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Effects of acute and chronic phencyclidine on neurotransmitter enzymes in rat brain*

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Phencyclidine (1-phenycylohexyl piperidine hydrochloride; sernyl, PCP) is an anesthetic drug which has psychotomimetic properties in man [1, 2] and produces increases in locomotor activity and stereotyped behavior in rats, mice and monkeys [3, 4]. The psychotomimetic effects of PCP in man have been described as resembling more closely those seen in schizophrenic psychosis than in the psychotic states produced by several other hallucinogenic drugs [5]. The clinical presentation of acute phencyclidine intoxication in man is often characterized by an acute confusional state with disorientation and by motor ataxia and rigidity; this has some resemblance to toxic states induced by atropine and other anticholinergics. Behavioral and biochemical experiments with PCP indicate that the drug has diverse effects on several neurotransmitter systems in the brain and periphery. Previously reported pharmacological effects of PCP in animals include: (a) inhibition of uptake of norepinephrine, dopamine and serotonin in rat brain [6], (b) decreased synthesis or release, and/or increased metabolism of catecholamines in mouse or rat brain [7], (c) behavioral and pharmacological evidence for competitive antagonism of acetylcholine in peripheral organs and the CNS [8, 9], and (d) competitive inhibition of red cell and brain acetylcholinesterase (AChE) activities *in vitro* [10]. *In vivo* effects of PCP on AChE or other brain cholinergic enzymes, however, have not been extensively investigated. There is only one published study of the effect of PCP on brain γ-aminobutyric acid (GABA) [11]. We have shown recently that chronic administration of PCP, at some doses, produces an apparent supersensitivity to the effect of the drug on stereotyped behavior in the rat [12]. As a part of a series of studies of the behavioral and biochemical effects of acute vs chronic administration of PCP, experiments were carried out to investigate the effects of a single dose as compared to multiple doses of PCP on several enzymes—choline acetyltransferase (CAT), acetylcholinesterase (AChE), acetyl CoA hydrolase (AcCoA-H) and the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD)—in several regions of rat brain.

Sprague–Dawley male rats with initial weights of 200–300 g were used. Rats were housed in groups of six, had access to Purina rat chow and water, and were maintained in rooms with a 12-hr light–dark cycle and temperature control of 76 ± 2°F. The three groups used in the experiment were designated as follows: saline controls [these rats received injections of saline for 30 days and saline on day 31], acute PCP [these rats received injections of saline for 30 days and PCP (10 mg/kg, i.p.) on day 31], and chronic PCP [these rats received daily injections of PCP (10 mg/kg i.p.) Monday–Friday, and PCP (1 mg/100 ml) in their drinking water on Saturday and Sunday, for a period of 30 days, and received PCP (10 mg/kg, i.p.) on day 31]. Rats were killed 45 min after the 31st injection, and the brains were dissected over ice and frozen at −20° until analysis.

Selected brain regions (cerebellum, hippocampus or hypothalamus) were homogenized in 5 vol. of ice-cold 0.05 M potassium phosphate buffer (pH 7.5); the various enzyme activities were determined in each homogenate.

AChE was assayed according to the method of Garry and Routh [13] with slight modifications. The coefficient of variation of duplicate assays on the same sample was 4.3 ± 3.2 per cent.

CAT activity was determined by the charcoal method of Brandon and Wu [14] with slight modifications, measuring the labeled acetylcholine produced. The coefficient of variation of duplicate assays on the same sample was 3.9 ± 4.4 per cent.

AcCoA-H activity was determined by a charcoal method, developed in our laboratory†, that is similar to the CAT assay except that (1) the reaction mixtures in a final volume of 60 μl of 0.5 M potassium phosphate (pH 7.5) contained only 2 mM EDTA, an aliquot of brain homogenate, and 200 μM AcCoA[1-^{14}C], and (2) the incubation time was 20 min. At the end of each incubation, the reaction was stopped by addition of 1 ml of 10% acetic acid, and 180 mg of charcoal Norit neutral was added to absorb unreacted AcCoA. The reaction mixture was allowed to stand for 20 min with occasional vortexing and then was centrifuged at 5000 rpm for 20 min. A 0.5-ml aliquot of the supernatant fraction was transferred to a scintillation vial for measurement of radioactivity. The coefficient of variation of duplicate assays on the same sample was 4.4 ± 4.2 per cent.

