12. Y. Lamboeuf, G de Saint Blanquat and R. Derache, *Clin. exp. Pharmac. Physiol.* **1**, 361 (1974).
13. Y. Lamboeuf and G. de Saint Blanquat, *Archs. Int. Pharmcodyn. Ther.* **243**, 17 (1980).
14. R. A. Deitrich, *Psychoneuroendocrinology* **1**, 325 (1976).
15. J. H. Thompson, *Res. Commun. Path. Pharmac.* **2**, 687 (1971).
16. O. Özturkcan, G. de Saint Blanquat and R. Derache, *J. Physiol. Paris* **64**, 355 (1972).
17. E. D. Jacobson, *Gastroenterology* **54**, 434 (1968).
18. R. K. Bonninschen and H. Theorell, *Scand. J. Clin. Lab. Invest.* **3**, 58 (1951).
19. F. Lundquist, *Biochem. J.* **68**, 172 (1958).
20. H. U. Bergmeyer and H. Möllering, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer) p. 1520. Academic Press, New York (1974).
21. H. W. Sippel, *Acta Chem. Scand.* **27**, 541 (1973).
22. C. J. P. Eriksson, *Alcoholism Clin. exp. Res.* **4**, 22 (1980).
23. E. A. Carter and K. J. Isselbacher, *Proc. Soc. exp. Biol. Med.* **138**, 817 (1971).
24. H. Wallgreen, in *International Encyclopedia of Pharmacology and Therapeutics* **20**, (Ed. J. Tremolieres) p. 161. Pergamon Press, London (1970).
25. B. Lichtenstein and D. Winne, *Naunyn-Schmiedberg's Arch. Pharmac.* **279**, 153 (1973).
26. D. R. Petersen, A. C. Collins and R. A. Deitrich, *J. Pharmac. exp. Ther.* **201**, 471 (1977).
27. H. Peterson and K. H. Kiessling, *Biochem. Pharmac.* **26**, 237 (1977).
28. O. Tottmar, H. Marchner and N. Karlson, *Br. J. Nutr.* **39**, 957 (1960).
29. H. Marchner and O. Tottmar, *Acta Pharmac. Tox.* **39**, 331 (1976).
30. T. M. Kitson and K,. E. Crow, *Biochem. Pharmac.* **28**, 2551 (1979).
31. R. A. Deitrich, P. A. Troxell, N. S. Worth and V. G. Erwin, *Biochem. Pharmac.* **25**, 2733 (1976).
32. U. Rydberg, *Opusc. Med.* **26**, 1 (1972).

Biochemical Pharmacology, Vol. 30, pp. 545–547.
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0006-2952/81/050545-03 $02.00/0

Effect of chronic administration of phencyclidine on hepatic mixed-function oxidases in the mouse

(*Received* 25 *April* 1980; *accepted* 15 *August* 1980)

Phencyclidine (PCP), a dissociative anesthetic, is known to be a potent psychotomimetic agent that resembles its pharmacologic analog, ketamine [1]. The recent literature suggests that PCP is one of the major drugs of abuse and also is used chronically [2, 3]. Phencyclidine is commonly sold in a mixture with other drugs of abuse [4, 5]. If the administration of PCP caused an alteration in the rate of metabolism of the compound that is mixed with PCP, both diagnosis and treatment of overdose cases would be complicated. For example, the acute administration of PCP has been reported to enhance both ethanol and hexobarbital sleeping time in the mouse [6]. On the other hand, significant shortening of hexobarbital [7] or pentobarbital [8] sleeping time was observed after chronic treatment of PCP. Radzialowski and Oppermann [7] further demonstrated that treatment of male rats for 4 days with PCP (50 $mg \cdot kg^{-1} \cdot day^{-1}$, i.p.) caused significant increases in hexobarbital, aminopyrine, and zoxazolamine metabolism *in vitro*. It has also been reported, however, that chronic administration of PCP (1 mg/kg) for 6 days failed to change cholesterol metabolism in rats [9]. In view of these findings, we systematically studied the effect of chronic administration of PCP on hepatic mixed-function oxidase systems.

