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# Structure-Activity Relationships of Phencyclidine Derivatives in Rat Cerebellum[1,2]

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PANG, K., S. W. JOHNSON, S. MAAYANI AND R. FREEDMAN. *Structure-activity relationships of phencyclidine derivatives in rat cerebellum.* PHARMACOL BIOCHEM BEHAV **24**(1) 127–134, 1986.—The depressant effects of phencyclidine [1-(1-phenylcyclohexyl) piperidine, PCP] and three of its analogs (m-amino-PCP, m-nitro-PCP, and PCP-methyliodide) on the spontaneous action potential discharge of cerebellar Purkinje neurons in urethane-anesthetized rats were examined in this study. Both intraperitoneal injection and micro-pressure ejection were employed as routes of drug administration. The relative potency after parenteral administration corresponded closely with previous findings in behavioral test paradigms. PCP and m-amino-PCP were equipotent, m-nitro PCP was less potent than either PCP or m-amino-PCP, and PCP-methyliodide showed almost no activity. After local administration onto neurons, m-amino-PCP was significantly more potent than PCP, while PCP, m-nitro-PCP, and PCP-methyliodide were equipotent. Tritiated PCP, m-nitro PCP, and m-amino PCP have similar distribution and metabolism in cerebellum. PCP-methyliodide, a quaternary ion, does not cross the blood brain barrier. M-nitro PCP is appreciably less ionized at pH 7.4 than PCP or m-amino-PCP and, therefore, may be more easily sequestered into lipids. Differences between PCP and its analogs found in experiments which employ parenteral administration may reflect differences in drug distribution. These differences are minimized when these drugs are administered directly onto neurons via pressure microejection.

Phencyclidine Rat Cerebellum Phencyclidine derivatives

THE psychotomimetic drug PCP[4] has been postulated to have several different effects in central neuronal circuitry. Interactions have been described with various neurotransmitters, such as norepinephrine [4,12], dopamine [6, 9, 15], acetylcholine [2, 3, 8] and opioids [19]. In addition, PCP has also been postulated to block sodium channels [18], in the manner of a local anesthetic agent [14], and to block potassium channels [1,2]. A potentially valuable tool in elucidating the neurobiological basis of the psychopharmacological effects of PCP is that of structure-activity relationships. PCP derivatives exist which differ in potency in a number of behavioral tests, such as rotorod performance [10] and spatial alternation paradigms [2]. Insofar as similar structure-activity profiles may be characterized for cellular actions of PCP, they may serve as a means to identify which actions may underlie behavioral effects of this drug.

Several previous communications from our laboratory have utilized rat cerebellar Purkinje neurons as a cellular target to study PCP actions [11,12]. Local administration of PCP by pressure microejection, or parenteral administration of this drug, elicits a dose-dependent and reversible slowing of Purkinje neuron spontaneous discharge. Several lines of evidence suggest that PCP depresses Purkinje neuron discharge by acting as an indirect noradrenergic agonist. In this study we compared electrophysiological effects of PCP and three of its derivatives, m-amino-PCP, m-nitro-PCP, and PCP methyliodide, in the rat cerebellum. In previous studies utilizing behavioral protocols and parenteral administration, the order of the potency for the derivatives was: Amino-PCP>PCP>>Nitro-PCP [10]. PCP methyliodide was virtually inactive. In order to examine the possibility of differential behavioral potency, drugs were administered both at the site of recording with multibarrel micropipettes, and parenterally. In addition, radiolabeled PCP or PCP derivatives

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[2]The abbreviations used are: PCP, 1-(1-phenylcyclohexyl) piperidine (phencyclidine); GABA, gamma aminobutyric acid; amino-PCP, 1-(1-m-aminophenylcyclohexyl) piperidine; nitro-PCP 1-(1-m-nitrophenylcyclohexyl) piperidine; meI-PCP, 1-(1-phenylcyclohexyl) piperidine methyliodide.

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TABLE 1

TLC SYSTEMS FOR PCP AND 5 OF ITS METABOLITES (Rf VALUES)*

| Solvent System | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | PCP |
|---|---|---|---|---|---|---|
| 1. Chloroform: Ethanol:$NH_4OH$ (90:10:1) | 0.60 | 0.52 | 0.32 | 0.26 | 0.68 | 0.9 |
| 2. Ethanol: iso-octane (13:3) | 0.86 | 0.83 | 0.80 | 0.27 | 0.9 | 0.86 |
| 3. Ethanol: acetic acid: water (90:1:10) | 0.71 | 0.69 | 0.69 | 0.51 | 0.77 | 0.86 |

Key: (No. 1) 1-(1-phenylcyclohexyl)-4 hydroxypiperidine, (No. 2) 4-phenyl-4 piperidinocyclohexanol, (No. 3) 4-(4'-hydroxypiperidino)-4-phenylcyclohexanol, (No. 4) piperidine, (No. 5) 1-phenylcyclohexylamine.

