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INTERACTION OF PHENCYCLIDINE WITH MOUSE NEUROBLASTOMA CELLS*

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Abstract—Growth of mouse neuroblastoma (Nb) cells (clone M1) was not affected by phencyclidine (PCP) concentrations of 10^{-6} M up to 2×10^{-4} M, whereas 10^{-3} M PCP caused a 100% inhibition of cell growth. Several PCP analogs, including the quaternary PCP methiodide, exerted effects similar to those of PCP. The uptake of [piperidyl-3,4-^{3}H]PCP ([^{3}H]PCP) by the Nb cells was studied using cell monolayers in Petri dishes. Non-specific entry of PCP into the cells was linear with added substrate but specific uptake exhibited saturation kinetics. The concentration for half-maximum specific uptake was 2×10^{-5} M, and the capacity of the cells at saturation was 2–3 nmoles [^{3}H]PCP/mg protein, at 22°. The uptake rate constant was $0.2 \pm 0.05 \times 10^5$ ($M^{-1}\,min^{-1}$) and the dissociation constant was 0.25 ± 0.05 (min^{-1}). Uptake was temperature dependent and was inhibited by 2,4-dinitrophenol (DNP). This may indicate that this binding represents (at least in part) an active uptake process of PCP into the cells.

Phencyclidine, 1-[1-(phenylcyclohexyl)]piperidine (PCP), was first recommended for clinical trials in 1957 as a promising anesthetic. While PCP is an effective anesthetic because it does not depress respiration [1–3], it produces adverse CNS side-effects, such as catalepsy, hallucinations, prolonged post-anesthetic confusion and, occasionally, convulsions. These effects prompted the American Food and Drug Administration to discontinue its clinical use in 1965. PCP produces a long-lasting psychosis that resembles schizophrenia more than those produced by other psychotomimetic drugs [4, 5]. These effects have contributed to the emergence of PCP as a drug of abuse [6], usually known as "angel dust", "hog", "monkey stuff", etc. PCP and its derivatives, synthesized in many laboratories, exhibit a very wide spectrum of effects, and there is controversy in the literature about the relationship between the clinical effects and the suggested PCP mechanisms of action, to which these effects should be attributed.

It has been shown that PCP interacts with various receptor systems. The interaction with the muscarinic cholinergic receptor was investigated by Gabriel-evitz *et al.* [7]. Our laboratory, as well as others, studied the interaction of PCP with the nicotinic cholinergic receptor. The effect of PCP was attributed to its interaction with the ionic channel of the acetylcholine receptor [8–13].

PCP and some of its derivatives are inhibitors of both acetyl- and butyrylcholinesterase [14, 15]. It interferes effectively with recognition processes at the level of the opiate receptors, but not with the glycine receptor, γ-aminobutyric acid receptor, glutamate receptor, dopamine receptor, serotonin receptor and β-adrenergic receptor [16].

PCP also affects serotonergic systems in the CNS [17], and it is an indirect dopamine agonist, as shown by Schlemmer *et al.* [18]. It has been suggested that these effects are caused by PCP inhibition of the re-uptake system for catecholamines; such an inhibition has been indeed shown in synaptosomal and slice preparations from rat brain [19–21]. We studied [22] the effects of PCP on granular storage of catecholamines, using bovine chromaffin granules as a model, and found that the PCP concentrations needed for 50% inhibition of (−)noradrenaline uptake were two to three orders of magnitude larger than those needed to inhibit synaptosomal uptake.

The existence of a specific PCP receptor was demonstrated in rat brain membranes by Vincent *et al.* [23] and Zukin and Zukin [24]. Maayani and Weinstein [25], however, suggested that this "PCP receptor" was an artifact caused by the binding of [^{3}H]PCP to GF/B glass fiber filters.

While many biochemical, physiological and pharmacological studies with PCP have been performed in whole animals, animal tissues and tissue slices or homogenates, little work has been done on the interaction of PCP with whole cells or whole cellular organelles. Fosset *et al.* studied the interaction of PCP with intact cardiac cell cultures *in vitro* [26] and found that PCP molecules are agonists of the post-synaptic muscarinic receptors of these cells. In our laboratory, we have studied in the past few years the interaction of PCP with whole cells and cellular organelles [8, 9, 22, 27–29].

Here we report studies on the effect of PCP and several analogs on neuroblastoma (Nb) cell growth and plating efficiency and on the retention of PCP by intact Nb cells grown in culture. Since the cells are intact, the retention of [^{3}H]PCP may comprise active and passive uptake processes as well as specific receptor binding and nonspecific binding. We characterized the interaction in general terms of uptake by the cells and showed that the large quantity of [^{3}H]PCP taken up by intact cells would obscure any

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receptor binding if present. In our studies, the M1 clone of mouse neuroblastoma was used. This is an adrenergic clone derived from the C1300 mouse neuroblastoma clone [30], which has a high content of catecholamines.

