_2$N. After adding the
samples, the column again was washed with the conditioning buffer. PCP and the metabolic products formed in the microsomal incubation mixtures then were eluted with 6 ml of 100% CH3CN containing 0.1% TFA and 1% (CH3)3N. The eluted PCP and metabolites were taken to dryness by vacuum centrifugation and were resuspended in 120 μl of 15% CH3CN:H2O containing 0.1% TFA and 0.1% (CH3)3N. PCP and metabolites (100 μl/injection) were separated by HPLC using a C18 steel column (Waters, Milford, MA; 10 μm, 3.9 × 300 mm, at a flow rate of 2 ml/min). A linear gradient from 15 to 20% CH3CN:H2O containing 0.1% TFA and 0.1% (CH3)3N was held for 3 min, followed by a 20 to 35% gradient over the next 4 min and isocratic conditions of 35% for the next 8 min. One-third-minute fractions were collected and analyzed for [3H]PCP and metabolic products by liquid scintillation spectrometry. Analytical recoveries and identity of metabolic products were determined by comparison with an exact amount of authentic external standards injected at the start of each analytical run. The elution of the external and internal standards was monitored at 254 nm. This procedure could separate PCP and five major PCP metabolites. These metabolites and HPLC retention times were t-diOH, 5.58 min; c-PPC, 6.06 min; t-PPC, 8.49 min; PCHP, 9.67 min; PCHAP, 10.56 min; and PCP, 11.38 min.

Pharmacokinetic Experiments. The experimental design for the pharmacokinetic studies matched that of a study previously conducted in our laboratory using male SD rats (Valentine et al., 1994). Briefly, four female SD rats were administered a 1-mg/kg dose of unlabeled PCP and a tracer dose of approximately 250 μCi of [3H]PCP in 0.9% sterile saline. The total injection volume was 1 ml/kg. Blood samples were collected frequently, and the times of collection can be seen in the serum concentration-time plots in Fig. 1. After each blood sample collection, the blood volume was replaced with the same amount of 0.9% sterile saline. Heparinized saline then was placed in the cannulas to prevent clotting. Blood samples were allowed to clot, and serum was collected after centrifugation. Urine samples were collected frequently from a container at the base of the metabolic cage. The serum and urine were stored at 4°C and analyzed within a few days. In some cases, serum was stored at 4°C and analyzed within a few days.

Analysis of Biological Samples. Details of sample analysis also are described in a previous report (Valentine et al., 1994). Briefly, both serum and urine samples were analyzed for total radioactivity ([3H]PCP plus [3H]-metabolites) by adding a small aliquot of the sample directly to a scintillation vial and counting in a liquid scintillation spectrometry counter. Serum [3H]PCP concentrations were determined according to the method of Owens and Mayersohn (1986). Serum sample aliquots were alkalized with 100 μl of 1 N NaOH in 12- × 75-mm test tubes. [3H]PCP then was extracted twice with 500-μl hexane aliquots after gentle rocking for 1 h. The two hexane layers were transferred to plastic scintillation vials already containing scintillation fluid. Percent recoveries of [3H]PCP after extraction of serum samples were determined based on recoveries of a standard amount of [3H]PCP that was added to duplicate blank serum from each animal. These recoveries ranged from 70 to 92%. This method was verified to separate [3H]PCP from other metabolites in serum samples by comparing the results with those obtained from HPLC separations performed in our laboratory.