*All systems were run with Silicagel (Kodak) at room temperature (23+1°C).

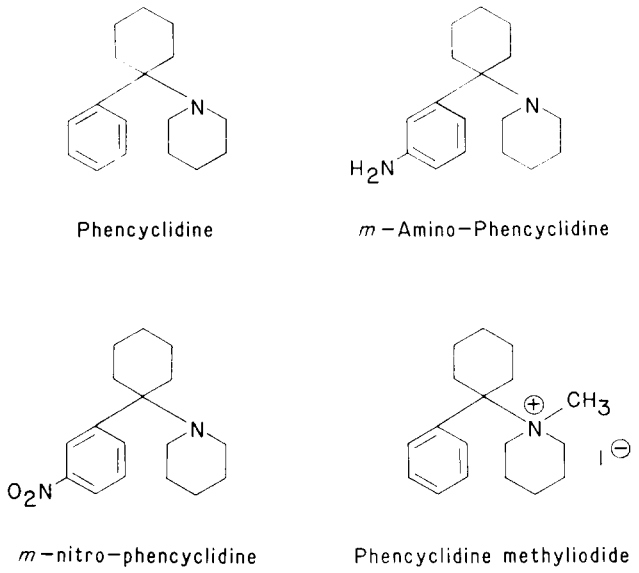


FIG. 1. Structure of phencyclidine and phencyclidine derivatives.

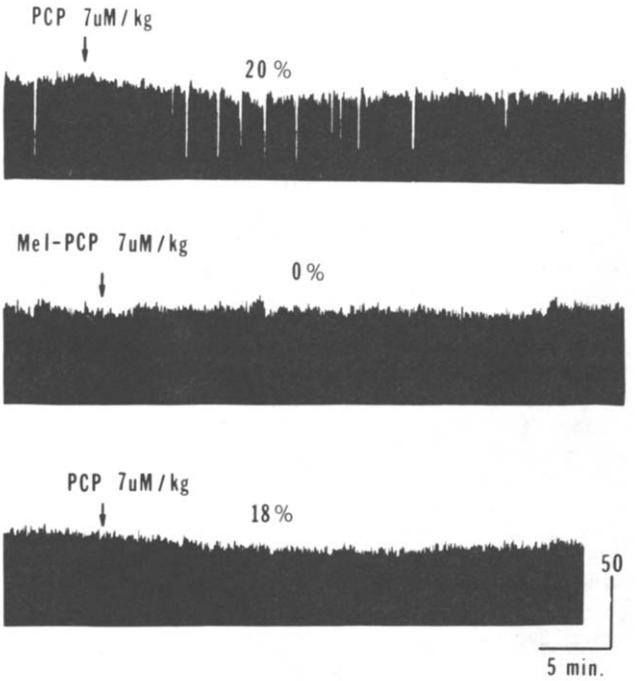


FIG. 2. Continuous ratemeter record which shows the effects of phencyclidine (PCP) and phencyclidine methyliodide (MeI-PCP) on a single cerebellar Purkinje neuron. Drugs were injected (IP) at the times indicated by the arrows. PCP produced an inhibition of Purkinje cell activity, while MeI-PCP, at the same dose, was inactive. Maximal inhibition induced by PCP occurred 10–20 minutes after drug administration. The percentages indicate percent inhibition of control activity. Calibration bars: Horizontal—5 minutes; vertical—50 spikes/bin (2 seconds/bin).

were injected parenterally in a separate set of experiments. The distribution of identified drug in various brain regions was measured to characterize the role of pharmacokinetic or metabolic factors in the differential potency of the derivatives.

## METHOD

### *Electrophysiology*

*Recording and drug applications.* Male Sprague-Dawley rats (200–350 g) were anesthetized with urethane (1.25 g/kg, IP), intubated, and placed in a stereotaxic frame. After removal of a portion of the skull and dura overlying the cerebellum, the brain surface was covered with 2% agar in saline. Body temperature was maintained at 37°C. Three-barreled micropipettes with 3–4.5 $\mu$m tips were used to record extracellular action potentials of spontaneously active single Purkinje neurons and to apply substances at the site of recording. Single-barrel pipettes were used to record spontaneous Purkinje cell discharge after parenteral drug injections. Methods for neuron identification and recording have been previously described in detail [7].

