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Short communication

Sexual dimorphic effects of chronic phencyclidine in rats

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Abstract

The behavioral effects of phencyclidine (PCP) were studied in male and female Sprague-Dawley rats to determine if chronic infusions would result in sexually dimorphic effects. Rats were trained to make operant responses for food during 30-min response periods that occurred 4 times each day. After attaining stable baseline behaviors, 10 mg of PCP/kg/day was infused s.c. for 10 days. Females were more profoundly affected than males. In the females, response rates were suppressed to 30–71% of control rates during the first 7 days of infusion. In contrast, response rate in male rats never fell below 77% of control during the infusion period. By the eighth infusion day both sexes had become tolerant to these behavioral effects. After stopping infusions there was clear evidence that behavioral dependence had developed; however, the abstinence effects in males and females were similar. Saturation studies of [^{3}H]dizocilpine (MK-801; (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine) binding to brain membranes were conducted to determine if there were sex-dependent receptor differences. There were no significant differences in $K_d \pm$ S.D. (7.6 ± 1.5 and 7.1 ± 0.9 nM for males and females, respectively) or $B_{max} \pm$ S.D. (4.1 ± 0.2 and 4.0 ± 0.5 pmol/mg protein for males and females, respectively).

Keywords: Behavioral pharmacology; Schedule-controlled behavior; Receptor binding; Phencyclidine; Drug dependence; Drug tolerance; Sex difference; (Rat)

1. Introduction

Sex-dependent differences in the pharmacological effects of acutely administered phencyclidine (PCP) have previously been described in rodents (Nabeshima et al., 1984a,b). Recent studies at our institution demonstrated profound differences in the profile of in vitro PCP metabolism and in PCP-metabolite covalent binding between male and female rats using liver microsomes (Owens et al., 1994). These studies suggested that there may be differences in the behavioral effects of chronically administered PCP in male and female rats and sexually dimorphic effects in the dependence-producing properties of PCP. To investigate these possibilities we examined the effects of chronic PCP in male and female Sprague-Dawley rats and the effects of cessation of chronic dosing using a regimen that had previously been shown to reliably produce behavioral dependence on PCP in male rats (Wessinger and Owens, 1991a). We also examined the binding of [^{3}H]dizocilpine (MK-801; (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine) to the NMDA-associated PCP binding site in naive male and female rats to determine if the differences in behavioral effects could be attributed to differences in PCP receptor binding affinity (K_d) or density (B_{max}).

2. Materials and methods

2.1. Subjects

Fifteen (7 male and 8 female) experimentally naive Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA, USA) served as the experimental subjects for the behavioral experiments. An additional 10 rats (5 of each sex) were used for the binding experiments.

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2.2. Behavioral studies

The behavioral experiments were conducted in standard rat operant test chambers (Model G7322, Gerbrands Corp., Arlington, MA, USA) located inside sound- and light-attenuating enclosures (Model G7210, Gerbrands) equipped with a fan which provided air circulation and masking noise. The test chambers were equipped with two response levers mounted below a pair of stimulus lights (28 VDC). The stimulus lights over the right lever were illuminated during behavioral response periods. An overhead pair of houselights (28 VDC) were illuminated from 7:00 a.m. to 7:00 p.m. to simulate the normal day/night cycle. Water was continuously available in a water bottle mounted on a side wall of the test chamber. When schedule contingencies were met a pellet dispenser (Model G5100, Gerbrands) delivered a 97 mg food pellet (Formula A, P.J. Noyes Co., Lancaster, NH, USA) into a food tray located between the levers. Experimental conditions and data acquisitions were controlled by an IBM-compatible microcomputer programmed using MEDSTATE NOTATION (MED-PC; MED Associates, East Fairfield, VT, USA) and operating through an interface (MED Associates).

