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# Enhancement of Brain Calcium Antagonist Binding by Phencyclidine and Related Compounds

GORDON T. BOLGER,*[1] MICHAEL F. RAFFERTY† AND PHIL SKOLNICK*

**Laboratory of Bioorganic Chemistry, NIADDK*
*National Institutes of Health, Bethesda, MD 20205*
*†Laboratory of Chemistry, NIADDK*
*National Institutes of Health, Bethesda, MD 20205*

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BOLGER, G T , M F RAFFERTY AND P SKOLNICK *Enhancement of brain calcium antagonist binding by phencyclidine and related compounds* PHARMACOL BIOCHEM BEHAV 24(3) 417–423, 1986 —The abilities of compounds structurally or pharmacologically related to phencyclidine to increase the apparent affinity of the [$^3$H]dihydropyridine calcium channel antagonist [$^3$H]nitrendipine were examined in lysed synaptosomal membrane preparations of rat brain The p-bromo analog of phencyclidine (1-(1-(4-bromophenyl)cyclohexyl)piperidine) was the most efficacious compound tested in enhancing the apparent affinity of [$^3$H]nitrendipine The efficacy of this compound was ~ two-fold greater than PCP The stereoisomers of PCMP (1-(1-phenylcyclohexyl-3-methylpiperidine) were also more efficacious than phencyclidine, although only a small degree of stereoselectivity was observed Levoxadrol, dexoxadrol and the enantiomers of ketamine did not potentiate [$^3$H]nitrendipine binding The enantiomers of SKF 10047 (n-allylormetazocine), dextrorphan, levorphanol and the ion channel toxins histrionicotoxin and pumiliotoxin-B also increased the apparent affinity of [$^3$H]nitrendipine, while several local anesthetics and $\mu$-opiate receptor ligands were without effect These studies suggest that the ability of phencyclidine and structurally related compounds to increase the apparent affinity of [$^3$H]nitrendipine is *not* mediated through an interaction with phencyclidine receptors, but may represent a unique site for allosteric modulation of neuronal dihydropyridine calcium channel antagonist binding sites

Nitrendipine Dihydropyridine Calcium antagonist Calcium channels Phencyclidine

PHENCYCLIDINE (PCP), initially developed as a general anesthetic [8,10], has become a widespread drug of abuse [13,37] due to its psychotomimetic actions The spectrum of pharmacologic effects produced by PCP are species and dose dependent [7] In man, low doses of PCP produce hypertension, tachycardia, gait ataxia, and a psychotomimetic syndrome which may include hostile behavior, auditory hallucinations and paranoid delusions [27] In rodents, PCP produces dose dependent hyperexcitability, stereotyped behaviors, motor incoordination, and ataxia [6, 31, 36, 43] The diversity of pharmacologic effects elicited by PCP may reflect the ability of this compound to interact with aspects of dopaminergic, cholinergic, noradrenergic, serotonergic, GABAergic and enkephalinergic neurotransmission [6, 29, 34–36, 39, 45] Further, PCP has been reported to alter the ion translocation properties of sodium, potassium and calcium ion channel complexes [2, 3, 16, 25, 44, 46]

The identification of stereospecific high affinity binding sites for [$^3$H]PCP in brain [37, 48, 49, 51] has facilitated the study of PCP and related compounds at a molecular level Structurally diverse classes of compounds which share pharmacologic actions with PCP (e g , ketamine, dexoxadrol, levoxadrol, cyclazocine, SKF 10047, (n-allylnormetazocine), dextrorphan and levorphanol) have been shown to interact with PCP binding sites in the central nervous system (CNS) [2, 26, 33, 40, 41, 51, 54] Recently we observed that PCP increases the affinity of [$^3$H]nitrendipine to dihydropyridine (DHP) calcium antagonist binding sites in rat brain [5] This effect of PCP was dose dependent, brain region specific and sensitive to inhibition by divalent cations. These observations suggest that the effect of PCP on DHP binding sites might represent a pharmacologically relevant site of action for PCP

In order to assess the pharmacologic specificity of the interaction of PCP with DHP binding sites we have investigated a series of drugs which are either structurally related to PCP or share some common psychopharmacologic properties with PCP We now report that several PCP derivatives as well as many compounds that are structurally unrelated to PCP (such as the opiates dextrorphan, levorphanol and SKF 10047, and the ion channel toxins histrionicotoxin and pumiliotoxin-B) produce a significant increase in the apparent affinity of [$^3$H]nitrendipine binding for DHP binding sites Further, PCP produced significant increases in the ap-

[1]Requests for reprints should be addressed to Dr G T Bolger, Laboratory of Bioorganic Chemistry, Bldg 4, Room 212, Bethesda, MD 20205

parent affinities of a small series of DHP calcium antagonists for the [$^3$H]nitrendipine binding site, while non DHP calcium antagonists were not similarly affected by PCP These findings suggest that although PCP and related compounds can alter the apparent affinity of [$^3$H]nitrendipine at the DHP binding site, this effect does *not* occur by occupation of PCP binding sites

## METHOD

### *Tissue Preparation*

Male Sprague-Dawley rats (Taconic Farms, Germantown, NY, 175–225 g) were used in all experiments A lysed synaptosomal enriched membrane fraction from rat forebrain was prepared according to Whittaker [52] Rats were killed by decapitation, the brains quickly removed and placed into ice cold 0 32 M sucrose The forebrain was isolated by making an oblique cut from the superior colliculus on the dorsal surface to the mammilary bodies on the ventral surface Tissue was homogenized in 20 vol tissue of 0 32 M sucrose using ten up and down strokes of a glass-teflon pestle (clearance 0 13–0 18 mm), motor driven homogenizer The homogenate was centrifuged at 1,100×g for 10 min, the pellet discarded and the supernatant recentrifuged at 24,000×g for 20 min The resulting pellet from this centrifugation was lysed by resuspending in 80 vol of 5 mM Tris buffer, pH 7 4 at 22°C, with a Brinkman Polytron (5 sec, setting 6–7)