Urine samples were analyzed by using the same method for two of the four animals. During the course of these experiments it was discovered that this extraction method, when performed on urine samples, allowed a very small percentage of monohydroxylated phencyclidine metabolites to be extracted with the [3H]PCP (as verified by HPLC analysis). Therefore, urine samples for three of the four female rats were analyzed for [3H]PCP by a different method. Five- to 20-μl aliquots of these urine samples (depending on the expected amount of [3H]PCP in the sample) were spotted directly on a TLC plate. Authentic PCP and metabolite standards also were spotted, and PCP was separated from its metabolites according to the TLC method of Law (1981). The spots from the urine samples corresponding to the PCP standard were cut out, added to scintillation vials, and covered with 10 ml of scintillation fluid. The samples were vortexed and then remained in the scintillation fluid for 24 h, to increase recovery, before counting. Percent recoveries of [3H]PCP were determined based on recoveries of a standard amount of [3H]PCP that was added to duplicate blank urine samples from each animal. The average recovery of [3H]PCP in six different analyses was low, but very consistent (28.5 ± 3.2%). As confirmation of the accuracy of the method, comparison of the results of the urine samples analysis by hexane extraction (5.2 ± 1.1%) or after TLC separation of metabolites (5.7 ± 0.4%) showed the results were not statistically different.

Pharmacokinetic Analysis. Serum [3H]PCP concentration-time data were analyzed by using the nonlinear least-squares curve-fitting routine WinNonlin (Statistical Consultants, Lexington, KY). Biexponential and triexponential curves were fitted with a 1/3 or 1/5 weight factor for each animal. The best-fit curve for each animal was decided after visual inspection of the fit of the curves to the data, analysis of the residuals, and a statistical F test for selecting between the alternative equations (Boxenbaum et al., 1974).

The pharmacokinetic values for male rats listed in Table 2 were determined by reanalyzing the data from Valentine et al. (1994), using the model-dependent methods described in the previous paragraph. The pharmacokinetic values for male rats published previously by Valentine et al. (1994) were determined by analysis using model-independent methods that were required for that particular study. The model-dependent values for T1/2β, systemic clearance (Cl), and volume of distribution at steady state (Vss) determined for the current study for male rats differed from the previously published, model-independent values by 6 to 13%. Values for the harmonic mean and “pseudo” S.D. of the T1/2β were calculated as described by Lam et al. (1985).

Statistics. Statistical analysis of the metabolism data was performed using Student’s t test. For the pharmacokinetic experiments, statistical significance between male and female groups were determined using Student’s two-tailed t test with unequal variances with statistical significance set at p < .05. The statistical software package SigmaStat (Jandel Corp., San Rafael, CA) was used for all statistical analyses.

Results

Effects of Rat Sex on In Vitro PCP Metabolite Irreversible Binding and Metabolism. The values for total CYP content for the females and males were not statistically
different in any of the strains (results not shown). The identity of the five major PCP metabolites was determined from their coelution from the HPLC column with authentic standards. The rate of formation of all PCP metabolites was significantly less in the females ($p < .05$; Table 1). In addition, the amount of PCP metabolized during the 20-min metabolic incubation period was much less in the females ($p < .05$; results not shown). All rats appeared to show a stereoselective preference for the formation of t-PPC over c-PPC. Males formed significantly more irreversibly bound metabolites than females (Table 1) in all strains. In fact, in vitro PCP irreversible binding in all females was barely above background levels.

**Serum PCP Pharmacokinetics.** During the pharmacokinetic experiments in SD females, standard methods were used to measure hematocrits. Hematocrits for each animal averaged $0.44 \pm 0.03$ before dosing and averaged $0.38 \pm 0.05$ 12 h after dosing. This showed the packed red blood cell volume did not change significantly during the first 12 h, the time when blood sampling was the most frequent.

Figure 1 shows the serum PCP concentration versus time plots for the four female rats. Model-dependent analysis showed that the concentration-time data were best described by a triexponential function for all females, with either a $1/y$ ($n = 2$) or $1/y^2$ ($n = 2$) weighting. For the male rats (figure not shown), the concentration-time data were best described by a triexponential ($n = 2$) or biexponential ($n = 3$) function with either a $1/y$ ($n = 3$) or $1/y^2$ ($n = 2$) weighting.