GAD activity was determined according to the method of Wu *et al.* [15] measuring the evolution of $^{14}CO_2$ from L-[1-^{14}C]glutamic acid under anaerobic conditions. The coefficient of variation of duplicate assays on the same sample was 2.3 ± 2.04 per cent.

Statistical analysis was a one-way analysis of variance with the three treatment groups for each region. The significance of the difference between specific groups contained in the overall analysis of variance in a specific region was determined by the Duncan Multiple Range Test. For some purposes two-way analysis of variance (region × drug treatment) was also performed.

A single *in vivo* dose of PCP significantly affected the activities of two cholinergic enzymes, CAT and AChE, and the GABA-synthesizing enzyme, GAD, in some of the brain areas studied (Fig. 1). CAT activity was decreased (50 per cent) in cerebellum ($P < 0.01$), increased (18 per cent) in hippocampus, and showed no significant change in hypothalamus. AChE activity was increased in cerebellum (41 per cent) and hippocampus (29 per cent); a similar percentage increase (44 per cent) was found in hypothalamus, although this result was not statistically significant. Acute PCP treatment increased GAD activity by about 20 per cent in hippocampus ($P < 0.05$), but did not significantly increase GAD activity in the two other brain areas. There was no change in AcCoA-H activity in any of the regions studied.

After chronic PCP treatment, the changes in CAT and AChE activity seen after a single PCP dose did not occur; the activities of these two enzymes, in the three brain regions of chronic PCP rats, were not significantly different from the saline controls (Fig. 1). Furthermore, in those brain regions that showed significant changes in CAT and

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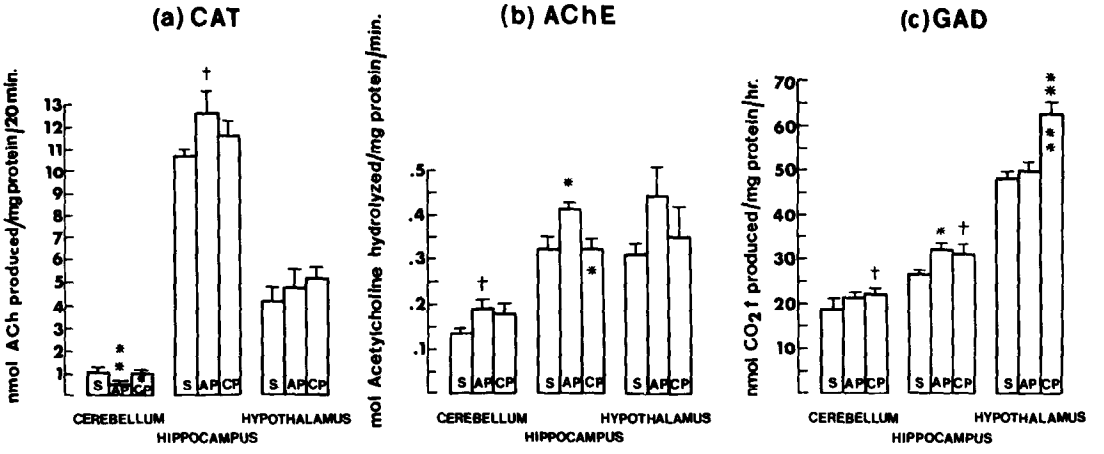

Fig. 1. Effects of acute and chronic phencyclidine on (a) CAT, (b) AChE, and (c) GAD activities in three regions of rat brain. Each bar represents mean (± S.E.M.) enzyme activity based on analysis of independent samples of four to five rats in each group. The activity of AcCoA-H is not shown in the graph because there were no significant effects due to PCP treatment. Overall mean AcCoA-H values in the three brain areas [expressed in nmoles·(mg protein)$^{-1}$·hr^{-1} were as follows: 8.44 (cerebellum), 6.92 (hippocampus) and 12.44 (hypothalamus). The statistical significance of the difference between specific groups was assessed by means of the Duncan multiple-range procedure after one-way analysis of variance. The level of significance of the difference between acute (AP) or chronic (CP) vs saline (S) is indicated *above* the bar; the level of significance of the differences between acute (AP) vs chronic (CP) PCP is indicated *within* the CP bar. Key: (†) $P < 0.10$; (*) $P < 0.05$; and (**) $P < 0.01$.