### *[$^3$H]Nitrendipine Binding*

[$^3$H]Nitrendipine binding was assayed in a volume of 2 ml in borosilicate glass tubes under fluorescent light The assay volume consisted of 1 ml of 5 mM Tris buffer containing drug(s), [$^3$H]nitrendipine and 1 ml of membrane suspension Incubations were initiated by addition of tissue and terminated after 1 hr (25°C) by rapid filtration through Whatman GF/B glass fibre filters followed by 2×5 ml washes with ice cold 5 mM Tris buffer using a Brandel Cell Harvester (Model 24R, Brandel Co , Gaithersburg, MD) The filters were placed in 8 ml of Beckman Ready-Solv, MP (Beckman Instruments Inc , Fullerton, CA) and counted by liquid scintillation spectrophotometry (Beckman Model LS 5800) Specific [$^3$H]nitrendipine bound was defined as the difference in [$^3$H]nitrendipine binding in the presence and absence of $10^{-6}$ M nifedipine

### *Protein Determination*

Protein was determined by the Miller modification [32] of the Lowry assay [28] using bovine serum albumin as a standard

### *Materials*

[$^3$H]Nitrendipine (70–80 Ci/mmol) was obtained from New England Nuclear The commercial preparation was diluted 2-fold with absolute ethanol and stored in the dark at −20°C Subsequent dilutions for use in binding experiments were prepared in 5 mM Tris Hcl, pH 7 4, and kept at 0°C in aluminum foil-wrapped containers Phencyclidine hydrochloride and 1-(1-(3-anilino)cyclohexyl)piperidine (m-$NH_2$-PCP) were obtained from the NIDA, Rockville, MD The following PCP analogues were supplied by Warner-Lambert/Parke-Davis Co , Ann Arbor, MI 1-(1-(4-bromophenyl)cyclohexyl)piperidine (p-Br-PCP), 1-phenylcyclohexylamine (PCA), N-(2-propyl)-1-phenylcyclohexylamine (N-propyl-PCA), N-methyl-1-phenylcyclohexylamine These compounds were supplied as the hydrochloride salts with the exception of p-Br-PCP which was the free base The stereoisomers of 1-(1-phenylcyclohexyl)-3-methylpiperidine (PCMP) were supplied by Dr Kenner Rice, National Institutes of Health (NIADDK/LC) Samples of dexoxadrol, levoxadrol, naloxone (all as hydrochloride salts) and morphine sulfate were obtained from Drs Kenner Rice and A E Jacobsen, National Institutes of Health The resolved stereoisomers of ketamine hydrochloride and SKF 10047 (N-allylnormetazocine) were supplied by Dr James Woods, University of Michigan Drugs were prepared as 1 mM stock solutions and stored at −20°C prior to use The aryl substituted DHPs, verapamil, diltiazem and flunnarizine were provided by Dr D J Triggle, State University of New York at Buffalo Nifedipine was obtained

TABLE 1

THE EFFECT OF DRUGS ON [$^3$H]NITRENDIPINE BINDING

| Drug | $K_D/K_D$ Exp | $B_{max}/B_{max}$ Exp |
|---|---|---|
| Opiates | | |
| Dextrorphan | 1 61† | 0 85 |
| Levorphanol | 1 61† | 0 85 |
| (−)SKF 10047 | 1 92† | 1 01 |
| (+)SKF 10047 | 1 88† | 0 88 |
| Local Anesthetics[a] | | |
| Lidocaine | 0 76* | 0 83 |
| Benzocaine | 0 98 | 0 89 |
| Procaine | 0 93 | 0 89 |
| Piperocaine | 0 90 | 0 82 |
| Cocaine | 0 74* | 0 85 |
| Neurotoxins | | |
| Pumiliotoxin B | 1 61† | 0 90 |
| Histrionicotoxin | 1 92† | 1 01 |
| Drugs Structurally Related to PCP[b] | | |
| PCP | 1 51† | 0 99 |
| (−)PCMP | 2 13† | 0 83 |
| (+)PCMP | 1 78† | 0 85 |
| p-Br-PCP | 3 22† | 1 09 |
| Dexoxadrol | 0 71* | 1 04 |
| Levoxadrol | 0 99 | 0 92 |
| (−)Ketamine | 1 12 | 0 95 |
| (+)Ketamine | 1 06 | 0 94 |

The $K_D$ and $B_{max}$ values for [$^3$H]nitrendipine binding were obtained by Scatchard analysis of the binding data, utilizing [$^3$H]nitrendipine in a concentration range of 35–800 pM The ratio of the $K_D$ or $B_{max}$ in the absence, to that in the presence of drug, $K_D$ Exp or $B_{max}$ Exp is presented A ratio significantly >1 indicates an increase in the apparent affinity of [$^3$H]nitrendipine All drugs were present at a concentration of 10 $\mu$M The results are the mean of three experiments [a]The local anesthetics listed were also assayed at concentrations up to 500 $\mu$M, with effects on [$^3$H]nitrendipine binding similar to those at 10 $\mu$M [b]When studied in the concentration range $10^{-7}$ M–$10^{-4}$ M, (±)PCMP produced similar biphasic concentration-effect relationships as did PCP (see Fig 2) (±)Ketamine at a concentration of 100 $\mu$M produced similar effects on [$^3$H]nitrendipine binding to those at 10 $\mu$M Significantly different from 1 00, *$p<0$ 05, †$p<0$ 005 paired $t$-test