Phencyclidine hydrochloride was obtained from NIDA, Washington, DC. m-Nitro-PCP (free base), m-amino-PCP

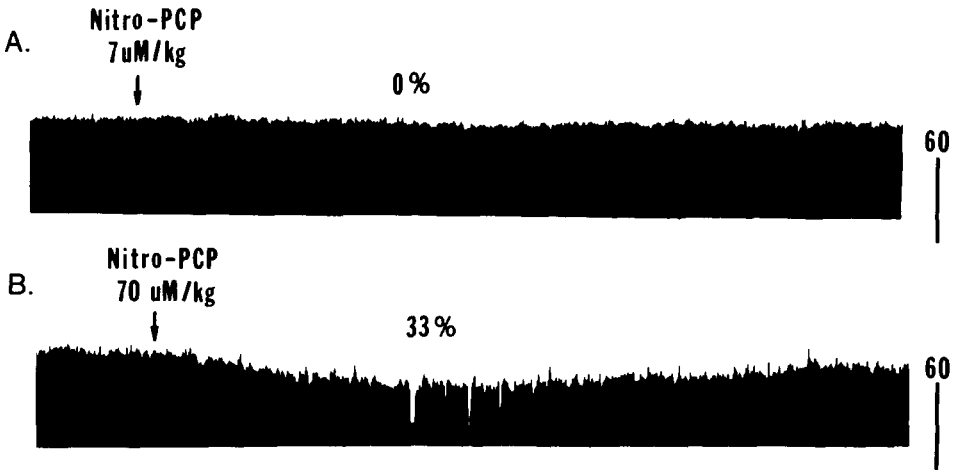


FIG. 3. Ratemeter records from two Purkinje neurons which illustrate dose-dependent effects of nitro-phencyclidine (nitro-PCP) on spontaneous activity. Drugs were injected (IP) at the times indicated by the arrows. Nitro-PCP, at a dose of 7 μmole/kg, was inactive (A). However, nitro-PCP inhibited neuronal activity at a dose which was 10-fold greater (B). Peak inhibition occurred 10–20 minutes after drug administration. The percentages represent percent inhibition of control activity. Vertical calibration bar: spikes/bin (2 seconds/bin). Horizontal time scale is the same as Fig. 2.

(free base), and PCP methyliodide (quaternary salt), were synthesized as described previously [10]. The structures of these drugs are shown in Fig. 1. All drugs were dissolved in 0.9% NaCl and adjusted to pH 7.4. For pressure ejection, the drugs were used at a concentration of 1 mM. In systemic administrations, the drugs were used at a concentration of 1.6 mM, except when a 70 μmole/kg dose was administered, in which case drugs were used at a concentration of 16 mM.

For initial experiments involving parenteral administration, PCP or one of its derivatives was injected IP and the time course of drug effects on a single Purkinje neuron's spontaneous firing rate was recorded. In subsequent trials, the spontaneous firing rates of six Purkinje neurons were determined before drug injection; these cells served as the control group. At the time of maximal drug effect, as had been identified by the initial time course studies, the spontaneous firing rates of another six Purkinje neurons were measured; these served as the experimental group. We have previously used this approach to study effects of amphetamine [7]. Control cells from all trials were grouped together as no significant differences were seen within this population. The experimental group for PCP and each derivative consisted of at least 25 Purkinje cells obtained from a minimum of 5 trials. When more than one drug injection was administered to the same animal, to compare two different drugs, the spontaneous firing rates of the Purkinje neurons were always observed to recover back to control levels before the next drug injection was administered. A counterbalanced design was employed to prevent order effects in drug comparisons.

Drugs were also administered by pressure ejection at the site of recording. The barrel of the pipette containing the drug to be ejected was connected to compressed nitrogen via a solid state-regulated solenoid valve (Medical Systems Corp., Great Neck, NY). Previous studies have shown that the amount of drug administered with this technique is linearly related to pressure and time of the ejection, and that pressure and time parameters are interchangeable [13]. Thus, the dose of drug can be expressed as the product of pressure and duration of ejection, or pounds per square inch-seconds (PSI-sec) (1 pound per square inch = 6.895 kilo pascal). To be considered valid, all responses to given doses of drug were required to show reproducibility and reversibility over several ejection trials. In order to minimize pipette variability when comparing dose-response relationships, one drug barrel was always filled with PCP, and the second with one of the 3 derivatives. For each Purkinje neuron, drug effects were compared by alternating between barrels containing PCP and the PCP-derivative.