Blood samples also were obtained for over 450 h in two of the female rats. Figure 2 shows a plot of total PCP equivalents (PCP plus metabolites) versus time for these animals. These data were best described by using a three-compartment model with a $1/y$ weighting function. The elimination of total PCP equivalents had a $T_{1/2a}$ of $96 \text{ h}$ in both animals. This is comparable to the $T_{1/2a}$ of $101 \text{ h}$ for total PCP equivalents in the male rats in a previous study (Valentine et al., 1994).

**Pharmacokinetic Parameters.** The average pharmacokinetic values in the female and male rats are shown in Table 2. The initial PCP concentration in serum ($C_0$) was not statistically different between the two sexes. The values for $C_{ls}$ and nonrenal clearance ($Cl_n$) of PCP was much lower in female rats ($p < .05$) than in male rats. The renal clearance ($Cl_r$) and $V_{ss}$ did not differ between males and females. In keeping with these differences in the pharmacokinetic profile, the female rats had a longer $T_{1/2a}$ of the drug.

Figure 3 shows a representative plot of the cumulative amount of PCP and total PCP equivalents appearing in female rat urine. Elimination of unchanged PCP in the urine was essentially complete at 30 h, with most of the drug appearing in urine in less than 12 h. Female rats excreted significantly higher ($p < .05$) amounts of unchanged PCP in the urine (5.5%) compared with male rats (2.6%).

**Discussion**

These studies were designed to determine the differences in PCP in vitro metabolism and pharmacokinetics between male and female rats. Our first series of experiments focused on the in vitro formation of a PCP irreversibly bound metabolite and the formation of five other major PCP metabolites (Mayersohn et al., 1985; Owens et al., 1993). In all of the

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**TABLE 1**

Total capacity for the formation of PCP-soluble and irreversibly bound metabolites in male and female rats

| Soluble Metabolites* | Males | | | Females |
|----------------------|-------|-------|-------|
|                      | SD    | F344  | DA    | SD    | F344  | DA    |
|                      | pmol/min/mg | pmol/min/mg | pmol/min/mg | pmol/min/mg |
| t-diOH               | $0.80 \pm 0.15$ | $0.78 \pm 0.11$ | $0.78 \pm 0.18$ | $0.03 \pm 0.02$ | $0.03 \pm 0.04$ | $0.07 \pm 0.03$ |
| c-PPC                | $0.17 \pm 0.03$ | $0.11 \pm 0.00$ | $0.12 \pm 0.01$ | $0.13 \pm 0.02$ | $0.03 \pm 0.00$ | $0.07 \pm 0.00$ |
| t-PPC                | $0.54 \pm 0.04$ | $0.40 \pm 0.02$ | $0.49 \pm 0.03$ | $0.17 \pm 0.02$ | $0.08 \pm 0.01$ | $0.18 \pm 0.01$ |
| PCHP                 | $2.21 \pm 0.18$ | $1.74 \pm 0.04$ | $2.06 \pm 0.06$ | $1.06 \pm 0.15$ | $0.33 \pm 0.06$ | $0.94 \pm 0.10$ |
| PCHAP                | $1.10 \pm 0.10$ | $0.66 \pm 0.06$ | $0.90 \pm 0.05$ | $0.24 \pm 0.08$ | $0.04 \pm 0.01$ | $0.13 \pm 0.01$ |
| Subtotal             | 4.82  | 3.69  | 4.34  | 1.63  | 0.51  | 1.40  |
| Bound metabolite(s)  | $1.25 \pm 0.02$ | $1.62 \pm 0.21$ | $1.51 \pm 0.29$ | $0.09 \pm 0.03$ | $0.00 \pm 0.00$ | $0.04 \pm 0.01$ |
| Grand total         | 6.07  | 5.31  | 5.85  | 1.72  | 0.51  | 1.44  |

* Solvent extractable metabolite determined by HPLC analysis and liquid scintillation quantitation of peak fractions.

* All values are means $\pm$ S.D.

* Female values were statistically different from male values (Student’s $t$ test, $p < .05$).

