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EFFECTS OF PHENCYCLIDINE ON [^{3}H]CATECHOLAMINE AND [^{3}H]SEROTONIN UPTAKE IN SYNAPTOSOMAL PREPARATIONS FROM RAT BRAIN*

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Abstract—Phencyclidine inhibited uptake *in vitro* of [^{3}H]norepinephrine (IC_{50} 0.52 μM), [^{3}H]dopamine (IC_{50} 0.73 μM) and [^{3}H]serotonin (IC_{50} 0.80 μM) in crude synaptosomal preparations from rat brain through a competitive mechanism. Phencyclidine was fairly similar in potency to *d*-amphetamine and methylphenidate in inhibiting catecholamine uptake but was 8 times more potent than *d*-amphetamine and 34 times more potent than methylphenidate in inhibiting [^{3}H]serotonin uptake.

Phencyclidine [1-(phenylcyclohexyl) piperidine hydrochloride] (sernyl, PCP) is an anesthetic drug which has psychotomimetic effects in man [1, 2]. Like *d*-amphetamine (d-AMP) and methylphenidate (MP), PCP produces stereotyped behaviour and increases in locomotor activity in animals [3–5]. Although some of these behavioral effects of PCP in animals and man are also shared by d-AMP and MP [6–8], there are important differences in the behavioral profiles of the three drugs. For example, although both PCP and d-AMP produce a similar pattern of stereotyped behavior and decreased social interaction in monkeys, the behavioral syndrome produced by PCP in these animals is not characterized by the hyperviglance seen after d-AMP [5, 9]. Similarly, there are important differences in the phenomenology of amphetamine-induced psychosis and psychotic-like states induced by PCP [1, 2]. One of the biochemical effects of d-AMP and MP is blockade of re-uptake of catecholamines in synaptosomal preparations *in vitro* [10, 11]. The studies of Kanner *et al.* [12] on the effect of PCP on the turning rate of rats with unilateral substantia nigral lesions strongly indicate that PCP promotes an increase in central dopaminergic activity. These studies suggested the possibility that PCP might have effects like d-AMP on brain dopamine metabolism. Hitzemann *et al.* [13] have suggested, on the basis of studies of the effects of PCP on the synthesis and metabolism of catecholamines from [^{14}C]tyrosine and [^{14}C]dopa in mouse brain, that one important mechanism of action of PCP might be an effect of the drug on the re-uptake of catecholamines, although they presented no specific data on synaptosomal uptake. In a preliminary report [14], we presented evidence that PCP was a potent inhibitor of biogenic amine uptake. This report presents more complete data on the effects of PCP on uptake *in vitro* of catecholamines and serotonin and compares the potency of phencyclidine with d-AMP and MP.

METHODS

Male Sprague–Dawley rats (200–300 g) were used in all experiments. Animals were decapitated and brains were quickly removed and chilled on ice. In some experiments, uptake in selected brain parts—caudate, nucleus accumbens or cortex—was studied, after these brain parts from 6 to 22 rats were dissected out and combined. Uptake experiments were performed with crude synaptosomes (nuclei free homogenates, P_2) using established methods, described in more detail in earlier publications [15–18], with minor modifications and specifications. In this series of experiments, the crude synaptosomal preparations were dissolved in the sodium-potassium-phosphate buffer to a final concentration of either 15 mg/ml for whole brain, 7.5 mg/ml for caudate, or 2.5 mg/ml for nucleus accumbens. The much lower concentration of tissue for nucleus accumbens was chosen because of the low weight of this brain part and the very large number of brains needed to achieve the concentration equivalent to that of caudate. One of three tritiated amines, all obtained from New England Nuclear, [^{3}H]*d,l*-norepinephrine (NE, sp. act. 5.96 Ci/m-mole), [^{3}H]dopamine (DA, sp. act. 7.84 Ci/m-mole) or [^{3}H]serotonin binoxalate (5-HT, sp. act. 5.0 Ci/m-mole) was added to the medium in amounts of either 5 μl/100 ml or 2.5 μl/100 ml of the synaptosomal-buffer mixture respectively. This yielded final concentrations of tritiated amines as follows: [^{3}H]DA: 0.0064 μM or 0.0032 μM; [^{3}H]NE: 0.0073 μM or 0.0037 μM; [^{3}H]5-HT: 0.005 μM or 0.01 μM. Most experiments were performed at the higher concen-

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Table 1. Comparative IC_{50} values for phencyclidine, d-amphetamine and methylphenidate on inhibiting accumulation of biogenic amines in rat brain synaptosomes*