AChE activities after a single dose of PCP, the enzyme activities after chronic PCP treatment were significantly lower than in the acute PCP group (Fig. 1). There was no change in AcCoA-H activity in the chronic PCP rats. In contrast to the reversal of the effects of acute PCP on the cholinergic enzymes, that was seen after chronic PCP treatment, the changes in GAD activity induced by the single dose of PCP were maintained or increased in the tissue from chronically treated animals.

PCP that was added *in vitro* (concentration 10^{-6} M) to homogenates of rat hippocampus substantially increased AChE activity (by 69 per cent) over control values. There was no change in CAT, GAD or AcCoA-H activities in rat hippocampus at this *in vitro* concentration of PCP.

Our results, which show that PCP significantly increased AChE activity in specific brain regions both *in vivo* and *in vitro*, differ from those reported by some other investigators. Leonard and Tonge [11] found no statistically significant effects of 10 mg/kg PCP on whole brain AChE activity in Wistar rats, although there was a non-significant trend for an increase in AChE activity at the 30-min time point. Pinchasi *et al.* [10] reported that *in vitro* PCP inhibited AChE activity in preparations from whole mouse brain. These differences in results may be due to differential effects of PCP on AChE activity in different brain regions, or differences in strain (Wistar vs Sprague–Dawley), species (rat vs mouse) or source of animals. Regional differences in the effects of drugs on neurotransmitter enzyme activity may be masked in enzyme activity investigated in whole brain homogenates. Even in the three specific brain regions that we studied, a two-way analysis of variance showed a significant ($P < 0.05$) "interaction effect" of the extent of the effect of PCP on AChE or CAT activity in different regions of rat brain. The variability of the neurochemical effects of PCP, even in a single strain of animals, is illustrated by the findings of Tonge [16]; she reported opposite effects of PCP on 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in Wistar rats obtained from two different sources. We have also previously reported different effects on brain catecholamines of another hallucinogenic drug, D-lysergic acid diethylamide (LSD) [17], in Sprague–Dawley rats, as compared to the results of Tonge and Leonard [18] in Wistar rats. The previously reported differences in the neurochemical effects of PCP and other hallucinogenic drugs in different strains or sources of rats make it understandable that similar differences in the biochemical effects of PCP may exist in two species (rat vs mouse). Although the significant effects of PCP that we reported on CAT and AChE activities in some regions of rat brain support the suggestion that the effect of PCP on brain cholinergic systems may be one mechanism involved in its effects in animals and man, it will be important to further investigate regional and species differences in these neurochemical effects of PCP on brain cholinergic enzymes.

This is the first report of significant effects of PCP on GABA enzymes *in vivo*; Leonard and Tonge [11] reported a decrease in GABA levels in whole brain preparations from Wistar rats 30–60 min after the administration of 10 mg/kg PCP. If a similar decrease in GABA levels also occurred in Sprague–Dawley rats, the increase in GAD levels that we report might be consistent with an increase in GABA turnover.

The failure of the 31st dose of PCP to affect CAT and AChE activities in hippocampus and cerebellum, to the same extent as a single dose of PCP, may indicate a tolerance of CAT and AChE to the effects of PCP. The lack of reversal of the effect of an acute dose of PCP on GAD activity after chronic treatment with the drug may indicate that tolerance or adaptation to the biochemical effects of PCP may characterize the effects of the drug on some, but not all, neurotransmitter systems in the rat brain. Tolerance to PCP has been demonstrated in its effects on operant behavioral responses in the monkey [19]; supersensitivity of stereotyped or ataxic behavior to the effects of PCP develops after chronic administration of the drug to the rat or monkey [4, 12].