After an overnight fast, rats were placed into the test chambers where they remained for the duration of the experiment. Beginning at 2:00 p.m. and every 6 h thereafter (i.e., 2:00 p.m., 8:00 p.m., 2:00 a.m. and 8:00 a.m.), a sonalert beeped and the lights over the right lever were illuminated signaling the start of a 30-min response period. The first response on the right lever turned off the sonalert tone until the next response period, but the stimulus lights remained on for the duration of the response period. During response periods only the right-hand lever was active; responses on the left lever were counted but had no scheduled consequences. For initial training the response requirement was set to a fixed-ratio 1 (FR1), thus during the response periods a food pellet was delivered after every lever response. Responding for food under the FR1 schedule of reinforcement was usually established within 1–2 days. During subsequent days the response requirements were gradually increased to the terminal value of FR30. The food pellets provided for a complete rodent diet. The subjects obtained all of their daily food during the four 30-min response periods and gained weight steadily. Routine chamber maintenance was performed daily, after the fourth response period, between the hours of 11:00 a.m. and 1:00 p.m. At this time the rats were removed from the chambers, weighed and observed for unusual behaviors or signs of ill-health.

Stable baseline rates of responding under the FR30 schedule were considered to be established when the range of daily session response rates varied by less than 15% for three consecutive 5-day periods. Approximately 2 h before the next daily session (noon), the rats were anesthetized with ether and implanted with an osmotic minipump (Alza Corp., Palo Alto, CA, USA) which delivered 10 mg of PCP/kg/day, s.c. (Wessinger and Owens, 1991b). Previous studies showed that this dose of PCP produced reliable behavioral dependence in male rats as evidenced by an abstinence syndrome that consisted of suppressed responding for 3–5 days after stopping chronic dosing (Massey and Wessinger, 1990; Wessinger and Owens, 1991a). Regular daily sessions were conducted over the next 10 days during the infusions. In order to stop the chronic dosing the pumps were removed, again under ether anesthesia, approximately 2 h before the next daily session. After removal of the pumps, regular daily sessions were conducted for another 10 days.

Phencyclidine hydrochloride was provided by the National Institute on Drug Abuse (NIDA, Rockville, MD, USA). It was dissolved in sterile saline to a concentration that provided a 10 mg/kg/day infusion via the osmotic minipumps. Doses are expressed as the salt.

Behavioral data are presented in terms of session response rate (responses/s) which refers to the response rate over a 24-h session and was determined by dividing the sum of the responses occurring during the four 30-min response periods by 7200 s. Data are also discussed in terms of percentage of control response rate. For each rat, the average of the session response rates for the 5 days prior to pump implantation was determined. Subsequent session response rates were then expressed as a percentage of these control values. Both of these parameters are presented as averaged values across groups for either the male or the female rats. Differences between male and female rats in the effects of chronic PCP on operant behavior were examined using repeated measures ANOVA.

2.3. Saturation binding studies

For the binding studies, naive rats (5 male and 5 female) were dispatched by decapitation and their brains rapidly removed. After removing the cerebellum, the rest of the brain was quickly frozen and stored at $-80°$ C until used. All solutions used in the binding assays were prepared fresh and used within 24 h. Reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA), except as noted. Brain tissues were thawed and then homogenized in 15 volumes of 0.32 M sucrose using a Tekmar homogenizer (setting 6.25 for 30 s). After the homogenate was centrifuged at $1000 \times g$ for 10 min at 4° C, the supernatant was retained and centrifuged at $20\,000 \times g$ for 20 min at 4° C. The resulting pellets were resuspended in 50 volumes of 50 mM Tris · HCl (pH 7.4 at 4° C) and then centrifuged at