MATERIALS AND METHODS

Chemicals. The following compounds were used: phencyclidine (PCP) [31, 32], 1-[1-(2-thienyl)-cyclohexyl]piperidine (TCP) [31, 32], PCP methiodide (PCPMeI) [31], 1-[1-(3-aminophenyl)cyclohexyl]piperidine (3-NH_2PCP) [33] and *N*-allyl-*N*-ethylphenylcyclohexylamine (unpublished results from our laboratory). Specifically labeled [piperidyl-3,4-^{3}H]PCP (sp. act. 23.7 Ci/mmole, radiochemical purity >99%) [34] was supplied by the Nuclear Research Center, Negev, Israel. Atropine and tubocurarine were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. *N*-Methyl-4-piperidyl benzilate (4-NMPB) was synthesized according to Biel *et al.* [35]; 3-quinuclidylbenzilate was synthesized according to Whitaker [36]. The concentrations of PCP solutions were checked by measuring u.v. absorption in the aqueous buffer solution using an index of absorption of $300 \pm 20\ M^{-1}\ cm^{-1}$ at $\lambda = 262$ nM.

Cell cultures. A clone of the mouse neuroblastoma cell line M1 [30] was obtained from Dr. E. Yavin of the Department of Neurochemistry, the Weizmann Institute of Science, Rehovot, Israel. Cells were grown in 75 cm^2 Falcon flasks in a high glucose, high pyruvate (110 mg/l) Dulbecco's Modified Eagle's Medium (DMEM) (GIBCO, Grand Island, NY, U.S.A.) supplemented with 10% fetal calf serum (Bio-Lab Ltd., Jerusalem, Israel) and with 0.5% of the following antibiotic mixture: penicillin, 5×10^4 units/ml; streptomycin, 10 mg/ml; and mycostatin, 10^4 units/ml.

Cells were grown in a 37° incubator, in a humidified atmosphere of 95% air and 5% CO_2. For cell growth and for binding experiments, dissociated cells were plated quantitatively into 35 mm plastic tissue culture plates (Corning, U.S.A.) or on Linbro Multiwell Tissue Culture Trays (24 × 16 mm wells, 2 cm^2 each). Multiwells were plated at a density of 50,000 cells/well and grown to confluency in 1 ml of medium per well.

Effect of PCP on cell growth. The effects of various concentrations of PCP on cell growth were studied over a period of up to 10 days. Cells ($1–1.5 \times 10^5$) were seeded onto 35 mm plates (Corning, U.S.A.) in a medium containing the drug. PCP is stable in growth medium as shown by Shahar *et al.* [27]. Every 24 hr thereafter, duplicate plates for each drug concentration were trypsinized, and the cells were counted in a hemocytometer. The effect of PCP on the plating efficiency of the cells was studied by seeding 300 cells onto 100 mm plastic plates (Falcon) in a medium containing the drug. After 10 days of growth, the cells were washed, fixed by methanol, and stained with Giemza.

Uptake of PCP by neuroblastoma cells. The growth medium was rapidly removed by aspiration, and the cells were covered with 0.5 to 1 ml of buffer, at either 22° or 37°, containing the labeled [^{3}H]PCP and any other drug, as specified. The buffer contained: 110 mM NaCl, 5.3 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, 25 mM glucose, and 70 mM sucrose. The pH was adjusted to 7.2 with HCl. Plates were then incubated for various periods of time. The process was stopped by removal of the incubation medium, followed by three washes with 0.5 to 1 ml cold (0°) buffer.

The total wash sequence was completed in 8–10 sec per well; the cells were in contact with the washing buffer for 5–6 sec only. Increasing the number of washes from three to six had no effect on the binding. Cells were then lysed with 0.5 ml of 1% sodium dodecylsulfate (SDS), and the wells were washed again with 0.5 ml of 1% SDS to give a lysate with a final volume of 1 ml which was used for protein determination and for radioactivity measurement. Protein was determined by the method of Lowry *et al.* [37]. Radioactivity was determined in a Packard Tricarb Scintillation Counter in 10 ml of scintillation fluid (1 liter toluene, 0.5 liter Triton X-100, 0.45 g dimethyl-POPOP, 8 g PPO)* containing 1 ml sample. Experiments were performed in duplicate. The reproducibility of the duplicates was usually 5–10%. In each experiment, the cell number per plate was determined in control plates.

