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Effect of Acute and Chronic Ethanol Pre-Treatment on the Disposition of Phencyclidine (PCP) in the Rat

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VADLAMANI, N. L., R. B. PONTANI AND A. L. MISRA. *Effect of acute and chronic ethanol pre-treatment on the disposition of phencyclidine (PCP) in the rat.* PHARMAC. BIOCHEM. BEHAV. 16(5) 847–850, 1982.—Disposition of [^{3}H] Phencyclidine in brain, plasma and adipose tissue of rats acutely and chronically-treated with ethanol was studied using a method possessing high sensitivity and specificity for PCP. In rats acutely-treated with ethanol (5 g/kg PO dose) and PCP (10 mg/kg IP dose), dispositional factors did not play a role in the intensified pharmacological and behavioral effects of PCP. However in rats chronically-treated with 2.5 g/kg PO dose of ethanol twice a day for 19 days, the disposition of PCP (5 mg/kg IP dose) was significantly altered and the values of PCP in brain, plasma and adipose tissue were significantly higher than those in the control group. Although inhibition of PCP metabolism and a comparatively slower rate of its elimination appear to account for the potentiation of drug effects in animals chronically-treated with ethanol, interaction of drugs at the level of the central nervous system cannot be ruled out.

[^{3}H] Phencyclidine disposition Acute and chronic ethanol pre-treatment Rat PCP-ethanol interaction

PHENCYCLIDINE (PCP, angel dust, crystal THC, peace pill) has become a major drug of abuse in the current drug subculture [16]. Its illicit abuse and the resultant psychotomimetic side effects pose a serious public health concern. The wide-spread increase in PCP abuse has been accompanied by a corresponding increase in multiple drug use and dangerous interactions between PCP and several other drugs e.g. morphine [21], barbiturates [4], Δ^9-tetrahydrocannabinol [5, 14, 17, 23], d-amphetamine [1,2] have recently been reported. Concurrent abuse of ethanol with PCP however, has been most frequently encountered in PCP overdose cases [8, 20, 24] and in two recent surveys of adult and youthful PCP users, 81% of the adults and 98% of the youths reported concurrent ethanol use [8,20]. Aside from two reports [3,22] dealing with the potentiation of lethality and toxicity with combined administration of PCP and ethanol, little information is available on the possible nature of this interaction.

Previous studies on the disposition and metabolism of PCP [6, 9, 10, 12, 15, 27] have shown that oxidative monohydroxylation in the 3 rings, conjugation, N-oxidation, dihydroxylation, N-dealkylation and the formation of N-(5-hydroxypentyl)-1-phenylcyclohexylamine were the metabolic pathways of PCP in experimental animals and man. The $t_{1/2\beta}$ of PCP in the plasma of mouse [10], rat [12], dog and monkey [26] and man [25] have been reported to be 0.6, 2.0, 2.86, 2.36 and 16 hr respectively. No information is available on the disposition of PCP in animals given acute or chronic doses of ethanol. In view of the clinical relevance and potential significance of the problem of PCP-ethanol interaction, this investigation was undertaken to determine if dispositional or pharmacokinetic factors play a role in the potentiation of PCP effects with ethanol in the rat.

METHOD

Samples of Drug

Phencyclidine hydrochloride was supplied by the Research Triangle Institute through the courtesy of National Institute on Drug Abuse, Rockville, Md. Phencyclidine [piperidyl-3,4-^{3}H(N)], specific activity 48 Ci/m mole was obtained commercially from New England Nuclear Inc., Boston, Mass. The labeled material was diluted with non-labeled PCP to a specific activity of approximately 10 μCi/mg for the injection solution.

Estimation of [^{3}H] PCP in Biological Materials

Aliquots (2 ml) of plasma (diluted 1:5 with distilled water) or tissue homogenates (10% ^{w}/v in 0.5 M HCl) containing 1 ml of nonradioactive PCP hydrochloride as carrier (500 μg/ml as free base) were adjusted to pH 9-9.5 with 1.5 M NH_4OH and the solution was buffered with 1 ml of 20% K_2HPO_4 solution and extracted with 15 ml cyclohexane as described previously [11]. The metabolites of PCP were not