45 000 × *g* for 20 min at 4° C. This high-speed centrifugation step was repeated 3 additional times (with resuspension and homogenization between each wash). After the final centrifugation, the pellets were resuspended and homogenized in approximately 16 ml of 0.32 M sucrose and stored at −80° C until used in the binding assays. On the day of the binding assay, the suspensions were thawed and 20 volumes of 0.08% Triton X-100 added. After incubating this mixture for 10 min at 4° C with gentle stirring, 25 volumes of 100 mM Tris · HCl (pH 7.4 at 4° C) were added and the suspension was centrifuged at 45 000 × *g* for 20 min at 4° C. Resulting pellets were then resuspended in 50 volumes of 50 mM Tris · HCl (pH 7.4 at 4° C). After a final 45 000 × *g* centrifugation, the pellets were resuspended and homogenized in 10 volumes of 5 mM Tris · HCl (pH 7.4 at 4° C). Protein concentrations were determined using the Coomassie Protein Assay (Pierce Chemical Co., Rockford, IL, USA) with an ELIZA plate reader. Saturation studies of the binding of [^{3}H]dizocilpine were conducted in triplicate as follows: Each assay tube contained 100 μl of various concentrations of [^{3}H]dizocilpine (diluted in 5 mM Tris · HCl buffer, pH 7.4 at 4° C) plus either 200 μl Tris buffer containing 10 μM L-glutamate and 10 μM L-glycine (total binding tubes), or 200 μl of the same buffer with 100 μM non-labeled PCP (NIDA; non-specific binding tubes). Eight concentrations of [^{3}H]dizocilpine (specific activity ~ 22.5 Ci/mmol; NEN Research Products, Wilmington, DE, USA) ranging from 0.3 to 60 nM were used. The binding reaction was initiated by the addition of 200 μl of membrane suspension. The total assay volume was 0.5 ml and the final protein concentration was between 0.3 and 0.5 mg protein/ml (average ± S.D., 0.43 ± 0.02 mg/ml). After thorough mixing, the assay tubes were incubated at 25° C for 4 h. Following this incubation period the reaction was terminated by rapid filtration onto glass-fiber filters (Whatman GF/B; Brandel, Gaitherburg, MD, USA; soaked for at least 2 h in 0.1% polyethylenimine) using a cell harvester (Model M-24R; Brandel). The filters were quickly rinsed twice with 3 ml of ice-cold incubation buffer. The filters were placed into 7 ml plastic scintillation vials, 5 ml of scintillation fluid (Ecoscint A, National Diagnostics, Manville, NJ, USA) was added and the filters were allowed to soak overnight before determining radioactivity using liquid scintillation spectrometry. Values for specific binding site density (B_{max}) and apparent dissociation constants (K_d) were determined by nonlinear analysis using the EDBA/LIGAND computer curve-fitting programs (McPherson, 1985) (Biosoft, Milltown, NJ, USA). Differences in binding parameters between male and female rats were examined for significance using an unpaired Student's *t*-test.

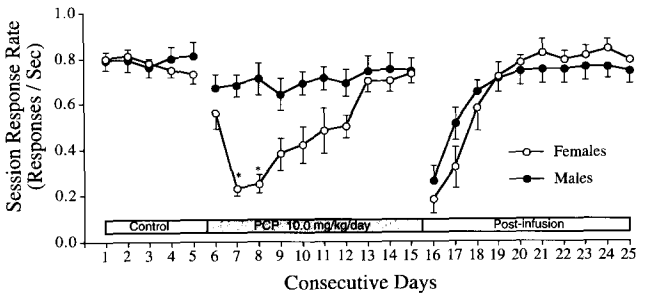

Fig. 1. Effects of chronic PCP on operant behavior of male (open points; averaged values for 7 rats) and female (closed points; averaged values for 8 rats) Sprague-Dawley rats. Error bars are S.E.M. Rats were trained to respond under a FR30 schedule of food reinforcement during daily sessions (shown above) that consisted of four 30-min trials occurring every 6 h. After stable baseline behavior was established (see control days 1–5), they were briefly anesthetized and implanted with osmotic pumps which infused 10 mg of PCP/kg/day over the next 10 days (days 6–15). After 10 days the pumps were removed and behavior was monitored for an additional 10 days (post-infusion days 16–25). Asterisks indicate days during which females differed significantly from males in their response to PCP.

3. Results

The behavioral effects of chronic PCP and PCP withdrawal in male and female rats are shown in Fig. 1. During the 5 days prior to pump implantation (days 1–5), control session response rates were stable and comparable between male and female rats. Control values for individual rats ranged from 0.57 to 1.00 responses/s. The average response rate over the 5 control days was 0.78 responses/s for the female rats and 0.79 responses/s for the male rats. The initial effect of chronic administration of 10.0 mg/kg/day of PCP was to decrease lever pressing for food in male rats to 0.67 responses/s or 85% of control session response rates (day 6). In the male rats, response rates gradually increased over the course of the 10-day infusion period and were up to 94% of control levels by the tenth day of the infusions (the average response rate on day 15 was 0.74 responses/s). In contrast, lever responding by females was somewhat more affected on the first day after starting PCP infusions (responding decreased to 71% of control levels on day 6) and dropped sharply on the second day of PCP infusion (day 7) to 30% of control levels or 0.23 responses/s. Over the remaining 8 days of the infusions the females' session response rate also gradually returned toward control levels so that during the last 3 days of the infusions the females showed essentially the same response to PCP infusion as the males (on day 15 the response rates were 94% of control rates in the females). Repeated-measures ANOVA showed a significant difference between male and female rats during

