

## THE RAT BRAIN PHENCYCLIDINE (PCP) RECEPTOR A PUTATIVE K<sup>+</sup> CHANNEL

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**Abstract**—Receptor binding studies were carried out to test whether the rat brain phencyclidine (PCP) receptor is part of a K<sup>+</sup> channel. [<sup>3</sup>H]PCP, and two analogs, [<sup>3</sup>H]TCP and *m*-amino[<sup>3</sup>H]PCP, labeled a single receptor on rat brain synaptic membranes. Each compound bound to a similar number of sites ( $B_{\max} = 2.7$  pmol bound/mg protein); the apparent dissociation constants for these compounds ( $K_D < 0.3$   $\mu$ M) decreased with increasing temperature. The following observations indicate that the PCP receptor is part of a K<sup>+</sup> channel: (1) aminopyridines (AP) and tetraalkylammonium ions blocked [<sup>3</sup>H]PCP binding; their respective orders of potency, 4-AP = 3,4-diAP > 2-AP  $\gg$  3-AP, and tetrabutylammonium (TBA) > tetraethylammonium  $\gg$  tetramethylammonium, paralleled their abilities to block K<sup>+</sup> channels, (2) the order of potency of PCP and its analogs for binding to the PCP receptor, TCP > PCE > *m*-amino-PCP > PCP > PCPY > *m*-nitro-PCP, paralleled their rank order for blocking brain K<sup>+</sup> channels, and (3) the stereospecific displacement of [<sup>3</sup>H]PCP binding by the isomers of the “sigma” ligands, (+)*N*-allyl-normetazocine (NANM) > (–)NANM, and (–)cyclazocine > (+)cyclazocine, and of the dioxolanes, dexoxadrol  $\gg$  levoxadrol, paralleled their abilities to block brain K<sup>+</sup> channels. Reciprocal plot and Schild plot analyses indicated that TBA, (+)NANM and dexoxadrol were competitive inhibitors at the PCP receptor, whereas 4-AP had an allosteric interaction.

Phencyclidine [1-(1-phenylcyclohexyl) piperidine, PCP]§ is a drug of abuse that produces a toxic confusional psychosis in man [1, 2]. As a first step toward elucidating the neuronal mechanism(s) of action of PCP, a high affinity (apparent dissociation constant,  $K_{app} < 1$   $\mu$ M) PCP receptor has been found in brain [3–6]. The relative affinities of PCP and its analogs for binding to this receptor correlate well with their relative behavioral potencies [6–8]. This suggests that the behavioral changes induced by PCP may be the result of modified neuronal function as a consequence of PCP binding to this receptor. The functional identity of this receptor is unknown.

Physiological studies indicate that PCP selectively blocks certain K<sup>+</sup> channels. In frog skeletal muscle, PCP prolongs action potentials and inhibits delayed rectification [9, 10], consistent with a blockage of K<sup>+</sup> conductance. PCP also prolongs action potentials and blocks K<sup>+</sup> conductance in neuroblastoma [11] and cardiac muscle cells [12]. In rat brain synap-

tosomes, PCP inhibits a component of <sup>86</sup>Rb efflux that corresponds to a voltage-gated, non-inactivating K<sup>+</sup> channel [13, 14]. Moreover, intoxication with the K<sup>+</sup> channel blocker 4-aminopyridine (4-AP) causes a psychotic syndrome in humans [15] that resembles the one produced by PCP [2]. These findings suggest that the PCP receptor is associated with a K<sup>+</sup> channel.

PCP has also been reported to block Na<sup>+</sup> channels [11], Ca<sup>2+</sup> channels [16, 17] and the non-selective monovalent cation channel of the nicotinic ACh receptor [10, 18]. In the present study, receptor binding studies were used to test whether the brain PCP receptor resides on, or is linked to, one of these ion channels. In support of a previous study which showed that PCP selectively blocks a voltage-gated, non-inactivating K<sup>+</sup> channel [14], the present binding data identified the rat brain PCP receptor as part of, or linked to, the same K<sup>+</sup> channel. Preliminary reports of some of these observations have been published in abstract form [19–21].