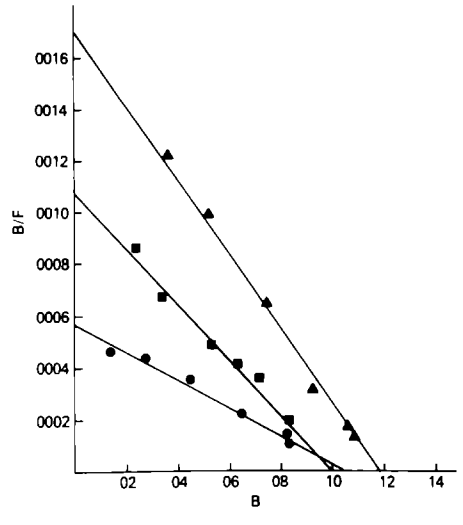



FIG 1 The effect of PCP and p-Br-PCP on [$^3$H]nitrendipine binding Scatchard analysis of [$^3$H]nitrendipine binding in control (●), PCP (10 $\mu$M), (■) or p-Br-PCP (10 $\mu$M) (▲) treated rat brain membranes using [$^3$H]nitrendipine is a concentration range of 35–1200 pM The $K_D$ and $B_{MAX}$ values were for control 191 1 pM, 0 107 pmoles/mg protein, PCP 94 6 pM, 0 102 pmoles/mg protein, and p-Br-PCP 63 9 pM, 0 115 pmoles/mg protein respectively The results are the mean of two experiments

from Pfizer, Inc (Groton, CT) The DHP derivatives were dissolved in ethanol as 1 mM stock solutions and stored in foil-wrapped containers at −20°C, all other calcium channel antagonists were taken up in 5 mM Tris HCl, pH 7 4, and stored frozen as 1 mM stock solutions Local anesthetics were obtained from the following sources cocaine, Merck and Co , Rahway, NJ, lidocaine and benzocaine, Dr L -Y M Huang, National Institutes of Health, procaine, E R Squibb and Sons, Inc., New Brunswick, NJ, piperocaine, Eli Lilly and Co , Indianapolis, IN All local anesthetics were prepared as 10 mM solutions and kept at −20°C Histrionicotoxin and pumiliotoxin B were generously provided by Dr John W Daly, National Institutes of Health, and stored as 1 mM solutions in ethanol at −20°C

## RESULTS

### *The Effect of Drugs on [$^3$H]Nitrendipine Binding*

Compounds structurally unrelated to PCP but possessing common psychopharmacologic properties with PCP were examined for their abilities to alter [$^3$H]nitrendipine binding in a synaptosomal-enriched membrane fraction prepared from rat forebrain The $K_D$ and $B_{max}$ values for [$^3$H]nitrendipine binding in untreated membranes (179±9 (n=3) pM and 0 0922±0 016 (n=3) pmol/mg protein, respectively) are in agreement with previously reported values [4,30] In preliminary experiments, the psychotomimetic opiates SKF 10047, dextrorphan and levorphanol [24, 26, 51] were all observed to increase [$^3$H]nitrendipine binding at a ligand concentration of 70 pM This increase in binding reflected a reduction in the apparent $K_D$ of [$^3$H]nitrendipine (Table 1) while the $B_{max}$ values were generally unaffected Neither morphine nor naloxone caused any significant change in the apparent $K_D$ of [$^3$H]nitrendipine in a concentration range of 0 1–100 $\mu$M (data not shown)

The local anesthetic properties of PCP derivatives [7,23] prompted an evaluation of the effects of local anesthetics on [$^3$H]nitrendipine binding None of the local anesthetics studied reduced the apparent $K_D$ of [$^3$H]nitrendipine while lidocaine and cocaine elicited a small but statistically significant increase in the apparent $K_D$ of [$^3$H]nitrendipine (Table 1)

In light of the reported actions with the frog skin neurotoxin histrionicotoxin with PCP receptors in brain [47], this compound and a second neurotoxin, pumiliotoxin B, were evaluated for their effect on [$^3$H]nitrendipine binding Both of these compounds significantly decreased the apparent $K_D$ of [$^3$H]nitrendipine binding at a concentration of 10 $\mu$M (Table 1)

The enantiomers of PCMP (structurally related to PCP) both decreased the apparent $K_D$ of [$^3$H]nitrendipine However, only a small degree of stereoselectivity was observed over a concentration range of 0 1–100 $\mu$M Dexoxadrol inhibited [$^3$H]nitrendipine binding while its enantiomer, levoxadrol, did not affect [$^3$H]nitrendipine binding (Table 1) The stereoisomers of ketamine (10 $\mu$M) did not significantly alter [$^3$H]nitrendipine binding The p-bromo derivative of PCP (p-Br-PCP) was the most efficacious compound tested in decreasing the apparent $K_D$ of [$^3$H]nitrendipine (Table 1, Fig 1) p-Br-PCP, like PCP (Bolger *et al* , 1984), reduced the microassociation and dissociation rate constants for [$^3$H]nitrendipine binding The values for the microassociation and microdissociation constants respectively were for control, $k_1$=3 20×10$^{-4}$ min$^{-1}$ pM$^{-1}$, $k_{-1}$=5 25×10$^{-2}$ min$^{-1}$, $K_D$ ($k_{-1}/k_1$)=164 06 pM and in the presence of 10 $\mu$M p-Br-PCP, $k_1$=8 07×10$^{-4}$ min$^{-1}$ pm$^{-1}$, $k_{-1}$=2 74×10$^{-2}$ min$^{-1}$, $K_D$ ($k_{-1}/k_1$)=33 95 pM, representing a 483% increase in affinity for [$^3$H]nitrendipine and in good agreement with the results obtained by Scatchard analysis In contrast, the m-amino derivative of PCP (m-$NH_2$-PCP) was much less active than PCP in increasing [$^3$H]nitrendipine binding (Fig 2)