RESULTS

Effects of PCP on cell growth. Figure 1 shows typical results for the effects of PCP. It was found that cell growth was not affected by PCP concentrations of 10^{-6} M, 10^{-5} M and up to 2×10^{-4} M. Between 4×10^{-4} M and 10^{-3} M a concentration-dependent inhibitory effect on cell growth was observed. Cells treated for 24 or 72 hr with 10^{-3} M PCP, and then grown in a fresh medium without PCP, began to multiply again.

The following analogs were also tested: 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP), *N*-allyl-*N*-ethyl-1-phenylcyclohexylamine, and the quaternary PCP methiodide. All analogs exerted effects similar

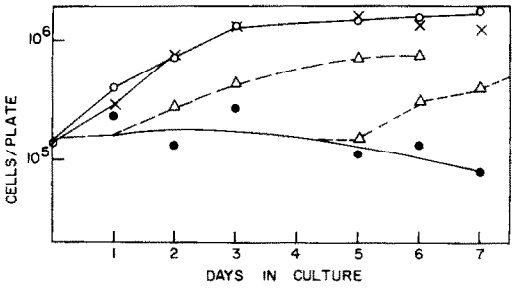

Fig. 1. Effect of PCP on the growth of Nb cells. Nb cells (1.5×10^5) were seeded onto 35 mm plates and grown in DMEM medium containing 10^{-4} M or 10^{-3} M PCP. Key: (○——○) control medium; (×——×) 10^{-4} M PCP; (●——●) 10^{-3} M PCP; and (△----△) cells grown in the presence of 10^{-3} M PCP and washed after 24 or 72 hr. PCP-free growth medium was then added, and cell growth was recorded.

* POPOP: 1,4-bis-[2-(4methyl-5-phenyloxazolyl)]-benzene; and PPO: 2,5-diphenyloxazole.

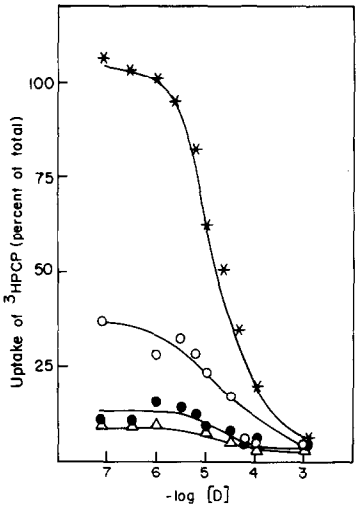

Fig. 2. Inhibition by PCP of [^{3}H]PCP binding to cells, and tissue culture plates treated with growth medium. Nb cells in 16 mm diameter wells were incubated for 10 min at 22° with 0.5 ml of 4–8 × 10^{-8} M [^{3}H]PCP (485,000 cpm per 0.5 ml), and increasing concentrations of PCP (*D*). Key: (★——★) inhibition by PCP of uptake of [^{3}H]PCP by Nb cells grown in wells (100 μg protein/well); (○——○) wells without cells or medium, untreated; (●——●) wells without cells, incubated for 6 hr at 37° with growth medium and (△——△) wells without cells, incubated for 2–3 days at 37° with growth medium.

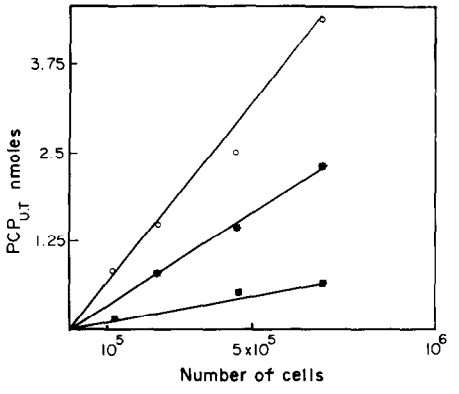

Fig. 3. Dependence of PCP uptake by Nb cells on the number of cells/plate. Nb cells were seeded at different densities. The experiment was performed at 37°, 30 hr after seeding. The total uptake of PCP ($PCP_{u,T}$) was calculated from the bound radioactivity and the specific activity of [^{3}H]PCP. Key: (○——○) 2 × 10^{-4} M PCP; (★——★) 4 × 10^{-5} M PCP; and (■——■) 5 × 10^{-6} M PCP.