the infusions ($F(1,13) = 14.11$, $P = 0.0024$) and a significant interaction between sex and days ($F(9,117) = 4.37$, $P = 0.0001$). An unpaired Student's t-test was used to determine on which days during the infusions the effects in males and females differed; Bonferroni's correction was applied to correct for multiple comparisons (the significance level was set at $P \leq 0.005$ to perserve an overal P value of ≤ 0.05). Compared to the effects in male rats, operant response rate in female rats was significantly suppressed on the second and third days of chronic infusion ($P < 0.0001$). When the PCP infusion was stopped by removing the infusion pumps, response rates in both males and females dropped dramatically. On day 16 the average response rate for the females was 0.18 response/s (23% of control) and for the males it was 0.26 responses/s (33% of control). Over the first 4 days of the post-infusion period response rates were similarly suppressed in both males and females, but gradually recovered toward baseline. By day 19 the male rats responded at 90% of control levels (0.71 responses/s) and the females responded at 94% of control (0.72 responses/s). During the last 5 days of the experiment (days 21–25) the female rats exceeded their control response rates slightly; percent of control values ranged from 102% to 108%. Over this same time period the male rats responded at 93% to 96% of control.

The data from the binding studies were best fit by a one-site model and the Hill coefficients ($\pm$S.D.) were near unity (1.08 ± 0.08 for females; 1.04 ± 0.04 for males). The apparent dissociation constant, $K_d \pm$ S.D., was 7.1 ± 0.09 and 7.6 ± 1.5 nM for the females and males, respectively. The number of binding sites, $B_{max} \pm$ S.D., was determined to be 4.0 ± 0.5 and 4.1 ± 0.2 pmol/mg protein for the females and males, respectively. There were no statistical differences (t-test, $P > 0.05$) in these parameters between male and female rats. An Eadie-Hofstee plot of the binding data of [^{3}H]dizocilpine to rat brain homogenates from a representative male and female rat is presented in Fig. 2.

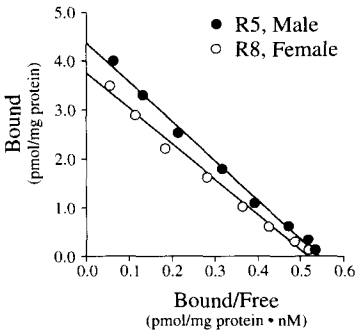

Fig. 2. Eadie-Hofstee plot of a representative male and female subject from saturation binding studies of [^{3}H]dizocilpine binding to rat brain homogenates. 'Well-washed' homogenates of whole brain, minus cerebellum, ($n = 5$ of each sex) were prepared. Binding assays were initiated by addition of brain homogenate (~ 0.5 mg protein/ml) to triplicate tubes containing maximally stimulating quantities of L-glutamate and L-glycine (10 μM) and various concentrations of [^{3}H]dizocilpine (0.3–60 nM). Non-specific binding was estimated in the presence of 100 μM PCP. After 4 h incubation at room temperature, the reaction was terminated by rapid filtration on glass fiber filters (pretreated with polyethylenimine), followed by 2 rinses. For the male and female groups the $K_d \pm$S.D. were 7.6 ± 1.5 nM and 7.1 ± 0.9 nM, respectively, and the $B_{max} \pm$S.D. were 4.1 ± 0.2 pmol/mg protein and 4.0 ± 0.5 pmol/mg protein, respectively.

4. Discussion

Relatively few studies have examined the effects of PCP or other non-competitive NMDA receptor antagonists in both male and female subjects. In the present study, which examined the effects of chronic s.c infusions, responding for food was profoundly suppressed in female rats during the first 7 days of a 10-day infusion of a moderate dose of PCP (Fig. 1). In contrast, this same 10 mg/kg/day dose produced very little behavioral disruption of response rate in male rats during the infusions. With continued infusion, however, these differences in the effects of chronic PCP on behavior of male and female rats dissipated and response rates were not suppressed during the last 3 days of infusions in either sex. After cessation of chronic infusions, both sexes exhibited an equivalent behavioral withdrawal syndrome. Such changes in operant behavior during withdrawal are evidence that drug dependence developed during the chronic infusions of PCP. The abstinence-induced behavioral suppression of response rates in both male and female rats was similar to that seen previously in male rats after cessation of chronic i.v. dosing with PCP at the same dose (Wessinger and Owens, 1991a).