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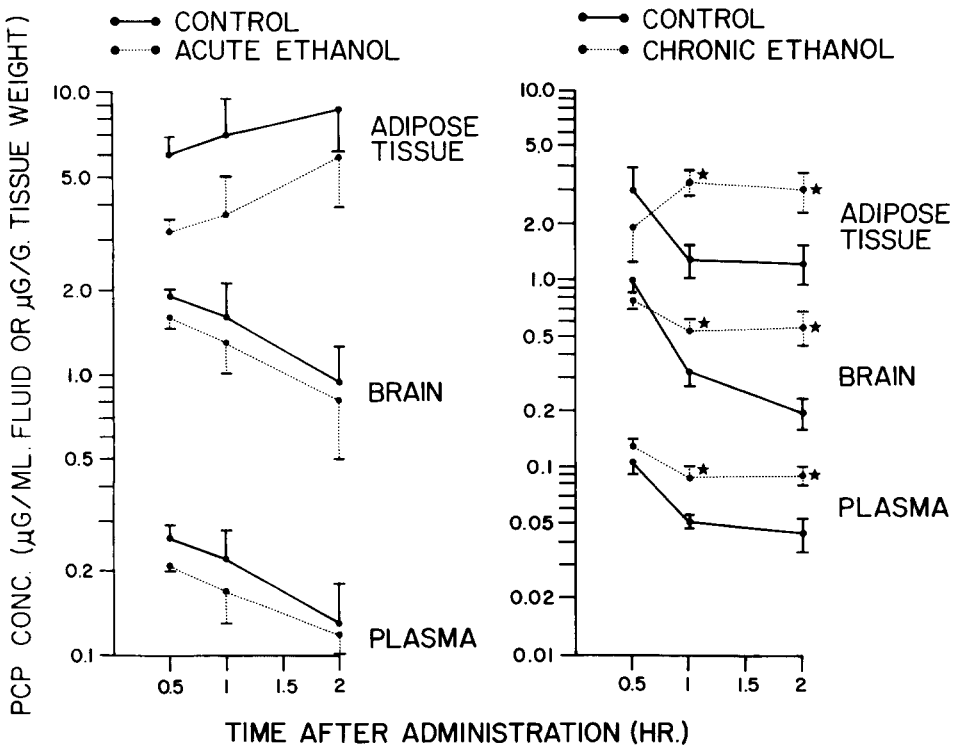

FIG. 1. Semi-logarithmic plot of PCP concentration as a function of time in rats acutely and chronically-treated with ethanol. In the acutely-treated group, the animals received a single 5 g/kg PO dose of ethanol and 15 min later a 10 mg/kg dose of [^{3}H] PCP by IP injection; in the chronically-treated group, the animals received a 2.5 g/kg PO dose of ethanol twice daily for 19 days and on the twentieth day a 5 mg/kg dose [^{3}H] PCP by IP injection. Data represent mean±S.E.M., microgram per g tissue or ml fluid (n=3 at each sampling time in the controls, acute and chronic ethanol groups) and * denotes significant differences from the control values at ($p<0.05$).

extracted by this procedure and the method was specific for PCP. *In vitro* recoveries of PCP from plasma or tissue homogenates were 100±5% (m±SD). Other details on extraction, counting and estimation of total radioactivity etc. have also been described earlier [11].

Animal Experiments

(a) Acute ethanol studies. Male Wistar rats (200–250 g) were administered a 5 g/kg dose of ethanol (20% w/v) orally and 0.25 hr later a 10 mg/kg dose of [^{3}H] PCP was injected intraperitoneally to the control and experimental animals. Control animals received a volume of water equivalent to the ethanol dose. After appropriate periods of time, the animals were sacrificed and blood and tissues were removed (N=3 at each sampling time). The tissues were homogenized in 0.5 M HCl to provide a 10% w/v homogenate and 2 ml aliquots analyzed in duplicate for PCP and total radioactivity [11]. Plasma samples diluted 1:5 with distilled water were similarly analyzed. Adipose tissue was included for analysis due to its very high uptake of lipophilic PCP [11,12].

(b) Chronic ethanol studies. Male Wistar rats were administered PO a 2.5 g/kg dose of ethanol twice daily (at 10 a.m. and 3 p.m.) for 19 days. Control animals received a volume of water equivalent to the ethanol dose at the same time for the same period. On the twentieth day, a 5 mg/kg dose of [^{3}H] PCP was injected intraperitoneally to the control and experimental animals. Tissues and plasma obtained at differed times (N=3 at each sampling time) were analyzed for PCP and total radioactivity as before [11]. The weights of animals in the control and chronic ethanol treated animals were in the range of 240–290 g.

(c) Statistical analysis. All data were subjected to statistical analysis and the statistical significance was determined using Student's *t*-test.

RESULTS

Acute Ethanol-PCP Studies

Data on the PCP values in brain, plasma and adipose tissue of the control and acute ethanol group appear in Fig. 1. No significant differences were observed in the concentrations of PCP in brain, plasma and adipose tissue at indicated times in the two groups. The brain to plasma PCP concentration ratios in the control and acute ethanol group at various times 7.3, 7.2, 7.1 and 7.7, 7.7, 6.8 respectively were also not significantly changed. Similarly no significant differences were seen in the total radioactivity values comprising free drug plus its metabolites in the two groups.