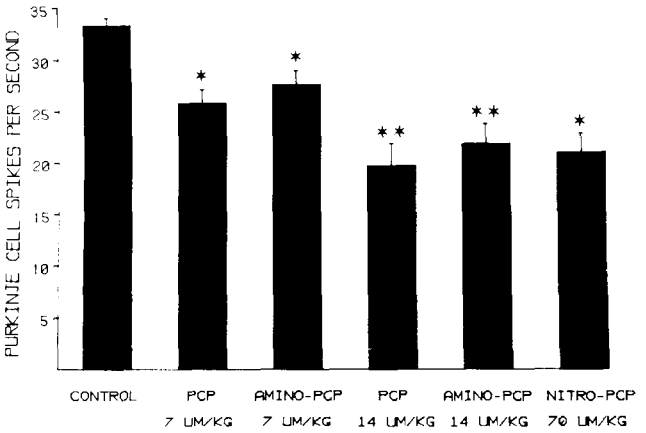


FIG. 4. Comparison of the effects of phencyclidine and phencyclidine derivatives on Purkinje neurons after IP injection. For each drug administration, the firing rates of six control cells were determined prior to drug administration. At the time of maximal drug effect, the firing rates of another six cells were recorded. Maximum inhibition occurred 10–20 minutes after PCP and nitro-PCP administration and 25–35 minutes after amino-PCP administration. Each bar represents the mean Purkinje cell firing rate S.E.M. for at least 24 cells. Neuronal activities after administration of drugs at the doses indicated were significantly lower than control rates. *Significantly different from control ($p<0.001$; two tailed Student's $t$-test). **Effects of PCP (14 μmole/kg) and amino-PCP (14 μmole/kg) were significantly greater than the effects of PCP (7 μmole/kg) and amino-PCP (7 μmole/kg), respectively ($p<0.05$; two-tailed Student's $t$-test).

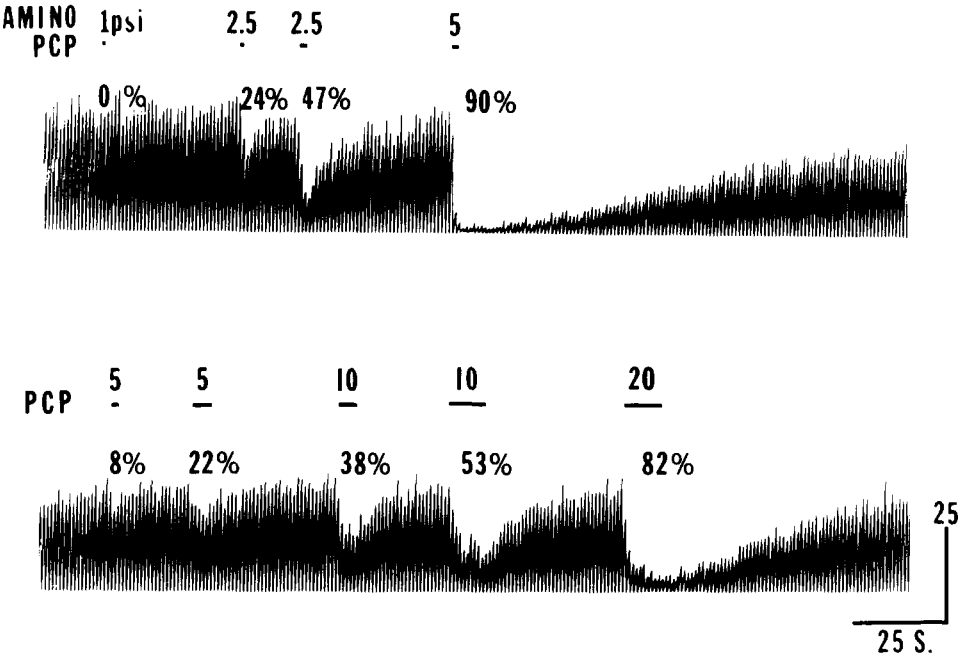


FIG. 5. Continuous ratemeter record which shows the responses of a Purkinje neuron to locally applied amino-phencyclidine (amino-PCP) and phencyclidine (PCP). Each drug was administered by pressure ejection from different barrels of the same pipette assembly. The bars represent the duration (seconds) of drug administration at the ejection pressure (pounds per square inch; PSI; 1 PSI=6.895 kilopascals), indicated. The percentages represent the percent inhibition of control activity. In this cell, amino-PCP was more potent than PCP. Calibration bars: Horizontal—25 seconds; vertical—25 spikes/bin (1 second/bin).

Extracellular action potentials were amplified by conventional means, monitored on an oscilloscope and converted to constant voltage pulses by a window discriminator. The pulses were fed to a ratemeter which summed them over one-second intervals for display on a strip chart recorder.

*Data analysis.* In all cases, the drug-induced changes in discharge were analyzed by computer; the data from ratemeter displays were digitized with a Tektronix graphics tablet and led into a NOVA 3/12 computer. The neuronal activity over sequential 1-second periods was stored and subsequently displayed on a Tektronix CRT computer terminal. CRT terminal-generated vertical vectors could then be superimposed over the display on the CRT and used to analyze neuronal activity before, during, and after local drug applications or parenteral injection. The area under the curve during drug administration, divided by the duration of administrations, was analyzed as a percentage of change from the averaged area (mean spikes per second) under a control epoch of the curve. The resulting computer output was quantified as percentage of depression (or excitation) elicited by drug application, for the dose-response curves. Linear transformations of the dose response curves were used to calculate the $EC_{50}$ values and 95% confidence limits for PCP and the various derivatives [5,17].

