

markedly different; (b) The toxicity of a single i.p. dose of 20 mg ADR/kg (LD<sub>50</sub> for single i.v. dose of free drug) encapsulated in anionic liposomes was comparable to a similar dose of the free drug administered as a 96-hr tail vein infusion. Based on the mortality of mice treated with a 20 mg/kg dose of free adriamycin, i.p. and i.v., either as single or divided bolus injections, the reduced toxicity observed with ADR encapsulated in the anionic liposomes with no selective targeting properties is probably due to slow drug release *in vivo*; (c) In preliminary studies with L1210 and P388 mouse leukemia *in vitro* and *in vivo*, the antitumor activity of ADR was not found to be compromised or potentiated after encapsulation in liposomes; and (d) Due to the variable interaction of drugs with liposomal lipid bilayers, future studies should be directed toward systematically evaluating the effect of lipid composition on encapsulation of a drug, and quantitatively comparing liposome vs infusion doses of the drug, to understand the potential for such methods of drug delivery in cancer chemotherapy. Further, due to the differences in antitumor activity of parent drug and metabolites of the anthracyclines [22, 23], pharmacokinetic studies of metabolite formation following treatment with slow drug release carriers and as infusion will be important.

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### Structure-function relationships in the inhibition of synaptosomal dopamine uptake by phencyclidine and analogues: potential correlation with binding site identified with [<sup>3</sup>H]phencyclidine

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Phencyclidine (PCP) exhibits psychotomimetic, anaesthetic, analgesic, stimulant and depressant effects which probably involve a variety of neuropharmacological mechanisms. Biochemical and electrophysiological studies have

shown that PCP interacts with a series of functional neuronal receptors. These receptors include the muscarinic cholinergic receptor, the  $\mu$ -opioid receptor [1], the Na<sup>+</sup> and K<sup>+</sup> channels [2] and the nicotinic receptor [3]. PCP also

interacts at lower concentrations with specific binding sites identified with [ $^3\text{H}$ ]PCP [reviewed in 1 and 4]. The affinities of phencyclidine and phencyclidine analogues for these specific binding sites in rat brain are well correlated with the corresponding pharmacological potencies determined by the rotarod assay and the rat discriminative stimulus test [reviewed in 1, 4, 5]. The function of this receptor, which might also be that of the benzomorphans [5] is not yet known. PCP has been reported to be an inhibitor of [ $^3\text{H}$ ]dopamine uptake [6–8]. This is particularly interesting since many investigators feel that PCP psychosis resembles acute schizophrenia and this in turn would imply the involvement of dopamine neurones. In this paper we describe the effects of PCP and seven of its derivatives on [ $^3\text{H}$ ]dopamine ([ $^3\text{H}$ ]DA) uptake by rat brain synaptosomes and show that this effect is reasonably well correlated with their binding properties to the PCP binding sites identified with [ $^3\text{H}$ ]PCP.

#### Materials and methods

Freshly prepared synaptosomes (whole rat brain minus the cerebellum) were first incubated at 30° for 15 min in the following medium: NaCl 140 mM; KCl 5 mM; CaCl<sub>2</sub> 2.8 mM; MgSO<sub>4</sub> 1.3 mM; Tris-Cl 10 mM pH 7.4 plus 0.2 mM pargyline (a monoamine oxidase inhibitor from Sigma) in the absence or in the presence of PCP or its derivatives. The uptake was initiated by the addition to the incubation of 10<sup>-8</sup> M [ $^3\text{H}$ ]DA (47 Ci/mmol, Amersham). Aliquots (200  $\mu\text{l}$ ) of the incubation medium were filtered on GF/B filters (Whatman) and rinsed twice with 5 ml of the incubation buffer. The radioactivity retained on the filter was determined by liquid scintillation spectrophotometry (Packard 2450) in 7 ml of biofluor (NEN). For all uptake experiments at 30° a parallel uptake measurement at 0° was carried out and subtracted from the uptake measured at 30°. The difference gives the specific [ $^3\text{H}$ ]DA uptake component of the total uptake measured. Uptake rates were determined after 10 min, a period corresponding to linearity in the kinetics of [ $^3\text{H}$ ]DA accumulation.

#### Results

The synaptosomal preparation accumulates [ $^3\text{H}$ ]DA with the following characteristics:  $K_m = 0.12 \mu\text{M}$  and  $V_m = 28$  pmoles/10 min/mg of protein. As it has been previously described, PCP is a potent inhibitor of the uptake process [6]. Figure 1(A) shows that PCP (1  $\mu\text{M}$ ) inhibits the [ $^3\text{H}$ ]DA uptake. The  $\text{IC}_{50}$  of this inhibition determined after an uptake period of 10 min is 0.46  $\mu\text{M}$  (Fig. 1B). This value is very similar to that obtained by others [8] and also to the affinity of [ $^3\text{H}$ ]PCP for its receptor (0.25  $\mu\text{M}$ ) [1]. Furthermore this value is equal to the affinity of PCP for its binding site in the same conditions of ionic composition of the incubation medium [4].