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§ Abbreviations: PCP, phencyclidine, 1-(1-phenylcyclohexyl) piperidine; TCP, 1-[1-(2-thienyl) cyclohexyl] piperidine; PCE, *N*-ethyl-1-phenylcyclohexylamine; PCPY, 1-(1-phenylcyclohexyl) pyrrolidine; NANM, *N*-allyl-normetazocine; AP, aminopyridine; diAP, diaminopyridine; TAA, tetraalkylammonium; TBA, tetrabutylammonium; TEA, tetraethylammonium; TMA, tetramethylammonium; ACh, acetylcholine; and HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

### MATERIALS AND METHODS

**Materials.** PCP and its analogs, and the stereoisomers of NANM (*N*-allyl-normetazocine, SKF 10047) and cyclazocine were obtained from the National Institutes for Drug Abuse (NIDA, Research Triangle Park, NC). The dioxolanes, dexoxadrol and levoxadrol, were gifts from the Upjohn Co. (Kalamazoo, MI). Tetrodotoxin and saxitoxin, D-600 and nisoldipine, and PCPY [1,1-phenylcyclohexyl] pyrrolidine] and perhydrohistrionicotoxin were gifts from Drs. B. K. Krueger, W. J. Lederer and E. X. Albuquerque,

respectively, of the University of Maryland School of Medicine. [ $^3\text{H}$ ]PCP was obtained from both New England Nuclear (Boston, MA; 49.9 Ci/mmol) and NIDA (15 Ci/mmol). [ $^3\text{H}$ ]TCP (64 Ci/mmol), the thienyl analog of PCP, was obtained from the Research Products International Corp. (Mt. Prospect, IL) and New England Nuclear. *m*-Amino[ $^3\text{H}$ ]PCP (18 Ci/mmol) was custom tritiated by Amersham (Arlington Heights, IL). HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), Tris, quinacrine, amantadine, the aminopyridine (AP) analogs, the tetraalkylammonium (TAA) ions, polyethyleneimine and poly-L-lysine were obtained from the Sigma Chemical Co. (St. Louis, MO).

**Ligand binding assays.** The binding of tritiated PCP analogs to synaptic membranes, prepared from rat forebrain [22], was measured by competition with their respective unlabeled analogs under two conditions. At 0°, the binding mixtures consisted of 50 mM Tris/HEPES, pH 7.0, containing 5 or 10 nM [ $^3\text{H}$ ]PCP or *m*-amino[ $^3\text{H}$ ]PCP, or 2.5 nM [ $^3\text{H}$ ]TCP, and the respective unlabeled compound. At 37°, the binding mixtures consisted of 20 mM Tris/HEPES, pH 7.0, containing 5 nM [ $^3\text{H}$ ]PCP or *m*-amino[ $^3\text{H}$ ]PCP, or 2.5 nM [ $^3\text{H}$ ]TCP, and the respective unlabeled compound. The total volume was 0.5 ml. Following a 10-min temperature equilibration, binding was initiated by the addition of the membranes (usually 0.6 mg protein). After equilibrium was reached (60 min at 0°, 30 min at 37°), 4 ml of cold wash buffer (50 mM Tris/HEPES, pH 7.0, at 0°; 20 mM Tris/HEPES, pH 7.0, at 37°) was added. The binding mixtures were then rapidly filtered under reduced pressure on Whatman GF/B filters that had been presoaked with 0.1% poly-L-lysine or 0.1% polyethyleneimine to reduce non-specific binding to the filter by the ligand [3]. The filters and retained membranes were washed twice with 4 ml of the same wash buffer, and the bound radiolabeled ligand was measured by liquid scintillation spectroscopy. Each assay was carried out in triplicate. Appropriate filter blank (protein-free) values for each data point (also in triplicate) were subtracted from the counts obtained in the presence of protein. Under these assay conditions, the filter blank values were little changed by the addition of the unlabeled ligand.