TABLE 1

CONCENTRATION* OF METABOLITES OF PCP IN RATS CHRONICALLY TREATED† WITH ETHANOL AFTER INTRAPERITONEAL INJECTION OF A 5 mg/kg DOSE OF [^{3}H]PCP

Tissue or fluid	Pretreatment	0.5 hr	1 hr	2 hr
Plasma	Control	1566 ± 9	1103 ± 256	724 ± 168
	Chronic ethanol	1862 ± 362	1519 ± 23	1436 ± 30‡
Brain	Control	1213 ± 98	760 ± 104	852 ± 3
	Chronic ethanol	1182 ± 151	1160 ± 89§	1293 ± 119§
Adipose tissue	Control	2241 ± 417	2529 ± 723	2574 ± 543
	Chronic ethanol	3068 ± 550	2839 ± 326	3117 ± 613

*Data represent concentrations of PCP metabolites mean ± S.E.M. (nanogram PCP-equivalents per g tissue or ml fluid) from 3 male Wistar rats at indicated times in the control and chronic ethanol groups. Nine animals each were used in the control and chronic ethanol groups. Duplicate determinations were performed on the individual tissues or fluids and the results averaged.

†Rats were administered PO a 2.5 g/kg dose of ethanol twice daily for 19 days, the control group received the same volume of water. On the twentieth day, a 5 mg/kg dose of [^{3}H]PCP was injected IP to the 2 groups of animals and the animals sacrificed at indicated times.

‡,§Denote significant differences from the control values at $p<0.01$ and $p<0.05$ respectively.

Chronic Ethanol-PCP Studies

The values of PCP in plasma, brain and adipose tissue at 1 and 2 hr were significantly higher in the chronic ethanol group as compared to the control (Fig. 1). The brain to plasma PCP concentration ratios in the control and chronic ethanol group at indicated times were 9.4, 6.2, 5.1 and 6.0, 6.1, 6.2 respectively. No significant differences were observed in the amounts of PCP metabolites in plasma at 0.5 and 1 hr or in adipose tissue at different times in the 2 groups (Table 1). Significantly higher values of PCP metabolites were seen in the plasma of chronic ethanol group at 2 hr and in brain at 1 and 2 hr as compared to the controls (Table 1).

DISCUSSION

Recent dose-response study [3] has shown that combined administration of PCP and ethanol resulted in a significant potentiation of lethal effects relative to either drug alone. In agreement with these observations, we found an enhancement of the depressant effects of acute doses of ethanol and PCP and these animals displayed varying degrees of ataxia and loss of hyperactivity, locomotion and stereotyped motor movements characteristic of PCP in the rat. A number of other CNS depressants also produce dangerous interactions with PCP [4, 14, 17, 21, 23]. The wide spectrum of pharmacologic interactions between ethanol and a wide variety of other drugs and the possible mechanisms underlying such interactions have been discussed in an excellent review [7]. Previous work [13,19] has shown that the acute doses of ethanol cause an *in vivo* inhibition of hepatic microsomal hydroxylase and N-demethylase activities thereby inhibiting metabolism and retarding the disappearance of a number of secondary drugs from blood in man and the rat. Ethanol intake also results in an increase in hepatic NADH/NAD ratio and alters the NAD-dependent conversion of uridine diphosphate glucose (UDPG) to uridine diphosphate glucuronic acid (UDPGA) thereby inhibiting the glucuronidation of the hydroxylated metabolites of secondary drugs [18]. The metabolism of PCP in the rat has been shown [6, 9, 10, 12, 15, 27] to occur via oxidative monohydroxylation in all the 3 rings, glucuronide conjugation, N-dealkylation, N-oxidation and dihydroxylation. Pharmacokinetic and dispositional factors however, do not play a role in the intensified drug effects seen in rats acutely-treated with ethanol and PCP in this study and the interaction of the two compounds at the CNS level apparently accounts for the observed potentiation of effects.

The deviations from proportionality in the concentrations of PCP in plasma and tissues in the controls of the acute ethanol experiment receiving 10 mg/kg dose of PCP and the controls of the chronic ethanol experiment receiving 5 mg/kg dose of PCP could conceivably be due to either the dose-dependent changes in disposition of PCP due to its high affinity for the fatty tissue [11,12] or different treatment schedules or body weight differences in the controls of the acute and chronic ethanol groups. Increased levels of PCP in brain, plasma in the chronic ethanol group could arise due to the inhibition of PCP metabolism and its slower rate of elimination. Chronic ethanol treatment however has been shown [13,19] to induce hepatic microsomal enzymes, which play an important role in the metabolism of secondary drugs and are only of secondary importance in the non-microsomal metabolism of ethanol via alcohol dehydrogenase. Although the plasma and brain $t_{1/2\beta}$ of PCP after IV injection in naive rats have previously been shown to be 2 and 3.3 hr respectively [12], the small number of data points in this study do not permit a correct estimation of $t_{1/2\beta}$. The comparison of the shape of the control and chronic ethanol-PCP decay curves however, points to a comparatively slower rate of elimination or decreased clearance of PCP in the chronic ethanol group. Alteration in the sensitivity of the CNS due to the chronic ethanol treatment or some degree of liver dysfunction may also be involved in the potentiation of toxic effects of PCP in animals in this group. A 2.5 g/kg PO dose of

ethanol twice a day did not however, severely affect the health of these animals, which fed well and their body weight gain was not significantly different from the control animals during the entire treatment. In spite of the complex nature of PCP-ethanol interaction, our results suggest that dispositional or metabolic factors play a role in part in the intensified effects observed with PCP in rats chronically treated with ethanol.

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