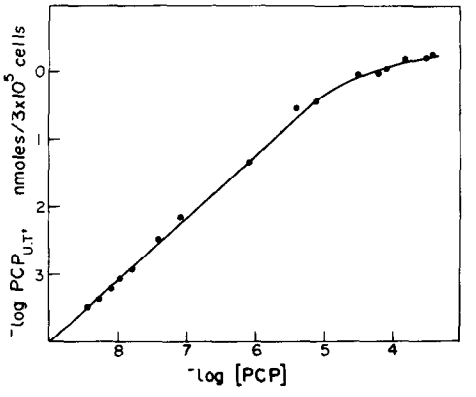

Fig. 4. Uptake of [^{3}H]PCP by Nb Cells. Nb cells were incubated for 10 min at 37° with different concentrations of [^{3}H]PCP at various isotopic dilutions. Thereafter the cells were washed and lysed, and the radioactivity was determined as described in Materials and Methods. Results are plotted on a double logarithmic scale. $PCP_{u,T}$: total uptake of [^{3}H]PCP by Nb cells.

to those of PCP, i.e. complete inhibition of cell growth at 10^{-3} M, and partial or no effect at 5 × 10^{-4} M to 10^{-4} M (not shown). PCP at concentrations of 10^{-4} M, 10^{-5} M and 10^{-6} M did not affect plating efficiency compared to control plates, but there was no cell growth in the presence of 10^{-3} M PCP.

Uptake of [^{3}H]*PCP by cells*. A possible artifact in binding experiments could arise from the binding of [^{3}H]PCP to the plastic material of the tissue culture plates [25]. We therefore measured the binding of [^{3}H]PCP to untreated tissue culture plates or to plates treated with growth medium for 1 hr, 6 hr or a few

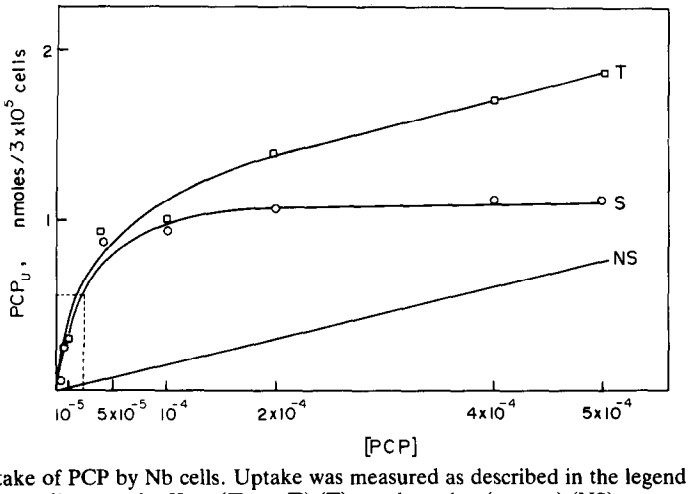

Fig. 5. Uptake of PCP by Nb cells. Uptake was measured as described in the legend to Fig. 4. Results are plotted on a linear scale. Key: (□——□) (T), total uptake; (———) (NS), non-specific uptake; and (○——○) (S), specific uptake.

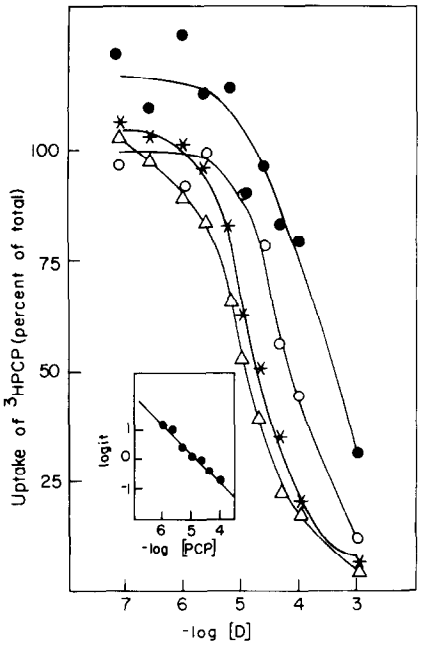

Fig. 6. Inhibition by PCP and PCP analogs of uptake by neuroblastoma cells of [^{3}H]PCP. Nb cells grown in 16 mm wells were incubated at 22° with 0.5 ml of 8×10^{-8} M [^{3}H]PCP (485,000 cpm) and increasing concentrations of the unlabeled drugs (D). The protein content of each well was 100 ± 20 μg, and results have been normalized to 100 μg protein/well. One hundred percent uptake was 30,000 cpm/100 μg protein. Key: (★——★) PCP; (●——●) PCPMeI; (○——○) ketamine; and (△——△) TCP. Insert: Logit-log plot [37] of inhibition data for PCP, plotted on a logarithmic scale. Ordinate: $\text{Log}_{10}\, P/(100 - P)$ (P = percent taken up specifically); Abscissa: Log_{10} [PCP].

days. The results are shown in Fig. 2. Untreated plastic tissue culture dishes showed significant binding of [^{3}H]PCP which was inhibited by increasing concentrations of PCP. Incubation of the plates in growth medium, however, abolished this binding. In our case, when the plates were seeded with cells in growth medium, at least 48 hr prior to the uptake experiment, this background binding was negligible.