The binding data collected by the above method were not corrected for non-specific binding (measured in the presence of a large excess of unlabeled ligand). The levels of non-specific binding were more accurately estimated by computer-assisted curve-fitting analysis of the total binding (see below). Protein was determined by the method of Markwell *et al.* [23].

To test for competitive inhibition, [ $^3\text{H}$ ]TCP binding was assayed at 37°, as described above, in the presence of increasing amounts of unlabeled TCP and a constant amount of a test compound. Total binding was analyzed by computer-assisted curve fitting [24].

To measure the displacement of [ $^3\text{H}$ ]PCP binding by a test compound, [ $^3\text{H}$ ]PCP binding was assayed as described above except that increasing amounts of the test compound, rather than unlabeled PCP,

were added to the binding mixtures. The data were corrected for non-specific binding by including a large excess of unlabeled PCP in the binding mixtures.

The procedure for determining the effect of pH on the affinities of the AP analogs was similar to the [ $^3\text{H}$ ]PCP displacement assay. Non-specific binding was determined by the inclusion of 500  $\mu\text{M}$  unlabeled PCP. The binding buffer was adjusted to the appropriate pH by varying the ratio of Tris to HEPES added, keeping the total buffer concentration constant (50 mM at 0°). Also, the stock solution of each AP analog (usually 1 M) was adjusted to the pH of the binding buffer.

**Data analysis.** The competitive displacement binding data were fitted by the program, EBDA, to obtain  $\text{IC}_{50}$  values. The ligand binding data were analyzed by the least-squares curve fitting program SCAFIT/LIGAND [24]. The latter method estimates receptor binding constants, receptor densities and non-specific binding levels from total receptor binding data. The binding parameters obtained by this method are more accurate than those calculated from binding data that are corrected for non-specific binding prior to data analysis [24]. The accuracy of the SCAFIT/LIGAND method is supported by the observation (see Results) that the several protocols and analogs we employed all yielded similar numbers for the receptor density.

Data obtained from the inhibitor studies were analyzed by two methods. First, the apparent dissociation constants ( $K_{\text{app}}$ ) for [ $^3\text{H}$ ]TCP binding measured in the presence of a test compound were plotted as a function of the concentration of that test compound, [I]. This graphical analysis is derived from the Lineweaver-Burk reciprocal plot, as used to determine competitive inhibition [25].

Second, the data were analyzed by the Schild plot [26], as exemplified by the analysis of the binding of calcium channel antagonists [27]. The dose ratio (DR) reflects changes in the  $K_{\text{app}}$  for [ $^3\text{H}$ ]TCP binding measured in the presence of the test compounds.

## RESULTS

**Binding of PCP and analogs.** Data from a representative experiment showing the binding of [ $^3\text{H}$ ]PCP to rat brain synaptic membranes at 37° are presented in Fig. 1. The Scatchard analysis indicates that [ $^3\text{H}$ ]PCP bound to two classes of sites. The high affinity sites represent a receptor site with  $K_D < 0.3 \mu\text{M}$  (a function of assay temperature and ionic strength; see below). Because the Scatchard curve asymptotically approaches a horizontal line, the apparent low affinity sites actually represent the level of non-specific binding estimated by computer analysis of the total receptor binding data [24].

The binding of two tritiated analogs of PCP, [ $^3\text{H}$ ]TCP and *m*-amino[ $^3\text{H}$ ]PCP, was analyzed similarly. At two assay temperatures, 0° and 37°, the order of potency of the analogs was: TCP > *m*-amino-PCP > PCP (Table 1). All three compounds bound to a similar number of high affinity sites (2.2 to 3.2 pmol ligand bound/mg protein). This suggests that TCP, *m*-amino-PCP and PCP bind to a common receptor.