Male ICR mice (30–36 g), obtained from the Charles River Breeding Laboratories (Wilmington, MA), were used. Three groups of fifteen mice each were treated as follows: group 1 (control) received saline daily for 7 days followed by Saline twice daily for 7 more days; group 2 (low dose, 1 week) received saline daily for 7 days, followed by the daily administration of PCP (40 mg/kg, i.p.) in the morning and saline in the evening for 7 days; group 3 (low dose, 1 week, then high dose, 1 week) received PCP (40 mg/kg, i.p.) daily for 7 days, followed by two daily doses of PCP (40 mg/kg, i.p.) for 7 more days. Body weight of each animal was monitored every day for the entire period of treatment.

All animals were decapitated 24 hr after the last injection. The livers were removed, weighed, and transferred to ice-cold isotonic (1.15 M) KCl solution. Three livers were pooled and assayed. All subsequent preparative procedures were carried out at 0–4°. Livers were homogenized in 3 vol. of KCl solution using a Potter–Elvehjem homogenizer (10 strokes). The homogenate was centrifuged successively to remove nuclear and mitochondrial fractions. Part of the resultant 9000 *g* supernatant fraction was centrifuged at 105,000 *g* for 60 min to obtain the microsomal pellet, which was resuspended in the original volume of KCl solution.

Each individual sample was assayed in duplicate incubations. Aniline [10], pentobarbital, and hexobarbital [11] hydroxylase activities, and aminopyrine [12] and ethylmorphine [10] demethylase activities were measured using the 9000 *g* supernatant fractions. Concentrations of cytochromes b_5 and P-450 were determined according to Omura and Sato [13]. NADPH dehydrogenase [14] and NADPH-cytochrome *c* reductase [15] activities were assayed using procedures described previously. Protein was measured

Table 1. Effects of chronic administration of phencyclidine on hepatic mixed-function oxidases in the mouse*

Variables	Saline control (Group 1)	PCP (40 mg·kg^{-1}·day^{-1} for 7 days) (Group 2)	PCP (40 mg/kg once a day for 7 days, then twice a day for 7 days) (Group 3)
Body weight (g)	31.8 ± 0.5	29.7 ± 0.4†	26.8 ± 0.4‡
Liver weight (g)	1.79 ± 0.05	1.75 ± 0.04	1.58 ± 0.05†
$\frac{\text{Liver weight}}{\text{Body weight}} \times 100$	5.65 ± 0.10	5.92 ± 0.10	5.89 ± 0.12
Hydroxylase activities [nmoles·(mg protein)$^{-1}$·30 min^{-1}]			
Pentobarbital	13.8 ± 1.0	20.0 ± 1.3†	24.6 ± 0.5‡
Hexobarbital	35.6 ± 4.5	53.6 ± 2.3†	66.0 ± 3.5§
Aniline	40.3 ± 0.6	45.7 ± 1.0§	49.0 ± 3.0†
N-demethylase activities (nmoles·(mg protein)$^{-1}$·30 min^{-1}]			
Aminopyrine	15.1 ± 0.3	24.0 ± 1.0‡	25.2 ± 0.8‡
Ethylmorphine	120 ± 4	209 ± 6‡	262 ± 15‡
NADPH-cytochrome *c* reductase [nmoles·(mg protein)$^{-1}$·3 min^{-1}]	301 ± 8	329 ± 28	385 ± 19§
NADPH dehydrogenase [nmoles·(mg protein)$^{-1}$·3 min^{-1}]	46.5 ± 1.1	54.7 ± 0.8§	60.2 ± 2.0§
Cytochrome P-450 (nmoles/mg protein)	0.79 ± 0.02	0.96 ± 0.05†	0.87 ± 0.04
Cytochrome b_5 (nmoles/mg protein)	0.48 ± 0.02	0.52 ± 0.02	0.49 ± 0.03

* All data are means ± S.E.; N = 6.
† $P < 0.05$, compared to control.
‡ $P < 0.001$, compared to control.
§ $P < 0.01$, compared to control.

according to the procedure of Lowry *et al.* [16]. All data were subjected to statistical analysis, and the statistical significance was determined using Student's *t*-test.

The data obtained are summarized in Table 1. Daily administration of PCP to Group 2 or 3 animals significantly reduced the body weights of the mice and significantly reduced liver weight in Group 3, but did not alter the liver-to-body weight ratio in Group 2 or 3.