In an attempt to further define the structural requirements necessary for increasing the apparent affinity of [$^3$H]nitrendipine binding by compounds structurally related to PCP, a series of N-substituted phenylcyclohexylamines (PCAs) were studied The ability of these analogs to increase [$^3$H]nitrendipine binding displayed only a marginal degree of structural dependence (Table 2)

The $EC_{50}$ for maximum potentiation of [$^3$H]nitrendipine binding by the PCP derivatives studied was in the concentration range of 1–3 $\mu$M From the concentration-effect curves generated for these compounds and the aryl substituted derivatives of PCP (Fig 1), it was observed that the structural dependence for the enhancement of [$^3$H]nitrendipine binding was due to changes in efficacy (i.e , maximal enhancement) rather than changes in potency between the test compound Compounds structurally related to PCP also produced a biphasic change in [$^3$H]nitrendipine binding (Fig 2) N-Propyl-PCA was particularly potent as an inhibitor of [$^3$H]nitrendipine binding at 10–100 $\mu$M (Fig 2, Table 2) $Ca^{2+}$ (1 mM) inhibited this effect of N-propyl-PCA on [$^3$H]nitrendipine binding

### *Effect of PCP on the Apparent Affinities of Other $Ca^{2+}$-Antagonists at the [$^3$H]Nitrendipine Binding Site*

The $K_i$ values for inhibition of specific [$^3$H]nitrendipine binding by other DHP $Ca^{2+}$ antagonists were measured in the presence of 10 $\mu$M PCP to investigate the structural dependence of the PCP-induced change in the dihydropyridine binding site The $K_i$ values for the 2-nitro (nifedipine), 2-cyano and unsubstituted DHP analogs were substantially decreased

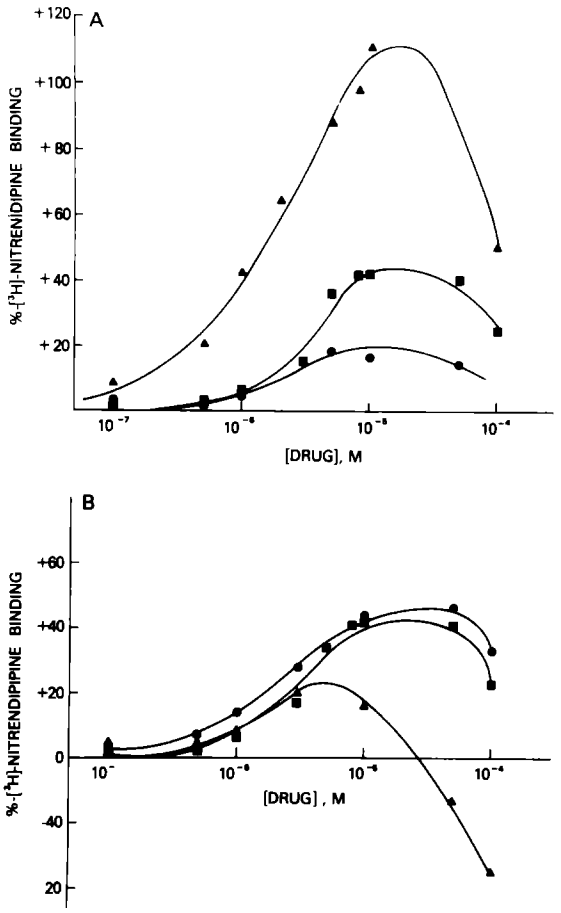



FIG 2 Potentiation of [$^3$H]nitrendipine binding by PCP derivatives The potentiation of [$^3$H]nitrendipine binding by (A) p-Br-PCP (▲), PCP (■), m-$NH_2$-PCP (●), and (B) PCA (●), PCP (■), and N-propyl-PCA (▲) The results are expressed as the percent increase (+) or decrease (−) in [$^3$H]nitrendipine binding in the absence of drug and represent the mean of two experiments

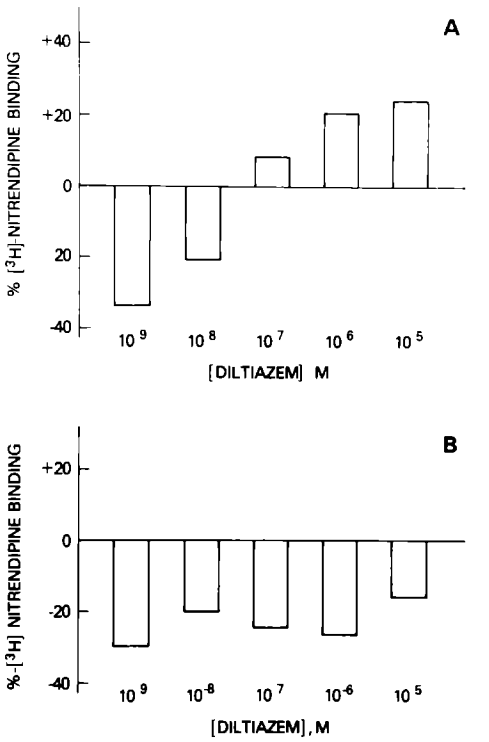



FIG 3 The effect of PCP on the alteration of [$^3$H]nitrendipine binding by diltiazem The ability of diltiazem to alter [$^3$H]nitrendipine binding (70 pM) was investigated in the absence (A) and presence (B) of PCP ($10^{-5}$ M) in a concentration range of $10^{-9}$–$10^{-5}$ M Each bar represents the percent increase (+) or decrease (−) of [$^3$H]nitrendipine binding mediated by diltiazem The results are the mean of two experiments