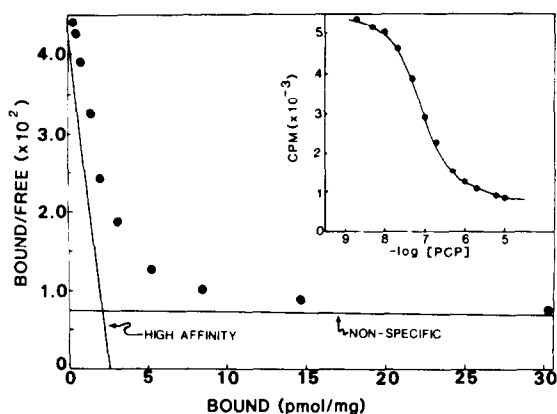


Fig. 1. Binding of [<sup>3</sup>H]PCP to rat brain synaptic membranes. Binding assays were performed at 37°. Data from a representative experiment are shown. Scatchard analysis (main figure) of the binding data (inset), uncorrected for non-specific binding, revealed two apparent classes of sites. These sites were determined by the computer program SCAFIT/LIGAND [24] to represent a high affinity receptor, with  $K_D = 75$  nM and  $B_{\max} = 2.6$  pmol PCP bound/mg protein in this experiment, and the level of non-specific binding. Curves for the high affinity receptor and for the level of non-specific binding are shown on the Scatchard plot.

**Displacement of [<sup>3</sup>H]PCP by various ion channel blockers and modulators.** A number of agents that block, selectively, Na<sup>+</sup>, Ca<sup>2+</sup>, voltage-gated K<sup>+</sup>, or Ca<sup>2+</sup>-activated K<sup>+</sup> channels, or the nicotinic ACh receptor-operated ion channel, were tested for their abilities to displace [<sup>3</sup>H]PCP from its binding sites on synaptic membranes. Two agents that inhibit or modulate Na<sup>+</sup> channel inactivation, aconitine and veratridine, were similarly tested. Neither the latter two compounds, nor any of the agents that block Na<sup>+</sup>, Ca<sup>2+</sup>, or Ca<sup>2+</sup>-activated K<sup>+</sup> channels, affected [<sup>3</sup>H]PCP binding when tested at their physiologically-relevant concentrations (Table 2). Capsaicin, which blocks certain rapidly-inactivating voltage-gated K<sup>+</sup> channels in the frog node of Ranvier [29], also had no effect on [<sup>3</sup>H]PCP binding.

Three compounds known to block the ionic channel of the nicotinic ACh receptor of *Torpedo* electroplex and to inhibit [<sup>3</sup>H]PCP binding to the *Torpedo* receptor displaced [<sup>3</sup>H]PCP from brain

synaptic membranes (Table 2). One of these compounds, amantadine, displaces [<sup>3</sup>H]PCP from the *Torpedo* receptor [34] and from brain membranes with similar affinities. However, the concentrations of quinacrine and perhydrohistrionicotoxin required to displace [<sup>3</sup>H]PCP from binding to brain membranes were at least 25-fold higher than the concentrations required to displace [<sup>3</sup>H]PCP from binding to the *Torpedo* receptor [35, 36].

Several aminopyridines (AP) and tetraalkylammonium (TAA) ions, including some [i.e. 4-AP, 3,4-diAP, tetrabutylammonium (TBA) and tetraethylammonium (TEA)] that block voltage-gated K<sup>+</sup> channels, also inhibited [<sup>3</sup>H]PCP binding. The orders of potencies of the AP analogs and the TAA ions for displacing [<sup>3</sup>H]PCP binding (Table 2) were, respectively: 4-AP = 3,4-diAP > 2-AP > 3-AP, and TBA > TEA > TMA (tetramethylammonium). The concentrations of 4-AP, TBA and TEA that displaced [<sup>3</sup>H]PCP were comparable to the concentrations that block voltage-gated K<sup>+</sup> channels in brain (Table 2).

**pH Dependence of aminopyridine potencies.** Unlike the quaternary amine TAA ions which are permanently charged, the aminopyridines can exist as either protonated or neutral molecules ( $pK_a = 9.25$ , 9.2 and 6.82 for 4-AP, 3,4-diAP and 2-AP respectively). The fraction of each AP molecule in its charged form decreases with increasing pH. As shown in Fig. 2, an increase in the pH of the binding mixture from 6.0 to 9.0 resulted in a 2-fold decrease in the potencies of 4-AP and 3,4-diAP for displacing [<sup>3</sup>H]PCP and in a 40-fold decrease in the potency of 2-AP.