*Distribution of Radiolabeled PCP Derivatives*

Female Sprague-Dawley rats weighing approximately 250 g were used in all distribution experiments. In these studies, either ($^3$H)-PCP (19 Ci/mmole), ($^3$H) nitro-PCP (31.5 Ci/mmole), or ($^3$H) amino-PCP (27.8 Ci/mmole) (obtained from Nuclear Research Center, Israel) was injected IP at a dose of 5 mg/kg (15–25 $\mu$Ci/animal). Labeled drugs were purified before injection by either distribution between two phases (($^3$H)-PCP and ($^3$H)-nitro-PCP)) or by combination of distribution steps and column chromatography ($^3$H-amino-PCP) as follows: ($^3$H)-PCP and ($^3$H)-nitro-PCP were purified by using a sodium borate buffer (0.1 M, pH=9.2). Various amounts of the stock ethanol solution (100 $\mu$l–1000 $\mu$l of 1 mCi/ml) were added to 2 ml of buffer and 2 ml of iso-octane (Fisher, spectrophotometric grade), then vortexed 2 min at room temperature (23±1°C.). Separation of the two phases was done by centrifuging for 2 min at 5000×g. The organic phase was transferred to a fresh 2 ml aliquot of buffer and the tritiated drugs were distributed between the phases, as described above. The procedure was repeated twice for each drug. The final organic phase was dried ($N_2$ at 50°C) and reconstituted in 400–1000 $\mu$l absolute ethanol. Recovery was about 50% for ($^3$H)-PCP, and 90% for ($^3$H)-nitro-PCP. Recovery was increased to about 70% for ($^3$H)-PCP by passing a fresh aliquot of iso-octane over the buffer aliquots used above, and combining the organic phases. ($^3$H)-amino-PCP was purified by three consecutive distribution steps between 1 M NaOH/iso-octane, 1 M HCl/iso-octane and again 1 M NAOH/iso-octane. After the third step, the organic phase was dried ($N_2$, 50°C) and reconstituted in ethylacetate:hexane (2:8). The solution was loaded on an alumina column made with 1.5 grams of aluminum oxide (Woelm, activity grade 1) and 8 ml of ethylacetate:hexane (2:8). Nonlabeled amino-PCP was added to the reconstituted ($^3$H)-amino-PCP as internal marker. The ($^3$H)-amino-PCP was loaded on the column just as the level of the solvent disappeared in the alumina. Aliquots of 500 $\mu$l were collected from the column as 500 $\mu$l aliquots of ethylacetate:hexane (2:8)

TABLE 2

TRITIATED PCP, M-NITRO PCP AND M-AMINO PCP IN WHOLE RAT BRAIN 40 MINUTES POST IP INJECTION

| Drug | Injected Solution: mg drug/kg animal weight | Injected Solution: cpm/mg drug ($\times 10^7$) | Radioactivity Found: cpm/brain ($\times 10^6$) | Radioactivity Found: moles/kg animal weight* |
|---|---|---|---|---|
| PCP | | | | |
| Exp 1 | 4.2 | 10.7 | 18.4 | 2.64 |
| Exp 2 | 5.0 | 8.5 | 18.0 | 3.52 |
| Exp 3 | 5.0 | 1.14 | 3.3 | 4.90 |
| m-nitro PCP | | | | |
| Exp 1 | 4.8 | 1.9 | 3.7 | 2.90 |
| Exp 2 | 5.5 | 1.9 | 3.3 | 2.80 |
| m-amino PCP | | | | |
| Exp 1 | 6.6 | 6.7 | 13.0 | 2.80 |
| Exp 2 | 2.3 | 13.7 | 15.3 | 2.54 |

*Concentration was derived from cpm/brain by TLC assay of the identity of radioactive species.