by PCP, while the 3-methoxy analog had a much smaller reduction in $K_i$ (Table 3) The sensitivity of these compounds to the effect of PCP paralleled their potencies at inhibiting [$^3$H]nitrendipine binding in smooth muscle [4] The affinities of the non-dihydropyridine $Ca^{2+}$ antagonists verapamil and flunnarizine were marginally increased by PCP The $IC_{50}$ value for inhibition of [$^3$H]nitrendipine binding by the inorganic calcium channel antagonist $La^{3+}$ was increased by PCP (Table 3) The interaction of diltiazem with the [$^3$H]nitrendipine binding site was also altered by PCP At concentrations of diltiazem that normally potentiated [$^3$H]nitrendipine binding [4], only an inhibition of binding was observed (Fig 3) Preliminary findings have indicated that in contrast to the actions of diltiazem at the [$^3$H]nitrendipine binding site, PCP did not inhibit [$^3$H]nitrendipine binding to brain membranes at low concentrations ($10^{-10}$–$10^{-7}$ M) [4] or display a temperature sensitive component of action [47]

## DISCUSSION

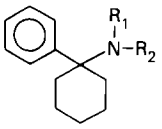
Phencyclidine has recently been observed to increase the apparent affinity of [$^3$H]nitrendipine for dihydropyridine calcium antagonist binding sites in rat brain [5] The present study was undertaken in order to determine whether a number of compounds that are structurally and/or pharmacologically similar to PCP were also capable of increasing the affinity of [$^3$H]nitrendipine, and whether this activation was related to an action at specific CNS binding sites for [$^3$H]PCP [11, 49, 51]

The psychotomimetic opiates levorphanol, dextrorphan, and (±)SKF 10047 are structurally unrelated to PCP, but have been reported to substitute for PCP in a number of behavioral paradigms [24] These compounds all potentiated [$^3$H]nitrenipine binding The lack of any marked stereoselectivity for the potentiation of [$^3$H]nitrendipine binding by these compounds is consonant with the small degree of stereoselectivity by these compounds both behaviorally and at PCP receptors *in vitro* [22, 40, 52] The inability of morphine and naloxone to potentiate [$^3$H]nitrendipine binding indicates that this effect is most likely unrelated to opiate receptor activity, but could be associated with the psychotomimetic properties of (±)SKF 10047, levorphanol and dextrorphan

The ion channel toxins histrionicotoxin and pumiliotoxin-B [12] also reduced the apparent $K_D$ of [$^3$H]nitrendipine Histrionicotoxin has been reported to interact with the [$^3$H]PCP binding site in brain [48] which is consistent with the ability of PCP to alter sodium and potassium ion channel function [1, 3, 16, 46] Characterization of the action of histrionicotoxin in frog nerve-muscle preparations indicates an interaction with both sodium and potas-

TABLE 2

THE EFFECT OF PCA DERIVATIVES ON [$^3$H]NITRENDIPINE BINDING

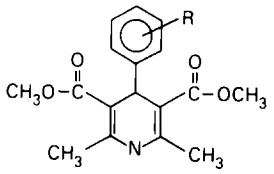


| Compound | % Change in [$^3$H]Nitrendipine Binding $10^{-5}$ M | % Change in [$^3$H]Nitrendipine Binding $10^{-4}$ M | $IC_{50}$ (nM) [$^3$H]PCP Binding in Rat Brain* |
|---|---|---|---|
| PCP | +41 0 | +21 7 | 83 0 |
| $R_1$=H, $R_2$=H (PCA) | +42 2 | +32 8 | 403 0 |
| $R_1$=H, $R_2$=$CH_3$ | +28 4 | +9 3 | 260 0 |
| $R_1$=H, $R^2$=$CH_2CH_2CH_3$ | +16 4 | −35 4 | 210 0 |
| $R_1$=H, $R_2$=$CH(CH_3)_2$ | +38 9 | −2 3 | 20 0 |
| $R_1$=$R_2$=$CH_2CH_3$ | +49 2 | +47 1 | 261 0 |

[$^3$H]Nitrendipine was present in the binding assay at a concentration of 70 pM The results are expressed either as the percent increase (+) or percent decrease (−) of [$^3$H]nitrendipine binding in the absence of PCA derivatives The results are the mean of two experiments *A E Jacobsen, Laboratory of Chemistry, NIADDK/NIH, personal communication

TABLE 3

ALTERATION OF CALCIUM ANTAGONIST BINDING BY PCP



| Compound | $K_i$ (M) Control | $K_i$ (M) +PCP (10 $\mu$M) |
|---|---|---|
| DHP Calcium Antagonists | | |
| R=2-$NO_2$ (nifedipine) | $4\ 85\times10^{-10}$ | $6\ 72\times10^{-11}$ |
| R=2-CN | $7\ 70\times10^{-10}$ | $2\ 04\times10^{-10}$ |
| R=H | $3\ 04\times10^{-8}$ | $9\ 50\times10^{-9}$ |
| R=3$CH_3O$ | $2\ 54\times10^{-8}$ | $1\ 01\times10^{-8}$ |
| Other Calcium Antagonists | $IC_{50}$ Control | $IC_{50}$ +PCP (10 $\mu$M) |
| Verapamil | $7\ 41\times10^{-8}$* | $5\ 75\times10^{-8}$* |
| Flunnarizine | $5\ 49\times10^{-7}$* | $3\ 98\times10^{-7}$* |
| $La^{3+}$ | $2\ 33\times10^{-5}$* | $6\ 16\times10^{-5}$* |

The inhibition of [$^3$H]nitrendipine binding by calcium antagonists was studied at a concentration of 70 0 pm [$^3$H]nitrendipine in the presence or absence of PCP (10 $\mu$M) The $K_i$ values were calculated from their corresponding $IC_{50}$ values by the equation of Cheng and Prussoff [9] The results are the mean of three experiments