The dependence of uptake on cell number is shown in Fig. 3. Plates were seeded at different densities and binding was measured 30 hr thereafter. Uptake was proportional to cell number between 10^5 and 7×10^5 cells, for the range of PCP concentrations used in the experiments.

Figure 4 shows the total uptake of PCP by Nb cells as a function of PCP concentration from 4×10^{-9} M to 5×10^{-4} M. The uptake is presented as a double logarithmic plot. Saturation of uptake was reached at about 10^{-4} M PCP. A more detailed study of the total uptake of PCP as a function of its concentration was performed and plotted linearly as shown in Fig. 5.

The non-specific (NS) uptake was determined by two methods. First, uptake was measured at concentrations of up to 5×10^{-4} M PCP, and a line parallel to the total uptake curve between 4×10^{-4} M PCP and 5×10^{-4} M PCP was drawn through the origin. This line represents a good approximation for NS uptake (Fig. 5). Second, the NS uptake was measured in the presence of 10^{-3} M PCP. The experimental NS curve was identical to the calculated one. At 5×10^{-4} M the NS uptake represented about 40% of the total uptake. At half-maximum saturation, however, the NS uptake was only 10% of the total uptake. The concentration at half-maximum specific transport (K_D) was about 2×10^{-5} M.

In order to complement the direct uptake measurements, the inhibition of uptake of [^{3}H]PCP by unlabeled PCP was determined. The results for the inhibition of the uptake of 8×10^{-8} M [^{3}H]PCP are shown in Fig. 6. Similar experiments using 4×10^{-9} M [^{3}H]PCP showed that increasing the PCP concentrations up to 10^{-7} M caused no inhibition of uptake of 4×10^{-9} M [^{3}H]PCP. This indicates that there was no saturable uptake below 10^{-7} M PCP.

The I_{50} value for PCP was calculated from the inhibition curve using the logit transformation [38], (insert, Fig. 6); I_{50} for PCP was $\sim 2 \times 10^{-5}$, in good agreement with the results from direct uptake experiments. (I_{50} is the concentration of PCP at half-maximum inhibition.)

To gain additional information on PCP uptake, we performed experiments on the kinetics of association and dissociation of [^{3}H]PCP to these cells. Figure 7 shows the rate of association of various concentrations of [^{3}H]PCP to the cells. (Note that even for the lowest concentration used, 2.8×10^{-7}, equilibrium was reached after 10 min, a 10-min incubation time was used in all our equilibrium experiments.)

In an uptake system where the law of mass action is valid the kinetic constants can be calculated from the following equation (1):

$$\ln (B_{eq}/B_{eq} - B_t) = k_{ob}.t \qquad (1)$$

where B_{eq} is the specific uptake at equilibrium, B_t is the specific uptake at time t, k_{+1} is the rate of association, k_{-1} is the rate of dissociation, and $k_{ob} = k_{+1} \cdot [D]_{\text{total}} + k_{-1}$.

A prerequisite for the use of equation 1 is that only a small percentage (<10%) of the total amount of the labeled drug present in the experiment be bound. This was the case in our experiments.

The results of Fig. 7 were drawn according to equation 1, in the Fig. 7 insert, and k_{ob} was calculated for various concentrations of PCP. Figure 8 is a plot of k_{ob} as a function of PCP concentrations. The points are from various experiments. Values for k_{+1} and k_{-1} were calculated from the slope and the intercept of the line drawn in Fig. 8. Values were: $k_{-1} = 0.2$ to 0.3 (min^{-1}) and $k_{+1} = 0.15$–0.25×10^5 ($\text{M}^{-1}\,\text{min}^{-1}$). The K_D value calculated from k_{-1}/k_{+1} was 1.2×10^{-5} M, in good agreement with the K_D value calculated from equilibrium experiments.

