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[^{3}H]MK-801 Binding to Well-Washed Rat Brain Membranes Following Cessation of Chronic Phencyclidine Treatment

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BURKE, T. F., S. BUZZARD AND W. D. WESSINGER. *[^{3}H]MK-801 Binding to well-washed rat brain membranes following cessation of chronic phencyclidine treatment.* PHARMACOL BIOCHEM BEHAV 51(2/3) 435–438, 1995.—Rats were implanted with osmotic minipumps SC that infused either saline or 10 mg/kg/day phencyclidine (PCP) for 10 days, a regimen that produces dependence to PCP. At the end of this 10-day infusion period, the pumps were removed and the rats were sacrificed either immediately or at various time points (12 h, 1, 2, and 7 days) after pump removal. The saturation binding parameters of [^{3}H]MK-801 were then determined in well-washed cortical/hippocampal membranes prepared from these rats. Neither the B_{max} nor the K_d of [^{3}H]MK-801 binding in membranes of PCP-treated rats differed from that determined using membranes from saline-treated rats at any time point studied. These results suggest that alterations in PCP receptors do not play a major role in the production of PCP dependence.

Phencyclidine Rats Drug dependence Receptor binding Chronic administration [^{3}H]MK-801 Dizocilpine

THE PHENCYCLIDINE (PCP) receptor is part of the *N*-methyl-D-aspartate (NMDA) receptor complex and is believed to be located in the cation channel, which is associated with this complex (6). Compounds that bind at this site, such as PCP, MK-801, and ketamine, block the channel and therefore act as noncompetitive antagonists of NMDA receptor-mediated responses (5). It is well established that cessation of chronic PCP administration can produce overt signs of withdrawal in rats (13,14) and monkeys (1), thus indicating that physical dependence develops to PCP. Previous studies in this laboratory showed that cessation of lower doses of chronic PCP administration resulted in disruptions in operant behavior, which was interpreted as evidence that dependence developed during the infusion (8,15). After administration was stopped, operant performance was disrupted for 3–4 days. The mechanisms underlying the development of PCP dependence are not clear. However, Massey and Wessinger (9) also showed that both the B_{max} and K_d of [^{3}H]TCP binding to whole rat brain membranes were increased significantly 24 h following cessation of chronic PCP administration. Although these receptor changes were modest in nature, they did occur at the time point at which the largest disruption of operant performance was observed in the above study (8), suggesting that changes in the number and/or affinity of PCP receptors may be related to dependence development.

The present study was designed to examine the time course of these receptor changes after cessation of chronic PCP treatment. Rats were infused with 10 mg/kg/day PCP for 10 days, a dosing regimen previously shown to result in behavioral dependence (8,15), and were sacrificed at various time points after PCP administration was stopped. The saturation binding parameters of the PCP receptor-selective radioligand [^{3}H]MK-801 were then determined in cortical/hippocampal brain membrane homogenates from these rats and compared to those obtained from control rats that received a 10-day saline infusion. The overall goal was to determine the degree to which these findings correlated with the time course of PCP withdrawal-induced behavioral disruptions.

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METHOD

Adult male Sprague–Dawley rats (National Center for Toxicological Research, Jefferson, AR) were chronically infused with PCP (10 mg/kg/day) or saline for 10 days using Alzet osmotic minipumps (Model 2ML2; Alza Corp., Palo Alto, CA) implanted SC in the back. At the end of this infusion period, the rats were sacrificed by decapitation either immediately or 12 h, 1, 2, or 7 days after removal of the pumps.

After sacrifice, the brains were removed and the cerebral cortex and hippocampus were dissected and quickly frozen at −70°C or −140°C. To prepare the tissues for the binding assay, thawed tissues were homogenized in 15 vol. of 0.32 M sucrose using a Tekmar Model SDT100EN (Tekmar Co., Cincinnati, OH) homogenizer (setting 6.25, 30 s). They were then centrifuged at 1000 × g for 10 min at 4°C. The buffy coat was loosened from the pellet by gentle swirling and the supernatant was retained and centrifuged at 20,000 × g for 20 min at 4°C. The resulting pellets were then resuspended by homogenization (setting 6.25, 5–8 s) in 50 vol. of 50 mM Tris-HCl (pH 7.4 at 4°C). This suspension was then centrifuged at 45,000 × g for 20 min at 4°C and the pellet resuspended as in the previous step. This high-speed centrifugation step was repeated three additional times (with resuspension by homogenization in between each step). After the final centrifugation, the pellets were resuspended in approximately 16 ml of 0.32 M sucrose and stored at −140°C until use. On the day of the binding assay, the suspension was thawed and 20 vol. of 0.08% Triton X-100 was added. These Triton-treated membranes are useful for studying the NMDA-dependent PCP binding site (16). After incubating this mixture for 10 min at 4°C with gentle stirring, 25 vol. of 100 mM Tris-HCl (pH 7.4 at 4°C) was added and the suspension centrifuged at 45,000 × g for 20 min at 4°C. Resulting pellets were then resuspended with the Tekmar (setting 6.25, 5–8 s) in 50 vol. of 50 mM Tris-HCl (pH 7.4 at 4°C). After a final 45,000 × g centrifugation step, the pellets were resuspended in 15 vol. of 5 mM Tris-HCl (pH 7.4 at 4°C), resulting in a final protein concentration of 0.3–0.5 mg protein/ml. Protein determinations were made using the method of Bradford (2). This "well-washed" membrane preparation has been shown to essentially eliminate endogenous glutamate (7).