**Binding of PCP to other membrane preparations.** The presence of PCP binding sites on various membrane preparations was determined.

Myelin-enriched and mitochondria-enriched fractions from brain were obtained during the preparation of synaptic membranes [22]. No significant binding of [<sup>3</sup>H]PCP to the myelin-enriched membranes was observed; the mitochondria-enriched preparation (which is approximately 10–15% contaminated with synaptic membranes) contained 6- to 7-fold fewer PCP binding sites per mg protein than did the synaptic membrane-enriched preparation.

Membranes prepared from human red blood cells [37] showed no binding of [<sup>3</sup>H]PCP, and membranes

Table 1. Binding of PCP and analogs to rat brain synaptic membranes

Ligand	Temp. (°)	$K_D$ (nM)	$B_{\max}$ (pmol ligand/mg protein)	N
[ <sup>3</sup> H]TCP	0	69 ± 9	2.3 ± 0.2	3
	37	21 ± 6	2.2 ± 0.2	3
<i>m</i> -Amino[ <sup>3</sup> H]PCP	0	148 ± 6	3.2 ± 0.2	3
	37	43	2.9	1
[ <sup>3</sup> H]PCP	0	290 ± 58	2.6 ± 0.1	7
	37	64 ± 5	2.8 ± 0.3	5

The binding of [<sup>3</sup>H]TCP, *m*-amino[<sup>3</sup>H]PCP and [<sup>3</sup>H]PCP to synaptic membranes was measured at both 0° and 37°. Scatchard plots of the binding data for each compound were analyzed by the computer-assisted curve-fitting program SCAFIT/LIGAND [24]. The estimated binding constants are summarized. Values are mean ± SE; N = number of determinations.

Table 2. Abilities of various channel blockers and modulators to inhibit [<sup>3</sup>H]PCP binding

Channel type	Test substance	[ <sup>3</sup> H]PCP binding	Physiological response	Ref.
		IC <sub>50</sub> (mM)	K <sub>i</sub> (mM)	
Voltage-gated K <sup>+</sup> channel	4-Aminopyridine (4-AP)	1.7 ± 0.2* (N = 3)	3	[28]
	3,4-Diaminopyridine (3,4-diAP)	1.9 ± 0.3 (N = 3)	†	—
	2-Aminopyridine (2-AP)	6.0 ± 0.4 (N = 3)	†	—
	3-Aminopyridine (3-AP)	13.5 ± 4.0 (N = 3)	†	—
	Tetrabutylammonium (TBA)	10.6 ± 1.4 (N = 3)	6–8	[28]
	Tetraethylammonium (TEA)	19.4 ± 1.2 (N = 3)	20	[28]
	Tetramethylammonium (TMA)	156 ± 13‡ (N = 2)	†	—
	Capsaicin	NE (0.1)	0.01	[29]
Ca <sup>2+</sup> -activated K <sup>+</sup> channel	Apamin	NE (0.1)	25 × 10 <sup>-9</sup>	[30]
	Quinine-SO <sub>4</sub>	≥0.1	0.001	[31]
Na <sup>+</sup> channel	Tetrodotoxin	NE (0.01)	1–5 × 10 <sup>-6</sup>	[32]
	Saxitoxin	NE (0.01)	1–5 × 10 <sup>-6</sup>	[32]
	Aconitine	NE (0.1)	0.01	[32]
	Veratridine	NE (1.0)	0.08	[32]
Ca <sup>2+</sup> channel	Verapamil	NE (0.1)	<1–10 × 10 <sup>-3</sup>	[33]
	D-600	NE (0.1)	<1–10 × 10 <sup>-3</sup>	[33]
	Nisoldipine	NE (0.01)	<0.001	[33]
Nicotinic ACh receptor	Amantadine	0.10	0.06	[34]
	Quinacrine	320 10 <sup>-3</sup>	7–14 × 10 <sup>-3</sup>	[35]
	Perhydrohistrionicotoxin	150 10 <sup>-3</sup>	0.4 × 10 <sup>-3</sup>	[36]