Sex-dependent differences in the acute behavioral effects of PCP have been previously reported. Female Sprague-Dawley rats are more sensitive to PCP as evidenced by increased locomotion, stereotypy (sniffing, head weaving, backpedaling and turning behaviors), motor incoordination, tremor and salivation (Nabeshima et al., 1984a,b). Sex differences have also been found in the behavioral response of rats to dizocilpine. Dizocilpine produces more marked and longer-lasting PCP-like behavioral effects (ataxia, increased locomotion and head weaving) in female Wistar rats than in male rats (Hönack and Löscher, 1993). Dizocilpine is a non-competitive NMDA receptor antagonist, thus it binds to the same binding site that is thought most relevant to the behavioral effects of PCP (Quirion et al., 1987). Although these drugs are structurally dissimilar, they both have similar effects in a variety of

animal models, including the capacity to produce behavioral dependence (see Wessinger (1994) for a review of other similarities).

A number of factors might contribute to these observed behavioral differences. Dispositional factors were examined by Nabeshima et al. (1984b). They found greater amounts of PCP in the plasma and brain tissue of female rats given the same dose of PCP as male rats. They also found lower levels of hepatic cytochrome *P*-450 content and aniline hydroxylase activity in male than in female rats and concluded that the sexually dimorphic effects were due to differences in ability to biotransform PCP (Nabeshima et al., 1984b). The Nabeshima group also investigated the role of sex hormone levels in the sexually dimorphic effects of PCP on stereotypy. Ovariectomy or chronic testosterone in females decreased the effects of PCP to produce stereotyped behaviors, while castration or chronic estradiol in male rats had the opposite effect. The administration of estradiol to male rats or castration resulted in decreased cytochrome *P*-450 activities (Nabeshima et al., 1984a). Owens et al. (1994) examined the in vitro metabolism of PCP and found significantly lower metabolite formation in female rat liver microsomes, compared to male rats of three different strains.

Hönack and Löscher (1993) suggest that their data on dizocilpine showing greater effects of dizocilpine in females do not simply reflect differences in drug metabolism because the onset of the behavioral effects occurred within just a few minutes of drug administration, although the observed differences in duration of action could be due to metabolic differences. Non-dispositional (i.e., pharmacodynamic) factors that could contribute to the disparity in the response to PCP between male and female rats have not been well explored. These factors could cause a difference in sensitivity to PCP, such that males and females would respond differently to the same drug levels at the site of action. Such factors could include differences in the number and/or affinity of PCP binding sites (either globally or in specific brain regions), or differences in endogenous modulators of this ionic-channel binding site.

To our knowledge, there have been no previous reports comparing the characteristics of the PCP binding site in male and female rats. One study examined dizocilpine binding in male and female mice. In this study, high and low affinity binding sites were found and there were significant differences in number and affinity between male and female mice in control animals. There was also a differential response of [^{3}H]dizocilpine binding after exposure to acute swim stress (Akinci and Johnston, 1993). In the present study in naive rats, only one binding site was found which was a high affinity site (K_d, 7–8 nM). No significant differences were observed in either K_d or B_{max} between male and female rats. In addition to the species differences, there were many methodological differences between the Akinci and Johnston study and the present study, which could explain these disparate results. For example, the present study used numerous wash-steps and an incubation period in detergent in order to eliminate endogenous ligands that affect PCP-receptor binding in the membrane preparation. Similar 'well-washed' preparations have been shown to essentially eliminate endogenous glutamate (Loo et al., 1986) and to be useful in studying the NMDA-dependent PCP binding site (Yoneda et al., 1991). Glutamate and glycine were added to the incubation buffer in quantities that maximally stimulate binding and a much longer incubation period was also employed.