The effects of PCP on aromatic and aliphatic hydroxylation were measured using aniline, pentobarbital, and hexobarbital as substrates. In the mice of Groups 2 and 3, both aromatic and aliphatic hydroxylase activities were enhanced significantly. The effects of PCP on *N*-demethylase activities were also determined, using aminopyrine and ethylmorphine as substrates. Demethylase activities were induced in both Groups 2 and 3.

The effect of PCP on the NADPH-dependent electron-transport system was also investigated. NADPH dehydrogenase and NADPH-cytochrome *c* reductase activities were assessed to indicate whether electron transfer activity along the microsomal electron transfer chain was enhanced. There was an increase in the NADPH dehydrogenase activity in both groups 2 and 3, which had been treated with daily PCP. NADPH-cytochrome *c* reductase was increased significantly only in group 3 [which had been treated with PCP (40 mg/kg) once a day for 7 days and, then, twice a day for another 7 days]. Both cytochrome P-450 and cytochrome b_5 levels, however, appeared to be slightly affected or unaltered by administration of PCP to Groups 2 and 3.

The effect of PCP on hepatic drug metabolism has received limited attention. The first study was reported in 1974 by Radzialowski and Oppermann [7] who demonstrated that treatment of male rats for 4 days with PCP, (50 mg·kg^{-1}·day^{-1}, i.p.) caused significant increases in the rates of metabolism of hexobarbital, aminopyrine, and zoxazolamine *in vitro* by the hepatic microsomal preparation. The present study substantiates this effect in the mouse and further reveals that the electron transfer activity along the microsomal electron transfer chain also can be enhanced.

Although the biotransformation of PCP has not been well studied, several studies have demonstrated that oxidative hydroxylation is one of the major modes of metabolism of PCP. Ober *et al.* [17] have reported that the dihydroxy metabolite is the main product of PCP excreted from urine in the monkey. Lin *et al.* [18] have reported that two conjugated hydroxylated compounds were found in urine of intoxicated human subjects and monkeys. Wong and Biemann [19] have also demonstrated that hydroxylation is the principal mode of PCP metabolism in the rat. They have further suggested that hydroxylation of the piperidyl moiety probably accounts for the formation of the *N*-dealkylated metabolites [19]. Based on these studies, it is apparent that PCP is also one of the pharmacologic agents that influence hepatic drug metabolism [20, 21].

Numerous research efforts have demonstrated that tolerance can develop to the behavioral effect of PCP in experimental animals [22–26] and in humans [3]. The exact mechanism for this tolerance is not clear. Pinchasi *et al.* [27] have shown that liver metabolism is not the major basis for tolerance development, since the kinetics of brain uptake of the drug in naive and in tolerant animals are similar. The present study, however, clearly demonstrates that PCP affects the hepatic biotransformation machinery. It appears that metabolic tolerance may play a significant role in the behavioral tolerance induced by this compound. This possibility is supported by our recent study that demonstrated cross-tolerance between barbiturates and PCP [8]. The cross-tolerance to PCP that developed with chronic

pentobarbital administration could be attributed to the increase in PCP metabolism. On the other hand, cross-tolerance to barbiturates that developed with chronic PCP administration could be attributed to both the metabolic and adaptive character of tolerance development; it was observed that pentobarbital-induced narcosis decreased by about 50 per cent in PCP tolerant animals. Yamamoto *et al.* [28] have demonstrated that pentobarbital degradation occurs predominantly by oxidative hydroxylation, which is particularly enhanced in tolerant animals. Barbital narcosis was also decreased significantly in PCP tolerant mice. Since barbital elimination has been shown to be mainly through urinary excretion of the parent compound [29], the tolerance to barbital observed in PCP tolerant mice is thus inferred to be mainly functional. Therefore, it appears that the cross-tolerance to barbiturate by chronic PCP administration is attributable to both an increase in barbiturate metabolism and to an adaptive response of the CNS.