The IC<sub>50</sub> values (column 3) for the representative ion channel blockers and activators listed in column 2 were determined from their abilities to compete with [<sup>3</sup>H]PCP for binding to synaptic membranes at 0°. NE indicates no effect on [<sup>3</sup>H]PCP binding by the test compound up to the concentration indicated in parentheses. Also shown are typical K<sub>i</sub> values for the physiological response (block or activation of the respective channel) elicited by the test substance (column 4).

\* Mean ± SE.

† Not reported in Ref. 28.

‡ Mean ± range.

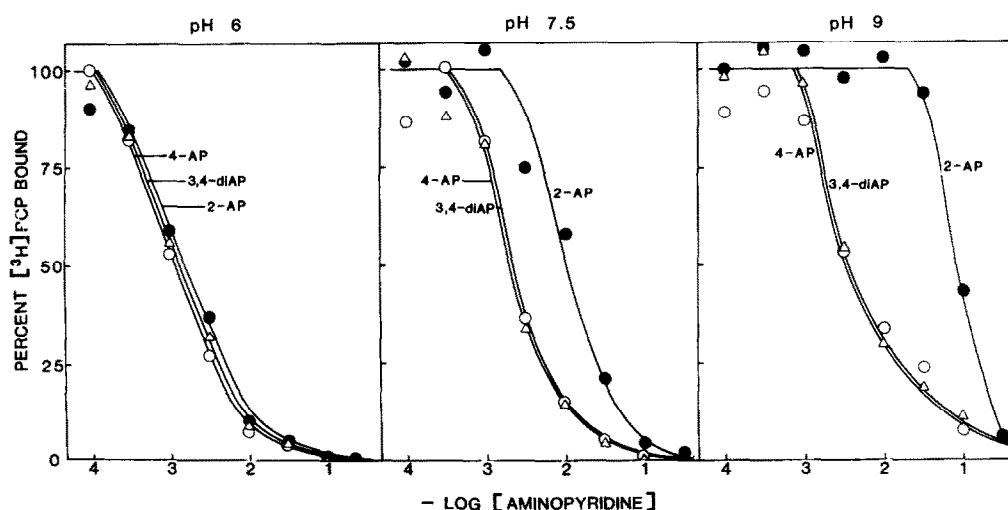


Fig. 2. Effect of pH on the ability of the aminopyridines, 4-AP, 3,4-diAP and 2-AP, to displace [<sup>3</sup>H]PCP from synaptic membranes. Binding assays were performed at 0°, at pH 6.0, 7.5 or 9.0 as indicated. "100% bound" is the amount of [<sup>3</sup>H]PCP bound in the absence of test compound, and "0%" is the amount of [<sup>3</sup>H]PCP bound in the presence of a large excess (500 μM) of unlabeled PCP. Key: (○) 4-AP; (△) 3,4-diAP; and (●) 2-AP.

Table 3. Binding properties of PCP and its analogs

Analog	Displacement of [ <sup>3</sup> H]PCP IC <sub>50</sub> (nM)	High affinity ligand binding K <sub>D</sub> (nM)	Block of brain K <sup>+</sup> channel K <sub>i</sub> (nM)
TCP	65 ± 8 (5)	69 ± 9 (3)	35 ± 15 (2)
PCE	137 ± 36 (6)	ND	ND
<i>m</i> -Amino-PCP	145 ± 39 (5)	148 ± 6 (3)	200 ± 100 (2)
PCP	290 ± 58 (7)	290 ± 58 (7)	1,400 ± 500 (6)
PCPY	315 ± 24 (5)	ND	ND
<i>m</i> -Nitro-PCP	7,300 ± 1,373 (5)	ND	42,500 ± 7,500 (2)