Amine	Phencyclidine	*d*-Amphetamine	Methylphenidate
Dopamine			
Caudate	0.73 (0.60–0.80)	0.34 (0.18–0.51)	0.36 (0.31–0.39)
Nucleus accumbens	0.47 (0.32–0.72)		
Norepinephrine			
Whole brain	0.52 (0.42–0.63)	0.31 (0.28–0.34)	0.17 (0.15–0.19)
Cortex	0.57 (0.48–0.72)		
Serotonin			
Whole brain	0.80 (0.57–1.00)	5.94 (3.38–10.20)	27.31 (17.49–42.13)

* Each number represents the IC_{50} value (expressed in μM) with 95 per cent confidence intervals in parentheses for the effects of the indicated drug on inhibition of accumulation of [^{3}H]DA, [^{3}H]NE or [^{3}H]5-HT in crude rat brain synaptosomes. All IC_{50} values were calculated from experiments using ^{3}H-amine concentration described in the legend of Fig. 1, and are based on results from two to four experiments.

tration of each amine except when two different concentrations were required to determine the K_i. Five ml of the amine-synaptosomal-buffer mixture was added to centrifuge tubes, all of which also contained pargyline at a final concentration of 10^{-4} M and either buffer or different concentrations of PCP, d-AMP or MP. Samples were incubated in their centrifuge tubes at 37°C with agitation. The following incubation times, which preliminary experiments had shown corresponded to the linear portion of amine uptake curve in our system, were used: [^{3}H]DA: 5 min; [^{3}H]NE: 10 min; and [^{3}H]5-HT: 6 min. Sample tubes with pargyline + buffer, which were not incubated at 37° and remained in ice, were used to obtain amine uptake values at 0° (blanks). After incubation, tubes were centrifuged and resulting pellets were washed with buffer, extracted with 0.4 N perchloric acid, homogenized with a Branson Sonifer (model 75), recentrifuged, and an aliquot of the supernatant was assayed by liquid scintillation counting. Triplicate samples were used for each drug concentration in each experiment, and the average error of triplicate samples was 6.2 per cent.

In the analysis of the data, dis./min values for uptake at 0° were subtracted from those obtained from samples incubated at 37° to obtain values relat-

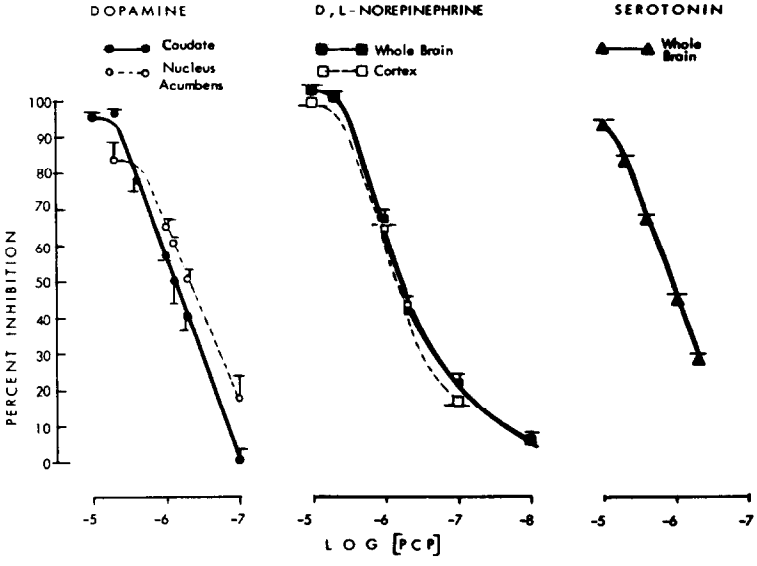
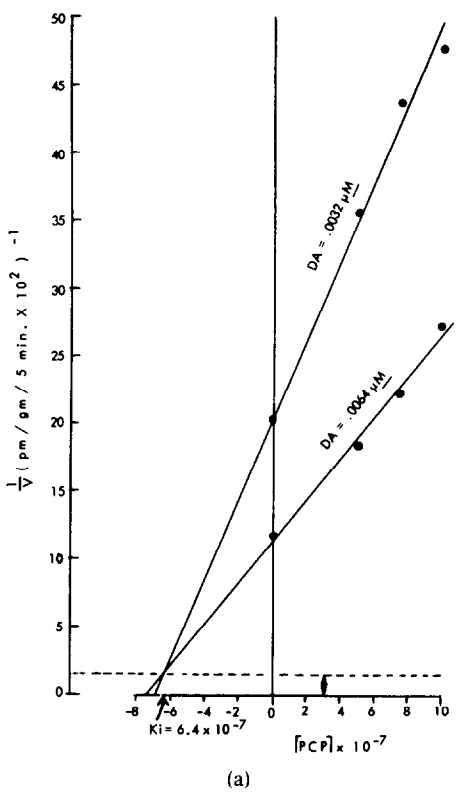
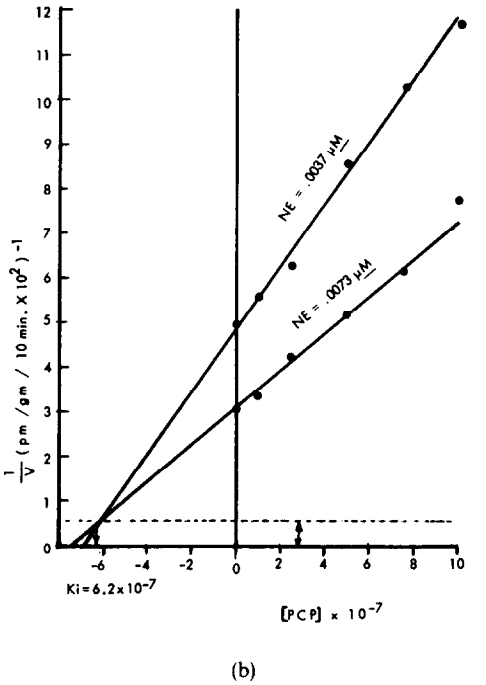