In summary, the effects of chronic administration of PCP on hepatic drug-metabolizing enzymes and several other variables of the multi-function oxidase system were investigated. Two groups of male ICR mice were given PCP, 40 $mg \cdot kg^{-1} \cdot day^{-1}$, or saline for 7 days respectively. Another group of mice was given PCP, 40 $mg \cdot kg^{-1} \cdot day^{-1}$, for 7 days followed by a twice daily dose of PCP, 40 mg/kg, for 7 more days. The body weight gain of mice treated with PCP was reduced significantly. Both the hydroxylation of aniline, pentobarbital, and hexobarbital and the *N*-demethylation of aminopyrine and ethylmorphine were enhanced significantly in groups 2 and 3. Both NADPH dehydrogenase and NADPH-cytochrome *c* reductase activities were also increased. Cytochrome P-450 and cytochrome b_5 content, however, were less affected. It is concluded that chronic administration of a high dose of PCP enhances the hepatic drug-metabolizing enzyme systems.

Acknowledgements—This study was supported by Grant DA-01403 from the National Institute on Drug Abuse.

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REFERENCES

1. E. D. Luby, J. S. Gottlieb., B. D. Cohen, G. Rosenbaum and E. F. Domino. *Am. J. Psychiat.* **119**, 61 (1962).
2. R. S. Burns and S. E. Lerner, *Clin. Toxic.* **9**, 477 (1976).
3. R. Stillman and R. C. Petersen, *Ann. intern. Med.* **90**, 428 (1979).
4. J. M. Rainey and M. K. Crowder, *New Engl. J. Med.* **290**, 466 (1974).
5. T. C. Tong, N. L. Benowitz, C. E. Becker, P. J. Forni and U. Boerner, *J. Am. med. Ass.* **234**, 512 (1975).
6. C. J. Stone and R. B. Forney, *Toxic. appl. Pharmac.* **40**, 177 (1977).
7. F. M. Radzialowski and J. A. Oppermann, *Toxic. appl. Pharmac.* **27**, 108 (1974).
8. I. K. Ho, B. A. Flint, K. Onoda and W. O. Berndt, *Pharmacologist* **20**, 195 (1978).
9. D. Kritchevsky, S. A. Tepper, L. M. Davidson and J. A. Story, *Proc. Soc. exp. Biol. Med.* **151**, 445 (1976).
10. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150**, 279 (1965).
11. J. R. Cooper and B. B. Brodie, *J. Pharmac. exp. Ther.* **114**, 409 (1955).
12. B. N. LaDu, L. Gaudette, N. Trousof and B. B. Brodie, *J. biol. Chem.* **214**, 741 (1955).
13. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
14. J. R. Gillette, B. B. Brodie and B. N. LaDu, *J. Pharmac. exp. Ther.* **119**, 532 (1957).
15. C. H. Williams and H. Kamin, *J. biol. Chem.* **237**, 587 (1972).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. R. E. Ober, G. W. Gwynm, T. Chang, D. A. McCarthy and A. J. Glazko, *Fedn. Proc.* **22**, 539 (1963).
18. D. C. K. Lin, A. F. Fentiman, R. L. Foltz, R. D. Forney and I. Sunshine, *Biomed. Mass Spectrom.* **2**, 206 (1975).
19. L. K. Wong and K. Biemann, *Biomed. Mass Spectrom.* **2**, 204 (1975).
20. A. H. Conney and J. J. Burns, *Adv. Pharmac.* **1**, 31 (1962).
21. A. H. Conney, *Pharmac. Rev.* **19**, 317 (1967).
22. D. P. Martin, C. C. Darrow, II, D. A. Valerio and S. A. Leiseca, *Lab. Anim. Sci.* **22**, 837 (1972).
23. R. L. Balster and L. D. Chait, *Clin. Toxic.* **9**, 513 (1976).
24. L. D. Chait and R. L. Balster, *J. Pharmac. exp. Ther.* **204**, 77 (1978).
25. I. Pinchasi, S. Maayani and M. Sokolovsky, *Psychopharmacologia* **56**, 27 (1978).
26. B. A. Flint and I. K. Ho, *Prog. Neuro-Psychopharmac.* **4**, 233 (1980).
27. I. Pinchasi, S. Maayani and M. Sokolovsky, *Biochem. Pharmac.* **26**, 1671 (1977).
28. I. Yamamoto, I. K. Ho and H. H. Loh, *Life Sci.* **20**, 1353 (1977).
29. A. G. Ebert, G. K. Yim and T. S. Miya, *Biochem. Pharmac.* **13**, 1267 (1964).