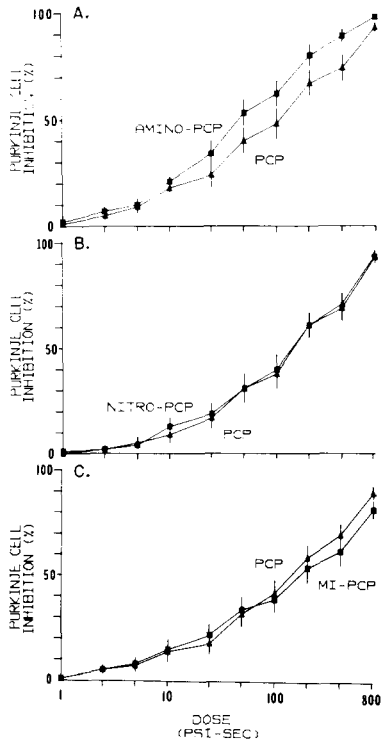


FIG. 6. Dose-related effects of locally administered phencyclidine (▲) and phencyclidine derivatives (■) on spontaneous activity of Purkinje neurons. PCP and one derivative were pressure ejected from different barrels of the same pipette. Only those cells which responded to both PCP and derivative were used in these results. Each point represents the mean response of at least 32 cells. Error bars represent±S.E.M. Amino-phencyclidine (amino-PCP) was significantly more potent than PCP as determined by 95% confidence limits (A). As calculated from Hillplots, the ED50 (95% confidence limits) were 40.9 (31.4–53.3) psi-seconds for amino-PCP and 80.6 (59.7–108.8) psi-seconds for PCP. Nitro-phencyclidine (nitro-PCP) and phencyclidine methyliodide (MI-PCP) were equipotent to PCP (B and C).

were added to the column. After 4 ml were collected, the solvent was changed to ethylacetate:hexane (3:7). Samples from each 500 μl were placed on silica plates (Eastman Kodak) for iodine visualization. The aliquots, which contained the cold marker, were checked for purity on silica plates (No. 13181, with fluorescent indicator) with iso-octane:acetone:$NH_4OH$ Rf.=0.38; purity was >94%. Recovery of ($^3$H)-amino-PCP was approximately 45% from the distribution steps and 50% from the column (about 25% of the original stock solution).

Forty minutes after injection of labeled PCP or derivatives, the animals were killed by decapitation, and their brains were removed quickly and dissected into 9 brain regions; cerebellum, thalamus, hypothalamus, frontal cortex, cingulate gyrus, caudate-putamen, hippocampus, retrosplenial cortex, and posterior cortex. The tissue was weighed and homogenized in 1 ml of saline. 100–200 μl aliquots were removed in triplicate and were counted in a Beckman LS9000 liquid scintillation counter using NEN 963 cocktail, with 43% efficiency.

In separate whole rat brain experiments, the radioactivity was extracted and identified forty minutes after IP injection of the labeled PCP or derivatives. Brains were homogenized in 10 ml HCl (0.1 M), at setting number 7 in a motor-driven teflon glass homogenizer. Approximately 0.3% of the injected radioactivity was found in the brain at this time, similar to that found in the distribution experiments. The homogenate was centrifuged at 3000×g for 2 min. Less than 5% of the counts were lost in the pellet. Three ml of supernatant was basified with 300 l 10 N NaOH (homogenate pH=12.6); then, approximately 5 grams of NaCl were added; to this, 3×3 ml iso-octane were added. It was vortexed (2 min) and centrifuged at 5000×g for 2 min. The phases were separated and the iso-octane fractions (9 ml total) were combined with 50–100 μl conc. HCl, then dried under $N_2$ at 50°C, and reconstituted in 200–500 μl of ethanol (amount dependent on radioactivity present). This procedure was used for all three tritiated drugs.

($^3$H)-PCP was identified and distinguished from five of its known metabolites, as shown below (Table 1), whereby 3

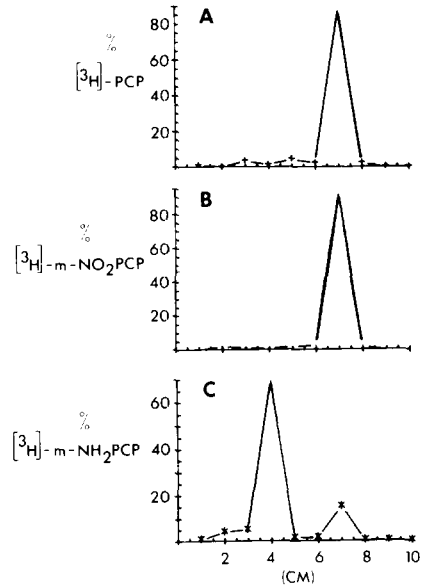


FIG. 7. Thin layer chromatography of tritriated PCP (A), m-$NO_2$-PCP (B), and m-$NH_2$-PCP (C) extracted from whole rat brain homogenate. Male rats were injected with 2.3–5.0 mg/kg (IP) (2–4 $\mu$Ci/animal). After extraction, the tritiated compounds were identified on Silicagel plate (25 $\mu$m, Kodak). X-axis describes running distance from the origin and Y-axis is % labeled compound for each peak out of 100% applied.

TLC systems resolvability was best achieved by the basic system. For ($^3$H)-nitro-PCP, silica plates were developed with acetone: iso-octane (1:5) Rf.=0.63, or ethanol:HAC (1:1) Rf.=0.72. For ($^3$H)-amino-PCP, silica plates were developed with acetone:hexane (1:10) Rf.=0.48, or iso-octane:acetone:$NH_4OH$ (8:1:5) Rf.=0.38, or hexane: hexane:ethylacetate:HAC (4:1:0.33) Rf.=0.57.