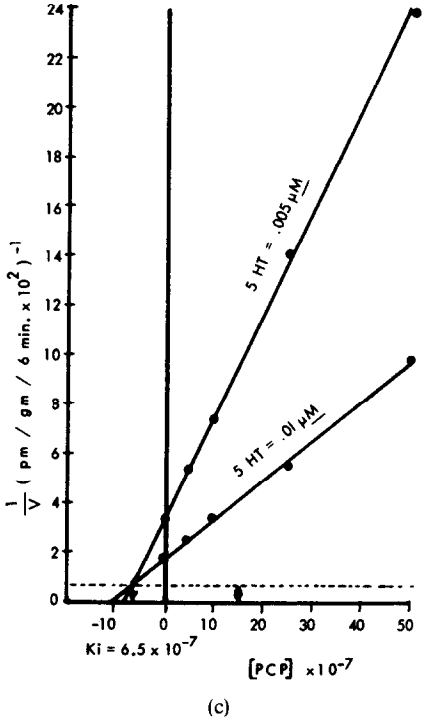
Fig. 1. Inhibition of accumulation of [^{3}H]DA, [^{3}H]NE and [^{3}H]5-HT into crude synaptosomes of rat brain. Each point represents the mean inhibition +S.E.M. compared to control samples, and means are based on values from two to four separate experiments. Five ml of the synaptosomal-^{3}H-amine-buffer mixture was used for each tube, with the following ^{3}H-amine concentrations: [^{3}H]DA = 0.0064 μM; [^{3}H]NE = 0.0073 μM; and [^{3}H]5-HT = 0.01 μM. Mean uptake values for control samples were: [^{3}H]DA: caudate, 87.0 pmoles/g, and nucleus accumbens, 114.9 pmoles/g; [^{3}H]NE: whole brain, 30.7 pmoles/g and cortex, 27.4 pmoles/g; and [^{3}H]5-HT: whole brain, 57.0 pmoles/g.



(a)



(b)



(c)

Fig. 2. Dixon plots for effects of phencyclidine on accumulation of (a) [^{3}H]DA in caudate, (b) [^{3}H]NE in whole brain, and (c) [^{3}H]5-HT in whole brain in crude synaptosomal preparations from rat brain. Linear regression lines were determined from mean values of points based on two experiments for each amine. The inhibition constant (K_i) was directly obtained from the plots and is indicated by the arrow (→). The dashed line (-----) represents the reciprocal of maximum velocity for the amine uptake.

ing only to the temperature sensitive uptake process. Per cent inhibition of uptake with drug was calculated by comparing dis./min for specific drug concentrations with control (buffer) samples in the same experiment. The IC_{50} values and 95 per cent confidence intervals were calculated by the method of probit analysis [18]. K_i values were determined from Dixon plots [19].

RESULTS

PCP was a potent inhibitor of uptake of [^{3}H]DA, [^{3}H]NE and [^{3}H]5-HT in crude synaptosomal preparations. Figure 1 shows log-dose response curves for the inhibitory effects of PCP on the accumulation of ^{3}H-amines in crude synaptosomal preparations, and Table 1 presents the IC_{50} values (with 95 per cent confidence intervals) for this inhibition; IC_{50} data for the two other drugs, d-AMP and MP, previously shown to be potent inhibitors of catecholamine uptake, are also presented.