*For verapamil, flunnarizine and $La^{3+}$, the $IC_{50}$ values for maximum inhibition of [$^3$H]nitrendipine binding are presented, with a maximum inhibition of 80%, 100% and 100% being observed for verapamil, flunnarizine and $La^{3+}$ respectively

sium ion channels [12,42], while pumiliotoxin-B acts at a site linked to calcium translocation, facilitating calcium mobilization in skeletal muscle [2,12] The concentrations of these toxins needed to alter ion conductances ($10^{-6}$–$10^{-5}$ M) are similar to those needed to alter [$^3$H]nitrendipine binding The finding that both PCP and these neurotoxins potentiate [$^3$H]nitrendipine binding suggests a common site for the modulation of ion channels by these compounds

The failure of local anesthetics to enhance the binding of [$^3$H]nitrendipine (Table 1) suggests that the local anesthetic properties of PCP and related compounds [7,23] do not play a role in the potentiation of [$^3$H]nitrendipine binding

The study of compounds structurally related to PCP provided considerable evidence that the potentiation of [$^3$H]nitrendipine binding occurs at a locus distinct from the [$^3$H]PCP binding site For example, the behaviorally inactive p-bromo derivative of PCP [41] was the most efficacious potentiator of [$^3$H]nitrendipine binding, while the meta-amino analog, which is a potent PCP mimetic [41], had a much smaller effect on [$^3$H]nitrendipine binding (Table 1, Figs 1 and 2) Alteration of the N-alkyl moiety of PCP yields compounds which are relatively inactive in eliciting PCP-like behavioral actions (PCA and the N,N-diethyl analog of PCP) These compounds were, however, more efficacious at increasing [$^3$H]nitrendipine binding than the N-isopropyl analog of PCP, which is an extremely potent PCP-mimetic *in vivo* and has a high affinity for [$^3$H]PCP binding sites *in vitro* [41] The lack of a marked stereoselectivity between the enantiomers of PCMP was yet another indication that the PCP binding site is not directly involved in the potentiation of [$^3$H]nitrendipine binding, since behavioral, electrophysiologic and receptor binding studies have demonstrated potency differences for these two enantiomers, the (+) isomer being the more potent [31,38] Further, dexoxadrol, levoxadrol and the isomers of ketamine which possess

PCP like actions and displace [$^3$H]PCP from its binding site *in vitro* [26,53] did not potentiate [$^3$H]nitrendipine binding

The biphasic nature of the concentration-effect relationship for PCP and structurally related compounds (Fig 1) suggest that there could be two sites at which PCP interacts with the [$^3$H]nitrendipine binding site In addition, the observation that $Ca^{2+}$ prevents the inhibition of [$^3$H]nitrendipine binding by N-propyl-(PCA), indicates that a site modulated by calcium is involved, possibly the same calcium sensitive site which regulates the potentiation of [$^3$H]nitrendipine binding by PCP [5] However, the local anesthetic properties of PCP [8,23] and structural derivatives might contribute to the inhibitory component of the biphasic concentration-effect dependence observed

The affinities of the non-DHP calcium antagonists verapamil and flunarizine were increased to a smaller degree by PCP than was [$^3$H]nitrendipine, which is consistent with evidence that these compounds interact with site(s) that are linked to the DHP binding site [4, 14, 15, 30] Diltiazem, a benzothiazepine, exerted a complex, concentration-dependent effect on [$^3$H]nitrendipine binding in brain, consistent with that observed in smooth muscle [4] PCP blocked the potentiative but not the inhibitory effect of diltiazem on [$^3$H]nitrenipine binding (Fig 2) Recent studies utilizing radiation inactivation to determine the molecular size of the [$^3$H]nitrendipine binding site in skeletal muscle indicated that diltiazem decreases the target size of this site, providing evidence for an oligomeric structure [19,20] It is possible that the effect of PCP on this complex is mediated via the subunit responsible for the positive heterotropic action of diltiazem

The inorganic calcium antagonist $La^{3+}$, was reported to inhibit [$^3$H]nitrendipine binding in rat brain [21] In contrast to the organic calcium antagonists, PCP reduced the potency of $La^{3+}$ for inhibiton of [$^3$H]nitrendipine binding Such a finding suggests that PCP could also interact at the cation coordination site(s) that is linked to the [$^3$H]nitrendipine binding site [4]

The structural diversity of compounds which potentiate [$^3$H]nitrendipine binding suggest that a number of sites may be linked to putative calcium channels to regulate [$^3$H]nitrendipine binding or that a single site of broad structural tolerance is involved The structural dependencies demonstrated within a series of PCP analogs strongly suggests that the site(s) mediating the potentiation of [$^3$H]nitrendipine binding possess specificity and are distinctly different from the PCP binding site Further evidence dissociating the PCP binding site from the [$^3$H]nitrendipine binding site arises from the observation that, while [$^3$H]PCP binding sites are present in "peripheral" tissues [50], PCP did not alter [$^3$H]nitrendipine binding to cardiac [5] and smooth muscle [4] membranes The ability of ion channel toxins to alter [$^3$H]nitrendipine binding provides evidence that the site modulating [$^3$H]nitrendipine binding may also be involved in the modulation of ion channels This might have important implications not only for the regulation of putative brain calcium channels, but other ion channel systems present in the CNS which may play a role in mediation of the psychotomimetic effects of PCP and pharmacologically related compounds

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## REFERENCES

1 Albuquerque, E X, L C Aguayo, J E Warnick, H Weinstein, S D Glick, S Maayeini, R K Ickowicz and M P Blaustein The behavioral effects of phencyclidines may be due to their blockage of potassium channels *Proc Natl Acad Sci USA* **78:** 7792–7796, 1981

2 Albuquerque, E X, J E Warnick, R Tamburini, F C Kauffman and J W Daly In *Disorders of the Motor Unit*, edited by D L Schotland Baltimore John Wiley and Sons, 1982, pp 611–625