* Total of all five soluble metabolites (determined by HPLC analysis) and the irreversibly bound metabolite(s) (determined by TCA precipitation of microsomal proteins).
experiments, the ability of the microsomal proteins to metabolize PCP was affected profoundly by the sex of the animal (Table 1). The in vitro dose of PCP used in our studies (1 μM) was similar to the serum concentration of PCP that would be expected after an 18-mg/kg/day infusion of PCP in male SD rats (Wessinger and Owens, 1991). In contrast, most studies of in vitro PCP metabolism have used a relatively high concentration of PCP (e.g., 100 μM in the studies of Hoag et al., 1984 and Owens et al., 1993 and 1000 μM in the studies of Hiratsuka et al., 1995). We chose this much lower dose because it better represented the PCP serum concentrations expected after an 18-mg/kg/day infusion of PCP in male SD rats (Wessinger and Owens, 1991). In contrast, most studies of in vitro PCP metabolism have used a relatively high concentration of PCP (e.g., 100 μM in the studies of Hoag et al., 1984 and Owens et al., 1993 and 1000 μM in the studies of Hiratsuka et al., 1995). We chose this much lower dose for the in vitro metabolic studies in our laboratory (unpublished observation). Furthermore, repeated attempts to detect this adduct in liver preparations from animals chronically dosed with PCP using this same antibody have been unsuccessful in our laboratory (unpublished observation). Moreover, Laurensana and Owens (1997) find no in vitro formation of this metabolite when using brain microsomes. Taken together, these data indicate that PCP metabolite irreversible binding to proteins does not play a role in the increased pharmacological sensitivity and neurotoxicity (Nabeshima et al., 1984; Olney et al., 1989; Wessinger, 1995) in male or female rats.

Our second series of in vitro metabolic experiments focused on the formation of five phase I PCP metabolites (Mayersohn et al., 1985). These data showed that the formation of all five PCP metabolites was decreased significantly in the females (Table 1). Previous studies show that CYP2C11 produces some of the increased metabolite production in SD male rats (Shelnutt et al., 1996, 1997), and we assume CYP2C11 also would be at least partially responsible for PCP metabolism in the other male strains. However, the isozyme(s) responsible for PCP metabolism in the females is not known. PCHP appeared at the greatest concentrations in both female and male rats in this study. Interestingly, PCHP has approximately one-third the pharmacological potency of PCP (Shannon, 1981).

In summary, the profound in vitro metabolic differences between the sexes could offer a possible mechanism for sex-related differences in male and female PCP effects. However, further studies of PCP pharmacokinetics in males and females were needed to help clarify the role of metabolism in PCP pharmacological effects.

Nabeshima et al. (1984) previously conducted an experiment to determine differences in PCP half-life between male and female SD rats, as part of their studies of sex-related differences of PCP behavioral effects. However, in their study, only four blood samples were collected over a 2-h period after dosing. As can be seen from the pharmacokinetic results in Table 2 and Fig. 1, blood samples must be collected for more than 2 h before the distribution phase is complete. Consequently, a true estimate of the terminal elimination half-life in females cannot be determined without approximately 16 to 20 h of blood sampling. Also, the values for $C_{\text{ss}}$, $Cl_{\text{s}}$, $V_{\text{ss}}$, $Cl_{\text{m}}$, and percentage of the dose in the urine were not determined in the Nabeshima study.

Our results showed that the $Cl_{\text{s}}$ of PCP in SD female rats

### Table 2

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<th>$V_{\text{ss}}$</th>
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*p < .05 compared with male values (Student’s t test).

Harmonic mean and “pseudo” S.D. (Lam et al., 1985).