The IC<sub>50</sub> values (column 2) for the analogs of PCP listed in column 1 were determined from their abilities to compete with [<sup>3</sup>H]PCP for binding to synaptic membranes at 0°. For comparison, the dissociation constants (K<sub>D</sub>) for [<sup>3</sup>H]TCP and *m*-amino[<sup>3</sup>H]PCP binding (taken from Table 1) are also shown (column 3). Several of the PCP analogs block a voltage-gated K<sup>+</sup> channel in rat brain [14]; the apparent K<sub>i</sub> values for block of the K<sup>+</sup> channel by these analogs are given in column 4. Values are means ± SE (numbers of determinations in parentheses). ND = not done.

prepared from rat kidney [38] had 10- to 20-fold fewer binding sites per mg protein than did synaptic membranes. Thus, it appears that preparations with few voltage-gated K<sup>+</sup> channels, namely, myelin, red blood cells and kidney, contain very few PCP binding sites.

**Correlations between PCP receptor binding and K<sup>+</sup> channel block.** The affinities of several analogs of PCP for binding to the brain PCP receptor were determined by measuring their abilities to displace [<sup>3</sup>H]PCP. TCP was the most potent, and *m*-nitro-PCP the least potent analog tested (Table 3). The IC<sub>50</sub> values obtained for TCP and *m*-amino-PCP (Table 3) were virtually identical to their apparent dissociation constants as determined when these unlabeled ligands were used to displace their respective tritiated ligands (Table 1).

Several of these PCP analogs have also been tested for their abilities to block a synaptosome K<sup>+</sup> channel [14]. As shown in Table 3, the order of potency of these compounds for block of the K<sup>+</sup> channel paralleled that for binding to the brain PCP receptor.

Some "sigma" ligands and dioxolanes also bind to the PCP receptor [39–41]. Table 4 shows the stereospecificities of NANM, cyclazocine and dioxadrol in displacing [<sup>3</sup>H]PCP from synaptic membranes: (+)NANM, (–)cyclazocine and diox-

adrol were more potent than their respective enantiomers (columns 2, 3 and 5). These three compounds were also more effective than their respective enantiomers in their abilities to block the synaptosome K<sup>+</sup> channel (see Table 4, columns 4 and 6).

**Nature of the inhibition at the PCP receptor.** The data in Tables 2 and 4 show that several classes of structurally dissimilar compounds, APs, TAA ions, "sigma" ligands and dioxolanes, displaced [<sup>3</sup>H]PCP binding. One representative compound from each of these classes was studied to determine the mechanism of displacement (e.g. competitive or non-competitive inhibition): the K<sup>+</sup> channel blockers, 4-AP and TBA; the "sigma" ligand, (+)NANM; and the dioxolane, dioxadrol. In these experiments, the effects of the test compounds on [<sup>3</sup>H]TCP binding were determined. For comparison, and as a control for competitive inhibition at the receptor, PCP was also included as a test compound.

The K<sub>app</sub> for [<sup>3</sup>H]TCP increased with increasing concentrations of the five test compounds, but there was little change in the estimated number of sites. The effects of (+)NANM and 4-AP on [<sup>3</sup>H]TCP binding are graphed in Fig. 3. The plot of K<sub>app</sub> versus concentration of (+)NANM was linear (Fig. 3A); this indicates competitive inhibition of [<sup>3</sup>H]TCP binding by (+)NANM. These data yielded a linear

Table 4. Effects of "sigma" opioids and dioxolanes on [<sup>3</sup>H]PCP binding

Compound	Displacement of [ <sup>3</sup> H]PCP		Block of brain K <sup>+</sup> channel K <sub>i</sub> (nM)	Relative potency of isomers:	
	0°	37°		Binding	K <sup>+</sup> channel
(+)NANM	890 ± 161 (5)	550 ± 52 (3)	100	6–2	6
(–)NANM	5,690 ± 640 (5)	930 ± 75 (3)	600	1	1
(–)Cyclazocine	530 ± 89 (5)	80 ± 10 (3)	30	2–6	2
(+)Cyclazocine	950 ± 219 (5)	490 ± 17 (3)	60	1	1
Dexoadrol	98 ± 34 (5)	45 ± 2 (3)	50	455–429	400
Levoxadrol	44,600 ± 8,318 (5)	19,300 ± 3,868 (3)	20,000	1	1