By equation 2, k_{-1} is related to the half-life ($T_{1/2}$) of uptake;

$$T_{\frac{1}{2}} = \frac{\ln 2}{k_{-1}} \qquad (2)$$

$T_{1/2}$ calculated from equation 2 and the k_{-1} value found in association experiments was 2–3 min. To determine k_{-1} independently of k_{+1} dissociation

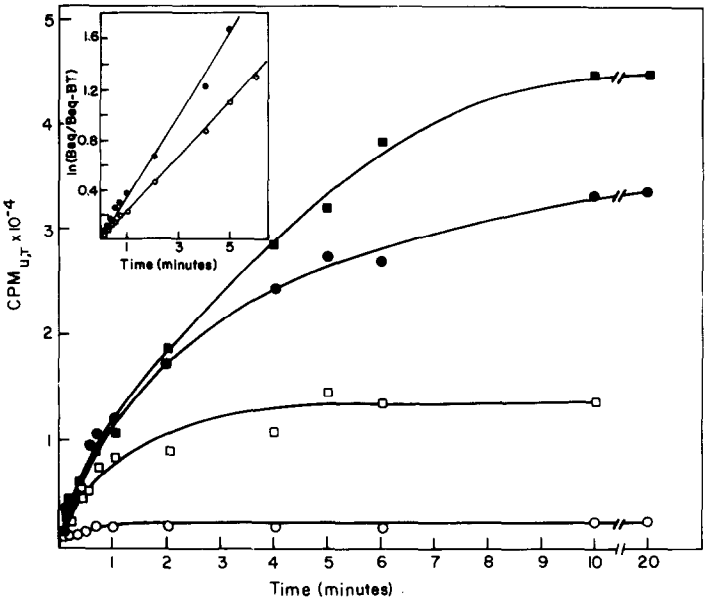

Fig. 7. Kinetics of [^{3}H]PCP uptake by Nb cells. Cells grown in 16 mm wells were incubated with 0.5 ml of 8×10^{-8} M [^{3}H]PCP (610,000 cpm) and various concentrations of PCP, for periods of time ranging from 5 sec to 20 min. Each well contained an average of 75 ± 15 μg protein. Key: (■——■) PCP = 2.8×10^{-7} M; (●——●) PCP = 10^{-5} M; (□——□) PCP = 5×10^{-5} M; and (○——○) PCP = 10^{-3} M. Insert: Calculated from uptake data presented in the figure. Specific uptake was defined as the difference between total uptake and uptake in the presence of 10^{-3} M PCP. B_{eq} is the specific uptake at equilibrium, B_t is the specific uptake at time t, and ln $(B_{eq}/B_{eq}-B_t)$ is presented as a function of time. Key: (○——○) PCP = 2.8×10^{-7} M; and (●——●) PCP = 10^{-5} M.

experiments were performed. In these experiments we diluted the reaction mixture with buffer (about 100-fold dilution). At 22° the $t_{\frac{1}{2}}$ for the bound [^{3}H]PCP is between 2 and 3 min. At 0°C no dissociation was detectable at 2 min. These results are consistent with our findings that three or six washes of the cells with 0° buffer, after incubation with [^{3}H]PCP, had no effect on the uptake. This result for $t_{\frac{1}{2}}$ from dissociation experiments is also consistent with the k_{-1} calculated from the association experiments.

To learn about the nature of the cellular uptake of PCP, the uptake was measured in the presence of various PCP analogs and of various cholinergic antagonists. Figure 6 shows the inhibition of [^{3}H]PCP uptake to Nb cells by the PCP analogs: TCP, ketamine and PCPMeI. Figure 9 shows the inhibition of [^{3}H]PCP uptake by tubocurarine, QNB and atropine. PCP and TCP were equally potent in inhibiting the uptake, with an I_{50} of 1–2 $\times 10^{-5}$ M. Ketamine was less efficient (I_{50} of 5×10^{-5} M) and PCPMeI was much less effective with an I_{50} of 2×10^{-4} M.

The cholinergic antagonists, QNB (Fig. 9) and 4-NMPB (results not shown), were as potent as PCP in inhibiting [^{3}H]PCP uptake. Atropine had an I_{50} of 2×10^{-4} M and tubocurarine was much less effective with I_{50} of 10^{-3} M.

The possibility that the retention of [^{3}H]PCP by intact cells represents an uptake process was studied by measuring its temperature dependence and its energy requirements.

The retention of PCP at 0–4° was only about 25% of the amount retained at 22°. When the energy uncoupler 2,4-dinitrophenol (DNP) was used, a marked dose-dependent inhibition of PCP uptake was observed. The results are summarized in Table 1.