Male data were published originally by Valentine et al. (1994) using a model-independent pharmacokinetic analysis, but were reanalyzed for these studies using model-dependent methods.
was much lower than that of SD male rats, but the Cl\textsubscript{r}\textsubscript{r} and V\textsubscript{m} were not different between the sexes. As a result, the female rats had a longer T\textsubscript{1/2a} of PCP because of decreased metabolic clearance of the drug. The magnitude of the differences in T\textsubscript{1/2a} and Cl\textsubscript{r} between male and female rats is consistent with the reports in the literature that PCP has a longer duration of action and a greater magnitude of behavioral effects and toxicity in female rats (Nabeshima et al., 1984; Olney et al., 1991; Wessinger, 1995). Although the apparent V\textsubscript{m} and the serum C\textsubscript{s} values after i.v. injection of PCP were not different between the sexes (Table 1), we cannot rule out the possibility that brain concentrations and the time course of PCP in the brain could be different. However, Laurennazza and Owens (1997) found no sexual dimorphism in PCP in vitro metabolism using brain microsomal enzymes.

Female SD rats also had a significantly lower Cl\textsubscript{r}\textsubscript{r} of PCP than male SD rats. Previous pharmacokinetic studies have shown the primary routes of excretion for PCP are via the kidney and the liver (Holsztynska and Domino, 1986). Liver clearance of PCP via biliary excretion accounts for only about 5 to 10% of a dose, and most of that is thought to undergo enterohepatic recirculation (Law, 1981). Because the percentage of a dose excreted unchanged in urine also was very small (Table 2), we think the calculated values for Cl\textsubscript{r}\textsubscript{r} primarily reflect hepatic metabolism. However, metabolism by other major clearing organs (like the kidney) has never been reported. We also followed the elimination of 18\textsuperscript{g}H-total radioactivity for about 20 days in two of the females and found a trace metabolite with a T\textsubscript{1/2a} of about 100 h (Fig. 2). A similar, long-lasting trace metabolite was found in male SD rats (Valentine et al., 1994).

Pharmacokinetic studies of PCP in humans using low doses of radiolabeled PCP show that the Cl\textsubscript{r} and T\textsubscript{1/2a} of PCP vary tremendously. In addition, there are large differences in their apparent ability to metabolize the drug. Studies by Cook et al. (1982a,b) show that the Cl\textsubscript{r} of PCP ranges from 140 to 770 ml/min, and the T\textsubscript{1/2a} of PCP ranges from 7 to 51 h. There is little variation in the apparent volume of distribution (as in the current study in rats). Cl\textsubscript{r} in humans accounts for about 10% of Cl\textsubscript{r}. Thus, the large variation in Cl\textsubscript{r} and T\textsubscript{1/2a} apparently is due to individual differences in hepatic PCP metabolism, which could result from genetic polymorphism in drug metabolism.

Because of the potential, serious, adverse reactions to PCP in humans, clinical studies of PCP at pharmacologically active doses of the drug have not been conducted in almost 40 years. Nevertheless, early clinical trials of PCP as a general anesthetic report that humans also differ widely in the duration of PCP effects. Although most patients recovered within a few hours, some subjects had reactions to PCP lasting for as long as 4 to 10 days (Greifenstein et al., 1958; Meyre et al., 1959). Thus, this prolonged duration of PCP effects could result from a decreased Cl\textsubscript{r} and a prolonged biological T\textsubscript{1/2a} in some individuals.

In animal experiments, most studies of the effects, toxicities, metabolism, and pharmacokinetics of PCP have been conducted in male rats (Nabeshima et al., 1984; Olney et al., 1991; Wessinger, 1995). Because the major difference in the T\textsubscript{1/2} of PCP in humans appears to be related to a possible genetic polymorphism in metabolism, the pharmacological comparison of female and male SD rats might be a suitable model for mimicking some aspects of human population differences in susceptibility to the effects of PCP.

In conclusion, female rats from three different strains showed a significantly reduced ability to metabolize PCP in vitro. Furthermore, pharmacokinetic studies in SD rats showed that the Cl\textsubscript{r}, Cl\textsubscript{an}, and T\textsubscript{1/2a} in female rats were significantly different from the values in male rats. These data suggest that the increased pharmacokinetic sensitivity to PCP in female rats (Nabeshima et al., 1984; Olney et al., 1989; Wessinger, 1995) primarily results from pharmacokinetic differences.

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References

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