To determine PCP receptor saturation binding, a preparation that was maximally stimulated with glutamate and glycine was used. To initiate the binding reaction, 200 μl of membrane homogenate was added to tubes containing 200 μl of either 5 mM Tris-HCl buffer (pH 7.4 at 4°C) with 10 μM L-glycine and 10 μM L-glutamate (total binding) or 200 μl of the same solution with 100 μM PCP added (nonspecific binding). All tubes contained 100 μl of varying concentrations of [^{3}H]MK-801 (0.3–60 nM). After a 4-h incubation at 25°C, the reaction was terminated by the addition of 5 ml ice-cold Tris-HCl (pH 7.4 at 4°C) and rapid filtration through glass-fiber filters (Whatman GF/B, Brandel, Gaithersburg, MD), which were presoaked in 0.05% polyethylenimine, using a cell harvester (Model M-24R, Brandel, Gaithersburg, MD). The filters were washed two additional times with 5 ml of the buffer solution and then placed into 7-ml plastic scintillation vials to which 5 ml of scintillation fluid (Ecoscint A, National Diagnostics, Inc., Manville, NJ) was added. The samples were allowed to sit overnight and then were counted by conventional liquid scintillation spectrometry using a Model 1500 counter (Packard Instrument Co., Downers Grove, IL).

B_{max} and K_d values were determined by computer-assisted nonlinear analysis using the EBDA/LIGAND package (Biosoft, Milltown, NJ) of programs (10). Differences between the saline-treated and PCP-treated groups at each time point were tested for significance using the Mann–Whitney U-test with Bonferroni's correction. Therefore, the significance level for each comparison (each of the five time points) was $p < 0.01$, which resulted in an overall significance level of $p < 0.05$.

[^{3}H]MK-801 was purchased from DuPont NEN (Wilmington, DE; specific activity 20.3–22 Ci/mmol). Phencyclidine hydrochloride (National Institute on Drug Abuse, Rockville, MD) was dissolved in 0.9% physiological saline. All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). All solutions were made fresh and used within 24 h.

RESULTS

Preliminary experiments determined the maximally stimulating concentrations of L-glutamate and L-glycine to be 10 μM. Under these conditions, which were used in all subsequent experiments, [^{3}H]MK-801 binding reached equilibrium after a 4-h incubation (data not shown). The [^{3}H]MK-801 saturation binding data were best fit by a one-site model. The individual and mean B_{max} and K_d values at each time point are presented in Table 1. There were no significant differences in B_{max} or K_d values between the saline- and PCP-treated groups at any time point. It should be noted that the differences in both B_{max} and K_d between the saline- and PCP-treated groups did approach significance ($p = 0.02$) at the 12-h time point. None of the other saline vs. PCP comparisons (0 h, 1, 2, or 7 days) generated a p value of less than 0.15. Figure 1 shows Scatchard plots for representative saline- and PCP-treated rats 12 h after cessation of chronic PCP infusion, the point at which the largest differences were observed. In no case did the Hill coefficients for any of the individual Scatchard analyses differ significantly from unity.

DISCUSSION

The present results do not agree with those of Massey and Wessinger (9) in that no significant alterations in PCP receptors were observed after discontinuation of chronic PCP administration. There are several methodological differences between the two studies that could explain this apparent discrepancy. One major difference is that Massey and Wes-

TABLE 1

BINDING TIME COURSE RESULTS

Treatment	B_{max}(pmol/mg protein)	K_d(nM)	n
Saline 0 h	4.31 ± 0.47	3.53 ± 0.14	4
PCP 0 h	4.48 ± 0.20	3.15 ± 0.28	4
Saline 12 h	4.00 ± 0.03	3.16 ± 0.14	4
PCP 12 h	4.38 ± 0.13	2.83 ± 0.09	4
Saline 1 day	4.70 ± 0.61	3.11 ± 0.05	4
PCP 1 day	4.92 ± 0.29	2.89 ± 0.11	4
Saline 2 day	4.88 ± 0.15	3.90 ± 0.23	4
PCP 2 day	5.00 ± 0.58	3.17 ± 0.39	4
Saline 7 day	5.27 ± 0.20	3.11 ± 0.11	4
PCP 7 day	4.72 ± 0.27	3.41 ± 0.31	4