3 Blaustein, M P and R K Ickowicz Phencyclidine in nanomolar concentrations binds to synaptosomes and blocks certain potassium channels *Proc Natl Acad Sci USA* **80:** 3855–3859, 1983

4 Bolger, G T, P T Gengo, R Klockowski, E Luchowski, H Siegel, R A Janis, A M Triggle and D J Triggle Characterization of binding of the $Ca^{++}$ channel antagonist [$^3$H]nitrendipine to guinea-pig ileal smooth muscle *J Pharm Exp Ther* **225:** 291–309, 1983

5 Bolger, G T, M F Rafferty and P Skolnick Phencyclidine increases the affinity of dihydropyridine calcium channel antagonist binding in rat brain *Naunyn Schmiedebergs Arch Pharmacol*, in press, 1985

6 Castellani, S and P Adams Acute and chronic phencyclidine effects on locomotor activity, stereotypy and ataxia in rats *Eur J Pharmacol* **73:** 143–154, 1981

7 Chen, G, C R Ensor, D Russel and B Bohner The pharmacology of 1-(1-phencyclohexyl)piperidine-HCl *J Pharm Exp Ther* **127:** 241–150, 1959

8 Chen, G M and J K Weston The analgesic and anesthetic effect of 1-(1-phenylcyclohexyl)piperidine on the monkey *Anesth Analg* **39:** 132–141, 1960

9 Cheng, Y C and W H Prussoff Relationship between the inhibition constant ($k_i$) and the concentration of inhibitor which causes 50 percent inhibition ($IC_{50}$) of an enzymatic reaction *Biochem Pharmacol* **22:** 3099–3105, 1973

10 Collins, V J, G A Gorospe and E A Rovenstine Intravenous nonbarbiturate, nonnarcotic analgesics Preliminary studies I Cyclohexylamines *Anesth Analg* **39:** 302–308, 1960

11 Cone, E F, R L McQuinn and H E Shannon Structure activity relationship studies of phencyclidine derivatives in rats *J Pharmacol Exp Ther* **228:** 147–153, 1984

12 Daly, J Biologically active alkaloids from poison frogs (dendrobatidae) *J Toxicol* **1:** 33–86, 1982

13 Domino, E F Some aspects of the pharmacology of phencyclidine In *The Psychopharmacology of Hallucinogens*, edited by R C Stillman and R E Wilhetle New York Pergamon Press, 1978, pp 105–117

14 Ehlert, F J, E Itoga, W R Roeske and H I Yamamura The interaction of [$^3$H]nitrendipine with receptors for calcium antagonists in the cerebral cortex and heart of rats *Biochem Biophys Res Commun* **104:** 937–943, 1982

15 Ehlert, F J, W R Roeske, E Itoga and H I Yamamura The binding of [$^3$H]nitrendipine to receptors for calcium channel antagonists in the heart, cerebral cortex and ileum of rats *Life Sci* **30:** 2191–2202, 1982

16 Eldefrawi, A T, R E Miller, D L Murphy and M E Eldefrawi [$^3$H]Phencyclidine interactions with the nicotinic acetylcholine receptor channel and its inhibition by psychotropic, antipsychotic, opiate, antidepressant, antibiotic, antiviral and antiarrhythmic drugs *Mol Pharmacol* **22.** 72–81, 1982