In conclusion, dramatic differences were observed in the response of male and female rats during the early phases of chronic PCP administration, but these differences disappeared with continued infusion and the behavioral abstinence syndrome seen upon stopping chronic dosing was similar in both sexes. Examination of the NMDA-associated PCP binding site characteristics with [^{3}H]dizocilpine in untreated rats did not reveal significant differences in either number or affinity between males and females. In addition to possible metabolic and pharmacokinetic differences between male and female rats, endogenous modulators of the PCP binding site (such as amino acids, polyamines and ionic regulators) could affect the pharmacodynamic response to PCP. These modulators could also be involved in the development of tolerance and dependence. Although the behavioral effects of PCP are believed to be mediated via the NMDA-associated PCP binding site, PCP is also well known to interact with many other neuronal systems including noradrenergic, dopaminergic, cholinergic and serotonergic systems (reviewed in Johnson, 1987). The system involved in the disparate effects of PCP in male and female rats remains to be identified.

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References

Akinci, M.K. and G.A.R. Johnston, 1993, Sex differences in acute swim stress induced changes in the binding of MK-801 to the NMDA subclass of glutamate receptors in mouse forebrain, J. Neurochem. 61, 2290.

Hönack, D. and W. Löscher, 1993, Sex differences in NMDA receptor mediated responses in rats, Brain Res. 620, 167.

Johnson, Jr., K.M., 1987, Neurochemistry and neurophysiology of phencyclidine, in: Psychopharmacology: The Third Generation of Progress, ed. H.Y. Meltzer (Raven Press, New York) p. 1581.

Loo, P., A. Braunwalder, J. Lehmann and M. Williams, 1986, Radioligand binding to central phencyclidine recognition sites is dependent on excitatory amino acid receptor agonists, Eur. J. Pharmacol. 123, 467.

Massey, B.W. and W.D. Wessinger, 1990, Effects of terminating chronic phencyclidine on schedule-controlled behavior in rats, Pharmacol. Biochem. Behav. 36, 117.

McPherson, G.A., 1985, Analysis of radioligand binding experiments: a collection of computer programs for the IBM PC, J. Pharmacol. Methods 14, 213.

Nabeshima, T., K. Yamaguchi, H. Furukawa and T. Kameyama, 1984a, Role of sex hormones in sex-dependent differences in phencyclidine-induced stereotyped behaviors in rats, Eur. J. Pharmacol. 105, 197.

Nabeshima, T., K. Yamaguchi, K. Yamada, M. Hiramatsu, Y. Kuwabara, H. Furukawa and T. Kameyama, 1984b, Sex-dependent differences in the pharmacological actions and pharmacokinetics of phencyclidine in rats, Eur. J. Pharmacol. 97, 217.

Owens, S.M., M.G. Gunnell and S.L. Sorrels, 1994, Sex is a major determinant of phencyclidine (PCP) metabolism and metabolite covalent binding in rats, in: Problems of Drug Dependence, 1993, Vol. 2, NIDA Research Monograph 141, ed. L.S. Harris (DHHS Publ. No. (ADM)94-3749, U.S. Govt. Print. Off., Washington, DC) p. 246.

Quirion, R., R. Chicheportiche, P.C. Contreras, K.M. Johnson, D. Lodge, S.W. Tam, J.H. Woods and S.R. Zukin, 1987, Classification and nomenclature of phencyclidine and sigma receptor sites, Trends Neurosci. 10, 444.

Wessinger, W.D., 1994, Tolerance to and dependence on MK-801 (dizocilpine) in rats, Pharmacol. Biochem. Behav. 49, 1049.

Wessinger, W.D. and S.M. Owens, 1991a, Phencyclidine dependence: the relationship between dose and serum concentrations to operant behavioral effects, J. Pharmacol. Exp. Ther. 258, 207.

Wessinger, W.D. and S.M. Owens, 1991b, Chronic administration of phencyclidine: pharmacokinetic comparison of intravenous and subcutaneous infusions in Sprague-Dawley rats, Drug Metab. Dispos. 19, 719.

Yoneda, Y., K. Ogita and R. Enomoto, 1991, Characterization of spermidine-dependent [^{3}H](+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine (MK-801) binding in brain synaptic membranes treated with Triton X-100, J. Pharmacol. Exp. Ther. 256, 1161.