## RESULTS

### *Electrophysiology*

*Parenteral administration.* PCP, amino-PCP, and nitro-PCP, when administered systemically, produced inhibitions in the spontaneous activity of cerebellar Purkinje neurons, while MeI-PCP was inactive (Fig. 2). PCP, administered at a dose of 7 $\mu$mole/kg (IP), produced about a 20% depression of Purkinje cell firing rate (Fig. 2, upper trace). The latency to onset of drug effect was 2–5 minutes with the maximal inhibition occurring 10–20 minutes after drug administration. Recovery from drug effect occurred 25–35 minutes after injection. In contrast, a dose of 7 $\mu$mole/kg (IP) of MeI-PCP did not inhibit the same neuron (Fig. 2, middle trace). The inactivity of MeI-PCP was not due to damage to the neuron, since subsequent injections of PCP produced inhibitions of activity in the same Purkinje cell (Fig. 2, lower trace). Nitro-PCP was also inactive when administered at a dose of 7 $\mu$mole/kg (IP; Fig. 3A). However, when the dose was increased ten-fold (70 $\mu$mole/kg), the activity of Purkinje neurons was inhibited (Fig. 3B). This drug effect had a latency to onset of about 5 minutes. The maximum drug effect occurred approximately 10–20 minutes after drug administration, and recovery was complete by 40 minutes.

Amino-PCP (7 and 14 $\mu$mole/kg, IP) also inhibited Purkinje neuron firing rates after systemic injections (not shown). The latency to onset of this drug effect was 15–20 minutes with the maximum inhibition occurring between 25–35 minutes. Activity of the cells was recovered to baseline levels by 55–65 minutes.

Figure 4 summarizes the data on the effects of systemically administered PCP and derivatives on Purkinje cell activity. PCP, at a dose of 14 $\mu$mole/kg, produced a 41% depression, while administration of 7 $\mu$mole/kg of PCP resulted in a 22% depression. Similarly, 14 $\mu$mole/kg of amino-PCP gave rise to a 34% depression, compared to a 17% depression after 7 $\mu$mole/kg of amino-PCP. Thus, amino-PCP was approximately equipotent with PCP for the inhibition of the activity of Purkinje neurons. Nitro-PCP produced only a 37% depression when administered at a dose of 70 $\mu$mole/kg, and thus, nitro-PCP was much less potent than PCP. MeI-PCP was inactive at doses up to 70 $\mu$mole/kg (N=3). In summary, the rank order of potency after systemic administration was as follows: Amino-PCP = PCP > nitro-PCP >> MeI-PCP (inactive).

*Local administration.* When administered locally at the site of recording, by pressure ejection from multibarreled pipettes, PCP and all three derivatives inhibited the activity of Purkinje cells. The responses of a single Purkinje neuron to amino-PCP and PCP are seen in the ratemeter record shown in Fig. 5. Amino-PCP and PCP produced similar dose-dependent inhibitions of activity of the Purkinje neuron shown here.

Dose-response curves for locally applied PCP and derivatives are seen in Fig. 6. Amino-PCP was approximately twice as potent as PCP (Fig. 6A). This difference was statistically significant at 95% confidence limits. However, the dose effects of nitro-PCP (Fig. 6B) and MeI-PCP (Fig. 6C) were not significantly different from PCP. Thus, the rank order of potency after local administration of drugs was as follows: Amino-PCP > PCP = nitro-PCP = MeI-PCP.

TABLE 3

REGIONAL DISTRIBUTION OF ($^3$H) PCP AND DERIVATIVES, 40 MINUTES POST INTRAPERITONEAL INJECTION

| Region | PCP (8)* | m-$NO_2$-PCP (4) | m-$NH_2$-PCP (2) |
|---|---|---|---|
| Cerebellum | 2.3 ± 1.0 | 3.3 ± 2.4 | 2.1 |
| Thalamus | 4.7 ± 2.3 | 8.1 ± 3.9 | 4.6 |
| Hypothalamus | 5.3 ± 2.1 | 8.7 ± 3.7 | 4.6 |
| Frontal Cortex | 3.7 ± 1.4 | 6.6 ± 4.1 | 4.4 |
| Cingulate Gyrus | 6.2 ± 3.1 | 10.8 ± 5.5 | 7.5 |
| Caudoputamen | 4.8 ± 2.0 | 6.2 ± 3.0 | 4.5 |
| Hippocampus | 3.5 ± 1.4 | 4.8 ± 2.9 | 3.6 |
| Retrosplenial Cortex | 7.3 ± 3.9 | 10.0 ± 5.1 | 6.8 |
| Posterior Cortex | 3.8 ± 1.6 | 4.0 ± 2.4 | 4.0 |

*Values are in $\mu$moles/kg tissue (±S.D.). Numbers in parentheses are number of animals.