Average B_{max} and K_d values ± SEM for the saturation binding of [^{3}H]MK-801 determined in cortical/hippocampal membranes from rats sacrificed either immediately after 10 days of SC infusion of 10 mg/kg/day PCP (0 h) or 12 h, 1, 2 or 7 days after pump removal.

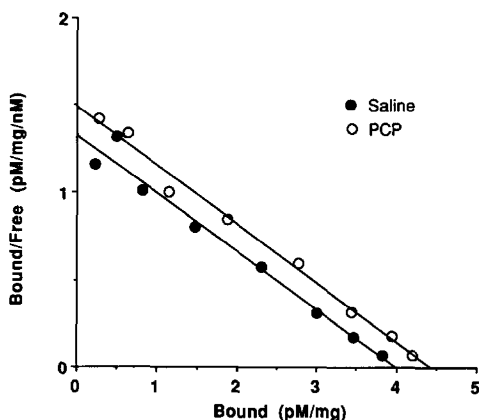

FIG. 1. Representative Scatchard plots of [^{3}H]MK-801 binding in cortical/hippocampal membranes from one rat administered saline (closed symbols) and one rat administered 10 mg/kg/day PCP for 10 consecutive days (open symbols), both sacrificed 12 h following pump removal. For the saline-treated rat, the B_{max} was 4.00 pmol/mg and the K_d was 2.99 nM and for the PCP-treated rat, the B_{max} was 4.42 pmol/mg and the K_d was 3.01 nM.

singer (9) used [^{3}H]TCP whereas the present study used [^{3}H]MK-801; however, it is unclear how this would produce dissimilar results. Another difference is that the present study used a tissue preparation comprised only of cortical and hippocampal membranes, the two brain regions with the highest PCP receptor densities (16), rather than the whole-brain membrane preparation used in the original study. This "enriched" preparation would be expected to amplify the signal-to-noise ratio of the assay, and thus increase the magnitude of receptor changes. However, it is possible that the PCP receptors in the cortex and hippocampus are not altered to a large degree in dependent animals and that receptor changes of a greater magnitude occurred in other brain areas that we did not study. Perhaps the most important difference was that the tissue used in the present study was subjected to a substantially more rigorous washing procedure than that used by Massey and Wessinger (9). One objective of the tissue preparation scheme was to eliminate as much endogenous glutamate and glycine as possible and to add back known, maximally stimulating quantities. This was accomplished by performing numerous tissue washes (centrifugation and resuspension), interposed by a freeze–thaw cycle, as well as by incubating with a detergent, Triton X-100, to disrupt the membranes. The previous study started with fresh brain tissue and used fewer wash steps with no detergent treatment and, therefore, may not have been able to remove as much endogenous glutamate and glycine as in the present study. The presence of residual endogenous modulators may also explain the change in K_d observed in that report. Interestingly, a recent report found that increased levels of extracellular brain glutamate were associated with naloxone-precipitated morphine withdrawal in rats (17). However, we are unaware of any reports measuring brain glutamate levels associated with PCP dependence and withdrawal.

Other studies that have attempted to assess PCP receptor changes in PCP-dependent animals have yielded mixed results. Itzhak and Alerhand (4) found no changes in the binding of a single concentration of [^{3}H]TCP to mouse brain removed 48 h after stopping chronic PCP administration (10 mg/kg/day, SC, for up to 21 days). However, this approach may not reveal changes in B_{max} or K_d, especially if such changes are not robust in nature. Similarly, Plunkett et al. (11) found no change in either the B_{max} or K_d of [^{3}H]TCP in brain tissues from rats sacrificed at the end of chronic PCP administration (17.8 mg/kg/day, SC, for 10 days). In contrast, an autoradiographic study by Quirion et al. (12) showed a decrease in the number of [^{3}H]PCP binding sites with no change in affinity in rat olfactory bulb tissues collected 24 h after stopping chronic PCP administration (10 mg/kg/day, SC, for 14 days).