PCP was a potent inhibitor of DA accumulation, with the drug having a slightly stronger effect in the nucleus accumbens ($IC_{50} = 0.47\ \mu M$) than in caudate ($IC_{50} = 0.73\ \mu M$). The drug was about 2.1 times less potent than d-AMP or MP. The potency of PCP in inhibiting NE accumulation in whole brain ($IC_{50} = 0.52\ \mu M$) and cortex ($IC_{50} = 0.58\ \mu M$) was in the same range as its potency in inhibiting dopamine in caudate or accumbens; as with DA, PCP was slightly less potent in inhibiting NE accumulation than d-AMP or MP. In the inhibition of 5-HT accumulation, however, PCP was considerably more potent ($IC_{50} = 0.80\ \mu M$); this is about 7.5 times more potent than d-AMP and 34.3 times more potent than MP.

Dixon plots (Fig. 2) showed that the effect of PCP on amine uptake of NE, DA and 5-HT was probably through a competitive type of inhibition. Graphical determination of K_i values for the effect of PCP on the three respective amines was: DA, $K_i = 6.4 \times 10^{-7}$ M; NE, $K_i = 6.2 \times 10^{-7}$ M; 5-HT, $K_i = 6.5 \times 10^{-7}$ M.

DISCUSSION

Our results indicate that PCP is a potent inhibitor of catecholamine and indoleamine uptake in crude synaptosomal preparations of rat brain, and that it acts as a competitive inhibitor. The addition of pargyline inhibits the metabolism of biogenic amines through the monoamine oxidase pathway, and previous chromatographic experiments by our own group [20] and other researchers [21–23] with similar uptake systems have shown that 80–95 per cent of radioactivity in the pellet of synaptosomes is unchanged NE, DA and 5-HT. Our results are similar to those recently reported by other investigators for the effects of phencyclidine and ketamine on inhibition of synaptosomal uptake of catecholamines [24, 25]. Ketamine, which is considerably less potent than PCP in its behavioral effects, is also about 100 times less potent than PCP in inhibiting synaptosomal NE accumulation [24]. The effect of PCP on 5-HT uptake has not been previously reported.

Other recent studies from our research group support the idea that dopaminergic mechanisms may play an important part in the behavioral effects of PCP. The stereotyped behaviors that PCP elicits in rats and monkeys are blocked by pimozide, a drug which is highly specific for blocking dopamine receptors [5, 26]. PCP also induces turning behavior ipsilateral to the side of the lesion in rats with unilateral nigra-striatal lesions and both α-methylparatyrosine and haloperidol significantly reduce this effect [12]. Some researchers have suggested that similar behavioral effects produced by amphetamine may be primarily mediated by the effects of this drug on releasing and/or blocking re-uptake of brain dopamine. Our finding that PCP has a potency for inhibiting synaptosomal dopamine accumulation that is in the same order of magnitude (10^{-7} M) as d-AMP and MP is consistent with the behavioral evidence suggesting that the effect of PCP on dopaminergic mechanisms may be important in mediating some of its behavioral effects in animals and man.

The biochemical effects of PCP on other neurotransmitters, however, may also have relevance for its behavioral effects. Reports by our own [4] and other [27] groups have shown that cholinergic drugs antagonize some of the behavioral effect of PCP, and that PCP is a competitive inhibitor of acetylcholinesterase. Carey and Heath [25] have suggested that the difference between PCP and d-AMP, both of which are potent inhibitors of synaptosomal catecholamine uptake, may be related to the anticholinergic effects of PCP. In our own experiments, the greatest difference between PCP and d-AMP or MP was in the potency of these compounds in inhibiting 5-HT accumulation. PCP was about 8 times more potent than d-AMP and about 34 times more potent than MP in this respect. Whereas PCP has roughly equal potencies in inhibiting accumulation of the three amines (DA, NE and 5-HT) in synaptosomal preparations, d-AMP and MP, on the other hand, were much more potent inhibitors of the catecholamines than of 5-HT. Neurochemical effects of PCP on other parameters of serotonergic function have not been extensively investigated. Tongue and Leonard [28] originally reported increases in 5-HT and decreases in 5-HIAA after administration of 10 mg/kg of PCP, but their later reports showed that this change varied widely in different groups of Wistar rats [29]. Experiments conducted in our laboratory, utilizing doses up to 20 mg/kg of PCP have not shown any significant increase in whole brain 5-HT in male Sprague–Dawley rats (R. C. Smith *et al.*, unpublished results). Further investigation of the effect of PCP on measures reflecting serotonin turnover, and the effect of drugs which deplete or antagonize brain serotonin on modifying behavioral response effects of PCP in animals, would help clarify the effect of PCP on brain serotonin metabolism. The relevance of the effect of PCP on brain serotonin metabolism to the differences in the behavioral profile of PCP, as compared to d-AMP and MP, could then be explored.

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