### *Distribution Studies*

*Identification of labeled drugs.* The concentration of ($^3$H) drugs found at 40 minutes in whole brain homogentates is summarized in Table 2. The thin layer chromatography procedure was used to identify the radioactive compounds present, so that calculations of drug concentrations could be

made (Fig. 7). Unmetabolized drug accounted for 70–90% of radioactivity present. The levels of the three compounds in the brain, 2–5 μmoles/kg, are similar to our previous findings with ($^3$H)-PCP. The distribution of the ($^3$H) drug 40 minutes after IP injection among nine brain regions is summarized in Table 3. These values are not expressed as drug concentration, since the percentage of unmetabolized PCP in the various brain regions was not identified, although it is probably similar to that found in whole brain. As can be seen by the comparison of the values in Table 2 to those summarized in Table 3, data obtained from whole brain were similar to those in specific dissected regions.

*Distribution of labeled PCP and derivatives.* The distribution of ($^3$H)-PCP, ($^3$H)-nitro-PCP, and ($^3$H)-amino-PCP were determined in nine brain regions 40 minutes after an intraperitoneal injection (Table 3). No significant differences, by analysis of variance, were found among the three compounds in any region.

## DISCUSSION

The striking correlation between the previously reported potency for behavioral effects of PCP and its derivatives and their potency for effects on Purkinje cell discharge after parenteral administration was a principal finding of this study. In behavioral protocols, the order of potency was amino-PCP > PCP >> nitro-PCP, with PCP methyliodide showing no activity [2,10]. In the present studies, we found that amino-PCP and PCP were not significantly different, but that both were markedly more potent than nitro-PCP, with PCP methyliodide inactive. Correlations between electrophysiological and behavioral potencies have also been found with comparisons of PCP and ketamine [12], and with comparisons of the stereoisomers of 3-methyl-PCP [11]. Similar differences after parenteral administration of PCP and the 3 derivatives studied here were found in hippocampus [16].

The present study is the first to assess the effects of these derivatives after local application to central neurons. Some of the differences in electrophysiological potency reported here are probably due more to central nervous system drug availability after parenteral administration than to neuronal sensitivity. Thus, when the PCP derivatives are administered at the site of recording by pressure microinjection, they appear approximately equipotent, except for the moderately increased potency of the m-amino derivative.

The finding of equipotency among the various derivatives with local, but not parenteral administration, is most easily explained for PCP-MeI. Given its permanent positive charge as a quaternary salt, it is unlikely to cross the blood brain barrier. The other derivatives obviously do enter the central nervous system, and, therefore, distribution and metabolic studies were performed to see if differential transport into the brain or metabolism could account for the observed potency differences with these compounds. No significant differences in whole brain metabolism or in distribution in the cerebellum were observed.

However, the differences in ionic dissociability of the various derivatives may also account for some of their differential potency after parenteral administration. At pH 7.4, 1% of PCP is un-ionized, compared with 12% of nitro PCP and 0.5% of amino-PCP [20]. Our experiments with local administration of PCP-MeI suggest that an ionized derivative of PCP is fully active, once the blood brain barrier is circumvented. Similarly, the relative distribution between ionized forms does not affect potencies of the other derivatives after local administration. The relative potencies of nitro-PCP, amino-PCP, and PCP after parenteral administration, however, would seem to parallel the differences in dissociation at pH 7.4. While nitro-PCP might have faster access to the brain as an undissociated molecule, it would also be much more rapidly sequestered into brain lipids because of the large undissociated fraction. This sequestration might account for its apparent lower potency in the parenteral experiments. Redistribution into lipid might also account for the recovery from effects of PCP of both behavior and Purkinje neuron discharge rate by 1 hour, despite the continued presence of high brain levels of drug.

In conclusion, distributional differences seem to account for much of the differences in potency between PCP and the three derivatives studied here. These differences in potency are minimized when the drugs are applied directly to neurons by pressure microejection. Biochemical data obtained with radioactive derivatives in this study and previous studies [10] confirm that most of the differences observed after parenteral administration can be accounted for by differences in drug distribution, rather than differences in agonist activity of the various molecular structures. The findings of other studies which have used PCP derivatives should be reviewed in light of these findings. Blockade of potassium channels in frog muscle [2]; [1] and the depression of the population spike in rat hippocampus [16] for example, may reflect similar distributional differences. The hippocampal study employed only parenteral administration, and the intracellular studies in frog muscle, although performed *in vitro*, probably require penetration of the drug into intracellular compartments [1] which means that similar distributional differences between the derivatives are likely to be present. Data about drug distribution are probably required in all studies which make use of series of derivatives to study drug structure-activity relations.

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