PCP as its analogues behave as competitive inhibitors of the uptake process (not shown) [7] with  $\text{IC}_{50}$  between 0.5 and 100  $\mu\text{M}$ . In our experimental conditions  $\text{IC}_{50}$  values obtained by competition represent the apparent dissociation constant between PCP or its analogues and the dopamine uptake system (the [ $^3\text{H}$ ]DA concentration is 10<sup>-8</sup> M, much lower than the  $K_m$  for dopamine uptake = 0.12  $\mu\text{M}$ ). Figure 1(C) shows that  $\text{IC}_{50}$  values for the inhibition of [ $^3\text{H}$ ]DA uptake by PCP and its derivatives are well correlated with their affinities for the PCP binding site ( $r = 0.93$ ,  $P < 0.01$ ).

#### Discussion

The results presented in this paper are in good agreement with those previously obtained by other groups [6, 7] in that they confirm that PCP inhibits synaptosomal dopamine uptake. The correlation between the affinities of different PCP analogues for the [ $^3\text{H}$ ]PCP binding site and the corresponding efficiencies in blocking dopamine uptake suggest that the binding of [ $^3\text{H}$ ]PCP might well be linked to the DA uptake system.

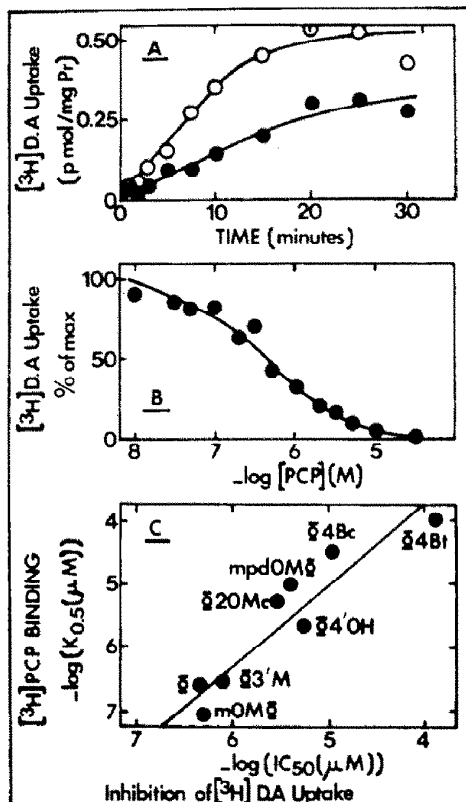


Fig. 1. Effects of PCP and its derivatives on [ $^3\text{H}$ ]DA uptake. (A) Kinetics of [ $^3\text{H}$ ]DA uptake in the absence and in the presence of PCP. (○) [ $^3\text{H}$ ]DA uptake in the absence of PCP; (●) [ $^3\text{H}$ ]DA uptake in the presence of 1  $\mu\text{M}$  PCP. (B) Dose-response curve for the PCP inhibition of [ $^3\text{H}$ ]DA uptake. (C) Correlation between  $\text{IC}_{50}$  values for the inhibition of [ $^3\text{H}$ ]DA uptake by PCP and its derivatives and their affinities for the PCP binding sites in competition with [ $^3\text{H}$ ]PCP [1] and expressed as the dissociation constant ( $K_{0.5}$ ). The equation of the straight line ( $y = -1.27x + 1.34$ ) was obtained by the least-squares method. The correlation coefficient and the statistical significance are  $r = 0.930$  and  $P < 0.01$  respectively. The different derivatives are:  $\Phi 4\text{Bc}$ ,  $\Phi 4\text{Bt}$ : *N*-(1-phenyl-4-tert-butylcyclohexyl)piperidine, *cis*- (c) or *trans*- (t) isomers;  $\text{mpdOM}\Phi$ : *N*-(1-phenyl-3,4-dimethoxycyclohexyl)piperidine;  $\Phi 20\text{Mc}$ : *N*-1-phenyl-2-methoxycyclohexyl)piperidine *cis*-isomer;  $\Phi 4'\text{OH}$ : *N*-(1-phenylcyclohexyl)4-hydroxypiperidine;  $\Phi 3'\text{M}$ : *N*-(1-phenylcyclohexyl)3-methyl-piperidine;  $\text{mOM}\Phi$ : *N*-(1-phenyl-3-methoxycyclohexyl)piperidine.

However the target for dopamine cannot be the permease system itself since DA is without effect on [ $^3\text{H}$ ]PCP binding [reviewed in 1]. On the other hand it is well known that the DA uptake process is Na<sup>+</sup>-dependent. This could suggest that PCP and analogues interact with the Na<sup>+</sup>-dependent part of the DA uptake system. This would be consistent with the antagonistic properties of Na<sup>+</sup> and other monovalent cations on [ $^3\text{H}$ ]PCP binding [4].

In conclusion, PCP binds to specific binding sites in the NS and is known to be a potent blocker of [ $^3\text{H}$ ]DA uptake by rat brain synaptosomes or slices. In this paper we show that PCP derivatives also block [ $^3\text{H}$ ]DA uptake with  $\text{IC}_{50}$  values that are well correlated with their affinities for the PCP receptor. If the binding site of PCP is not the permease

system itself of [<sup>3</sup>H]DA uptake, it might well be linked to the Na<sup>+</sup>-dependent part of this system in agreement with the antagonistic action of Na<sup>+</sup> and monovalent cations on the binding of PCP to its receptor.

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