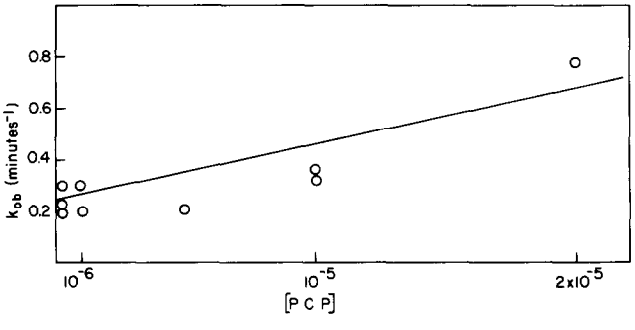

Fig. 8. Kinetics of [^{3}H]PCP uptake by Nb cells. This figure was drawn as calculated from the data of Fig. 7. Key: k_{ob} is the slope of the line ln$(B_{eq}/B_{eq}-B_t)$.

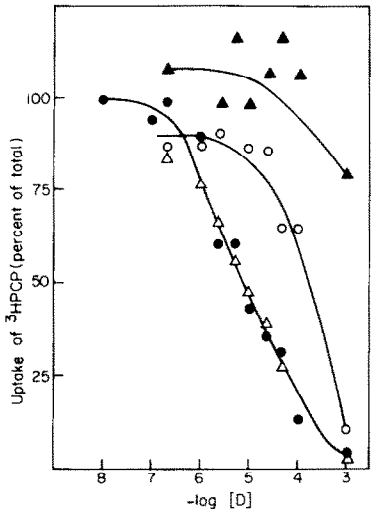

Fig. 9. Inhibition by various drugs of uptake of [³H]PCP by neuroblastoma cells. Nb cells grown in 16 mm wells were incubated at 22° with 0.5 ml of 8×10^{-8} M [³H]PCP (575,000 cpm) and increasing concentrations of the unlabeled drugs. One hundred percent uptake was 32,000 cpm/100 μg protein. Key: (●——●) PCP; (△——△) 3-quinuclidyl; benzilate (QNB); (○——○) atropine; and (▲——▲) tubocurarine.

DISCUSSION

In the present study, we characterized the uptake of PCP by Nb cells grown in culture. Uptake studies were performed on cells in plates, using three complementary approaches. First, equilibrium experiments were done, in which a component of the uptake was found to be saturable at concentrations higher than 10^{-4} M, with half-maximum saturation at 2×10^{-5} M PCP. This component was termed the specific uptake, and the non-saturable component was termed the non-specific uptake. Second, kinetic experiments of two kinds were performed. The kinetics of association were studied by determining the association constant, k_{+1}, and the dissociation constant, k_{-1}. The kinetics of association were compatible with a system that obeys the law of mass action, in which one drug molecule interacts with one uptake site in a reversible manner. The equilibrium dissociation constant, K_D, calculated from the ratio of the kinetic constants, is in good agreement with the K_D estimated from half-maximum saturation in equilibrium experiments. The maximum time needed to reach equilibrium at the lowest concentrations of PCP used (4×10^{-8} M) was 10 min. Dissociation experiments using the procedure of chase of bound [³H]PCP by a 100-fold dilution with buffer showed a dissociation half-life of $T_{\frac{1}{2}} = 2$–3 min, in good agreement with k_{-1} values from kinetics of association experiments. The dissociation half-life is much larger than the time required for the washing procedure used (6–10 sec). However, dissociation experiments done in the presence of various concentrations of unlabeled drug showed an increase in the rate of dissociation with increasing drug concentrations, an effect that can be kinetically described as negative cooperativity [39]. This might have been due to a change induced in the cell membrane by the high concentrations of unlabeled PCP ($5 \times 10^{-4} - 10^{-3}$ M) used in this procedure. Third, studies of the inhibition of uptake of [³H]PCP (8×10^{-8} M), by PCP and analog drugs, were done. Again the results were compatible with a system obeying the law of mass action in which

$$K_I = \frac{I_{50}}{1 + [D^*]/K_D}$$

where K_I is the affinity of inhibitor, $[D^*]$ is the concentration of bound radioactive ligand and K_D is the equilibrium dissociation constant of drug.

In our case, $[D^*] = [[^3H]PCP]$. In the inhibition experiments, [[³H]PCP] was two orders of magnitude smaller than the estimated K_D; therefore, I_{50} derived from inhibition experiments equals K_I [40]. I_{50} was calculated from the logit-log linear transformation of the inhibition curve (Hill plot) [38]. The logit-log curve was linear with a slope of 1 in agreement with a system of one class of non-interacting binding sites. It should be noted that the linearity of the Hill plot excludes positive cooperativity, and does not exclude negative cooperativity [39].

The K_I for PCP calculated from these experiments is in good agreement with K_D, thus indicating that both the labeled and unlabeled PCPs had the same binding affinities to Nb cells.