Based on the results of the present study and those described above, it would appear that a distinct role for PCP receptors in the development of PCP dependence has yet to be established. Although significant changes in receptor binding parameters were obtained in a previous study (9), it is unclear how, or even if, these alterations are functionally related to the dependence-producing properties of PCP. For example, we do not know whether this change in receptor density is a result of, or a prerequisite for, dependence development. Perhaps the PCP receptor itself is relatively unaffected and the changes are occurring at other sites that are associated with the NMDA receptor complex (e.g., NMDA, glycine). There is some recent evidence that glutamate binding to NMDA receptors increases in rat hippocampus after a single dose of PCP (3), but it is still not known how chronic PCP administration affects this site or other related sites or systems. In summary, the disparate results of the present study and similar previous studies suggest that PCP receptor alterations are not associated to a large degree with the behavioral disruptions observed upon cessation of chronic PCP administration.

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REFERENCES

1. Balster, R. L.; Woolverton, W. L. Continuous-access phencyclidine self-administration by rhesus monkeys leading to physical dependence. Psychopharmacology (Berlin) 70:5–10; 1980.
2. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. Anal. Biochem. 72:248–254; 1976.
3. Gao, X.-M.; Tamminga, C. A. An increase in NMDA-sensitive [^{3}H]glutamate and [^{3}H]kainate binding in hippocampus 24 hours after PCP. Neurosci. Lett. 174:149–153; 1994.
4. Itzhak, Y.; Alerhand, S. Differential regulation of sigma and PCP receptors after chronic administration of haloperidol and phencyclidine. Fed. Am. Soc. Exp. Biol. J. 3:1868–1872; 1989.
5. Lodge, D.; Aram, J. A.; Church, J.; Davies, S. N.; Fletcher, E.; Martin, D. Electrophysiological studies of the interaction between phencyclidine/sigma receptor agonists and excitatory amino acid neurotransmission on central mammalian neurones. In: Domino, E. F.; Kamenka, J. M., eds. Sigma and phencyclidine-like compounds as molecular probes in biology. Ann Arbor, MI: NPP Books; 1988:239–250.
6. Lodge, D.; Johnson, K. M. Noncompetitive excitatory amino acid receptor antagonists. Trends Pharmacol. Sci. 11:81–86; 1990.
7. Loo, P.; Braunwalder, A.; Lehmann, J.; Williams, M. Radioligand binding to central phencyclidine recognition sites is depen-

dent on excitatory amino acid receptor agonists. Eur. J. Pharmacol. 123:467–468; 1986.
8. Massey, B. W.; Wessinger, W. D. Effects of terminating chronic phencyclidine on schedule-controlled behavior in rats. Pharmacol. Biochem. Behav. 36:117–121; 1990.
9. Massey, B. W.; Wessinger, W. D. Alterations in rat brain [^{3}H]-TCP binding following chronic phencyclidine administration. Life Sci. 47:PL139–PL143; 1990.
10. McPherson, G. A. Analysis of radioligand binding experiments: A collection of computer programs for the IBM PC. J. Pharmacol. Methods 14:213–228; 1985.
11. Plunkett, L. M.; Owens, S. M.; Gunnell, M.; Owens, R. B. The effect of chronic phencyclidine (PCP) and phenylcyclohexene (PC) dosing on [^{3}H]TCP and [^{3}H]Haloperidol ([^{3}H]Hal) binding in rat brain. Fed. Am. Soc. Exp. Biol. J. 4:A329; 1990.
12. Quirion, R.; Bayorh, M. S.; Zerbe, R. L.; Pert, C. B. Chronic phencyclidine treatment decreases phencyclidine and dopamine receptors in rat brain. Pharmacol. Biochem. Behav. 17:699–702; 1982.
13. Spain, J. W.; Klingman, G. I. Continuous intravenous infusion of phencyclidine in unrestrained rats results in the rapid induction of tolerance and physical dependence. J. Pharmacol. Exp. Ther. 234:415–424; 1985.
14. Stafford, I.; Tommie, A.; Wagner, G. C. Effects of SKF-10,047 in the phencyclidine-dependent rat: Evidence for common receptor mechanisms. Drug Alcohol Depend. 12:151–156; 1983.
15. Wessinger, W. D.; Owens, S. M. Phencyclidine dependence: The relationship between dose and serum concentrations to operant behavioral effects. J. Pharmacol. Exp. Ther. 258:207–215; 1991.
16. Yoneda, Y.; Ogita, K.; Enomoto, R. Characterization of spermidine-dependent [^{3}H](+)-5-methyl-10,11-dihydro-5H-dibenzo[a, d]cyclohepten-5,10-imine (MK-801) binding in brain synaptic membranes treated with Triton X-100. J. Pharmacol. Exp. Ther. 256:1161–1172; 1991.
17. Zhang, T.; Feng, Y.; Rockhold, R. W.; Ho, I. K. Naloxone-precipitated morphine withdrawal increases pontine glutamate levels in the rat. Life Sci. 55:PL25–31; 1994.