17 Eldefrawi, M E , E F El-Fakahany, D L Murphy, A T Eldefrawi and D J Triggle High affinity binding of phencyclidine (PCP) to crayfish muscle Displacement by calcium antagonists *Biochem Pharmacol* **31:** 2549–2551, 1982
18 El-Fakahany, E E , M E Eldefrawi, D J Triggle, A T Eldefrawi and M E Eldefrawi Distinction between high-affinity [$^3$H]phencyclidine binding sites and muscarinic receptors in guinea-pig ileum muscle *J Pharmacol Exp Ther* **229:** 447–455, 1984
19 Ferry, D R , A Goll and H Glossman Calcium channels Evidence for oligomeric nature by target size analysis *EMBO J* **2.** 1729–1732, 1983
20 Goll, A , D R Ferry and H Glossman Target size analysis of skeletal muscle calcium channels *FEBS Lett* **157·** 63–69, 1983
21 Gould, R J , K M M Murphy and S H Snyder [$^3$H]Nitrendipine-labelled calcium channels discriminate inorganic calcium agonist and antagonists *Proc Natl Acad Sci USA* **79:** 3656–3660, 1982
22 Hampton, R Y , F Medzihradsky, J H Woods and P J Dahlstrom Stereospecific binding of [$^3$H]phencyclidine in brain membranes *Life Sci* **30·** 2147–2154, 1982
23 Hardie, J W R , J Hildalgo, I F Halverstadt and R E Allen 4-(2-Piperidyl-1,3-dioxolanes with local anesthetic, spasmolytic and central nervous system activity *J Med Chem* **9·** 127–136, 1966
24 Herling, S , R E Solomon and J H Woods Discriminative stimulus effects of dextrorphan in pigeons *J Pharmacol Exp Ther* **227.** 723–731, 1983
25 Kloog, Y , A Kalir, O Buchman and M Sokolovsky Specific binding of [$^3$H]phencyclidines to membrane preparation *FEBS Lett* **109.** 105–128, 1980
26 Leander, J D Comparison of phencyclidine, etoxadrol and dexoxadrol in the pigeon *Subst Alcohol Actions Misuse* **2:** 197–203, 1982
27 Lerner, S E and R S Burns Phencyclidine abuse, an appraisal In *NIDA Research Monograph 21,* edited by R C Petersen and R C Stillman Rockville, MD, National Institutes of Drug Abuse, 1978, pp 66–118
28 Lowry, O H , N J Rosebrough, A L Farr and R J Randall Protein measurement with the folin phenol reagent *J Biol Chem* **193:** 265–275, 1951
29 Malave, A , M P Holsapple and K W Yim Reduction by atropine of phencyclidine hypertension and apneusis *Life Sci* **30·** 929–934, 1982
30 Marangos, P F , J Patel, C Miller and A M Martino Specific calcium antagonist binding sites in brain *Life Sci* **31:** 1575–1583, 1982
31 Marwaha, J , M Palmer, B Hoffer, R Freedman, K C Rice, S Paul and P Skolnick Differential electrophysiological and behavioral responses to optically active derivatives of phencyclidine *Naunyn Schmiedebergs Arch Pharmacol* **315:** 203–209, 1981
32 Miller, G L Protein determination for large numbers of samples *Anal Chem* **31·** 964, 1959
33 Murray, T F and M E Leid Interaction of dextrorotary opioids with phencyclidine recognition sites in rat brain membranes *Life Sci* **34:** 1899–1911, 1984
34 Nabeshima, T , S P Siram, C Y Tai and I K Ho Development of dispositional tolerance to phencyclidine by osmotic minipump in the mouse *J Pharmacol Methods* **7·** 239–253, 1982
35 Nabishima, T , M Hiramatsu, M Amano, H Furakama and T Kameyama Decrease of methionine-enkephalin levels in mouse brain induced by phencyclidine *Eur J Pharmacol* **86:** 271–282, 1983
36 Nabishima, T , K Yamada, Y Kazumasa, M Hiramas-Ku, H Furukama and T Kameyama Effect of lesions in the striatum, nucleus accumbens and medial raphae on phencyclidine-induced stereotyped behaviors and hyperactivity in rats *Eur J Pharmacol* **91:** 455–462, 1983
37 Petersen, R C and R C Stillman Phencyclidine An overview In *Phencyclidine Abuse An Appraisal,* edited by R C Petersen and R C Stillman Rockville, MD, National Institute of Drug Abuse Research Monograph 21, DHEW Pub 81, 1978, No 78-728
38 Quirion, R , K C Rice, P Skolnick, S Paul and C B Pert Stereospecific displacement of [$^3$H]phencyclidine (PCP) receptor binding by an enantiomeric pair of PCP analogues *Eur J Pharmacol* **74:** 107–108, 1981
39 Raja, S N and P G Guyenet Effects of phencyclidine on the spontaneous activity of monoaminergic neurons *Eur J Pharmacol* **63:** 229–233, 1980
40 Shannon, H E Phencyclidine-like discriminative stimuli of (+)- and (−)-N-allylnormetazocine in rats *Eur J Pharmacol* **84:** 225–228, 1982
41 Solomon, R E , S Herling, E F Domino and J H Woods Discriminative stimulus effects of N-substituted analogs of phencyclidine in rhesus monkeys *Neuropharmacology* **21·** 1329–1336, 1982
42 Spivak, C E , M A Maleque, A C Olivera, L M Masukawa, T Tokuyama, J W Daly and E X Albuquerque Actions of the histrionicotoxins at the ion channel of the nicotinic acetylcholine receptor and at the voltage-sensitive ion channels of muscle membranes *Mol Pharmacol* **21:** 351–361, 1981
43 Sturgeon, R D , R G Fessler and H Y Meltzer Behavioral rating scales for assessing phencyclidine-induced locomotor activity, stereotyped behavior and ataxia in rats *Eur J Pharmacol* **59.** 169–179, 1979
44 Temma, K , T Akera, T M Brody and R H Rech Negative chronotropic and positive inotropic actions of phencyclidine on isolated atrial muscle in guinea pigs and rats *J Pharm Exp Ther* **226:** 885–892, 1983
45 Tonge, S R and B E Leonard The effects of some hallucinogenic drugs upon the metabolism of 5-hydroxytryptamine in the brain *Life Sci* **8:** 805–814, 1969
46 Tourneur, Y , G Romey and M Lazdunski Phencyclidine blockade of sodium and potassium channels in neuroblastoma cells *Brain Res* **245:** 154–158, 1982
47 Triggle, D J and R A Janis Calcium channel antagonists New perspectives from the radioligand binding assay In *Modern Methods in Pharmacology* edited by A R Liss New York A R Liss Inc , 1984, pp 1–28
48 Vignon, J , J -P Vincent, J N Bidard, J -M Kamenka, P Geneste, S Manier and M Lazdunski Biochemical properties of the brain phencyclidine receptor *Eur J Pharmacol* **81·** 531–542, 1982
49 Vincent, J P , B Kartolovski, P Geneste, J M Kamenka and M Lazdunski Interaction of phencyclidine (''angel dust'') with a specific receptor in rat brain membranes *Proc Natl Acad Sci USA* **76:** 4678–4682, 1979
50 Vincent, J P , J Vignon, B Kartalovski and M Lazdunski Compared properties of central and peripheral binding sites for phencyclidine *Eur J Pharmacol* **68.** 79–82, 1980
51 White, J M and S G Holtzman Three-choice drug discrimination Phencyclidine-like stimulus of opioids *Psychopharmacology (Berlin)* **80·** 1–9, 1983
52 Whittaker, V P The application of subcellular fractionation techniques to the study of brain function *Prog Biophys Mol Biol* **15:** 41–60, 1965
53 Zukin, S R and R S Zukin Specific [$^3$H]phencyclidine binding in rat central nervous system *Proc Natl Acad Sci USA* **76:** 5372–5376, 1979
54 Zukin, S R , K T Brady, L S Barbara and R L Balster Behavioral and biochemical stereoselectivity of sigma opiate/PCP receptors *Brain Res* **294:** 174–177, 1984