The binding capacity of these Nb cells for PCP was about 2 nmoles/mg cell protein ($\sim 2 \times 10^6$ cells) at a PCP concentration which equalled the K_D. This number is higher by five orders of magnitude than

Table 1. Inhibition by 2,4-dinitrophenol of uptake by Nb cells of [³H]PCP and of [³H]thymidine*

Ligand	Uptake (% of control) DNP (5×10^{-4} M)	DNP (10^{-3} M)	DNP (2×10^{-3} M)
PCP (10^{-7}M)	75	25	10
PCP (10^{-6}M)		25–40	
PCP (10^{-5}M)		25–40	
[³H]Thymidine	80–100	50–60	10

* Cells were pretreated for 1 hr with various concentrations of 2,4-dinitrophenol (DNP). Uptake was then measured at 22° (10 min) for [³H]PCP and 10 and 30 min for [³H]thymidine) in the presence of DNP. [³H]Thymidine uptake served as a positive control for the effect of DNP.

the capacity of homogenates of various tissues for PCP binding to putative specific PCP receptors [41].

The high capacity of Nb cells for PCP seems to be a general property of intact cells. We have similarly shown for muscle cells grown *in vitro* [9] that, at a PCP concentration which inhibits 50% of the carbamyl-choline-induced ion fluxes to the cells, the concentration of retained PCP is about three orders of magnitude larger than the number of acetylcholine receptors. The affinity of the uptake, 2×10^{-5} M, is close to the affinity of PCP for the muscarinic receptor [7]; it is 10-fold lower than the affinity for the cholinergic ionophore [11] and 50 to 100-fold lower than the affinity for the specific PCP receptor [41]. Nevertheless, the high capacity of the intact Nb cells for PCP would obscure any binding of those receptors, if such binding is present.

Several drugs were tested for their abilities to compete with PCP for interaction with the cells. The PCP analog, TCP, which is pharmacologically more potent than PCP and has a much higher affinity for the PCP receptor [41], was as potent as PCP in displacing [^{3}H]PCP. The quarternary analog, PCPMeI, however, was much less potent than PCP. The results with PCPMeI are interesting, since this compound, contrary to PCP, exists only in the ionized form. Kalir *et al.* [33] have suggested that the existence, at low concentrations, of non-ionized species which can penetrate through membranes is required for the central activity of PCP and analogs and found that PCPMeI was completely inactive in the rotarod test. However, the affinities of PCPMeI for the muscarinic and nicotinic acetylcholine receptors as measured in brain homogenates, and for the enzyme acetylcholinesterase, were comparable to those of PCP [42]. Also, 8×10^{-4} M PCP caused a 160% enhancement of *in vitro* oxidation of pyruvates by rat brain mitochondria. The quaternary amine PCPMeI was inactive [43], which could be explained by the poor penetration of PCPMeI through the mitochondrial membrane. Thus, the poor ability of the quarternary PCP derivative to compete with bound [^{3}H]PCP may indicate that PCP penetrates the cell membrane, or that we are dealing with an uptake process. Of the other drugs tested, QNB, 4-NMPB and atropine competed with this PCP uptake. Tubocurarine (again a quarternary drug which does not penetrate membranes) did not interfere with PCP uptake. The possibility that the penetration is an energy-dependent process (active uptake) is suggested by the fact that the binding is both temperature dependent and inhibited by DNP.

Despite the large capacity of the cells for PCP, there was no effect of PCP on cell growth at concentrations of up to $2–5 \times 10^{-4}$ M. At concentrations between 10^{-3} M and 2×10^{-4} M the effect of the drug was concentration dependent. The inhibition of cell multiplication has been shown to be reversible for both PCP and PCPMeI. The effects of PCP and of PCPMeI on cell growth were similar. This is not in contradiction to the findings that PCPMeI does not compete with PCP for its cellular uptake sites, since the effects of both drugs on cell growth were expressed only at very high drug concentrations ($\sim 10^{-3}$ M). At these concentrations PCPMeI inhibited most of the PCP uptake by the cells; therefore, the effects on cell growth may be attributed to a direct toxic effect on the plasma membrane of the cells.

Shahar *et al.* [27] studied the effects of PCP on myelinated cultures of dissociated dorsal root ganglion and found a membranal effect of the drug at concentrations of about 10^{-3} M. In their case, the effects were irreversible.

Work is now in progress trying to synthesize a labeled PCPMeI preparation free of PCP. Studies with this preparation with intact cells will help to shed light on the interaction of PCP with receptors on the cell surface membrane. Interference by PCP uptake which may obscure any other binding process, if present, will thus be negligible.

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