The IC<sub>50</sub> values for the stereoisomers of NANM, cyclazocine and dioxadrol, determined from their abilities to compete with [<sup>3</sup>H]PCP for binding to synaptic membranes at both 0° and 37°, are presented in columns 2 and 3 (means ± SE; number of determinations in parentheses). These compounds have also been reported to block a voltage-gated K<sup>+</sup> channel in rat brain [19, 42]; the K<sub>i</sub> values of the compounds for block of this channel are given in column 4.

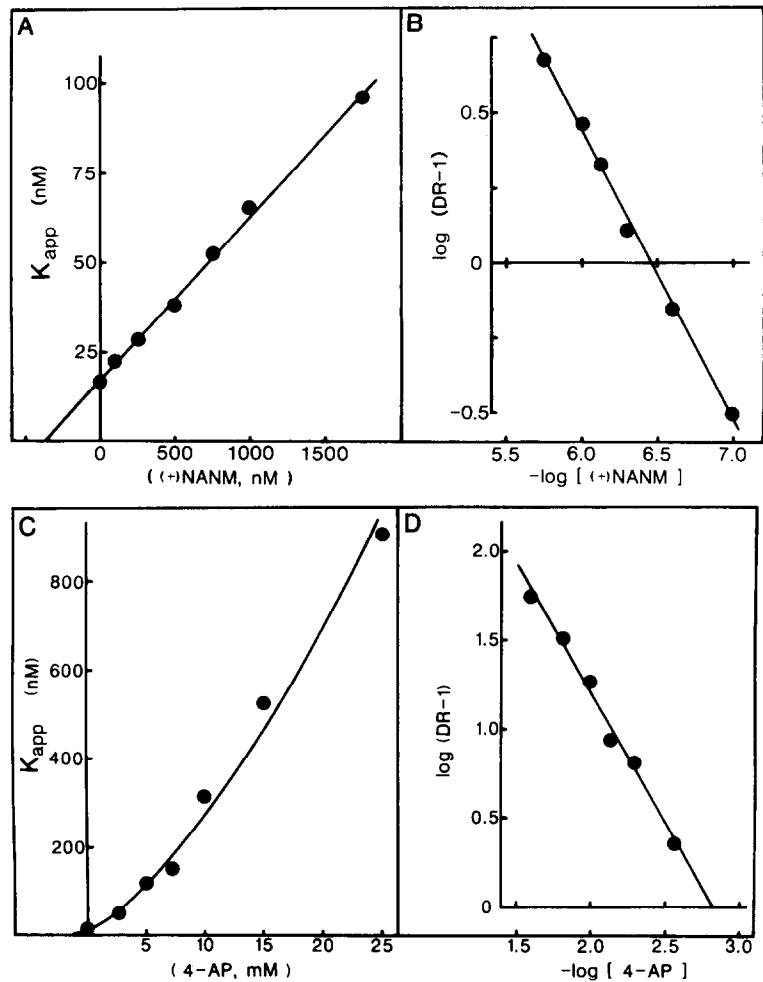


Fig. 3. Effects of (+)NANM (A and B) and 4-AP on [<sup>3</sup>H]TCP binding to synaptic membranes. Graphs A and C are plots of  $K_{app}$  vs inhibitor concentration where  $K_{app}$  represents the apparent dissociation constant for the binding of [<sup>3</sup>H]TCP measured in the presence of the inhibitor (test compound). Graphs B and D are Schild plots where the dose ratio (DR) is defined as the ratio of  $K_{app}(D)$  to  $K_{app}$ .  $K_{app}$  and  $K_{app}(D)$  are, respectively, the apparent dissociation constants for [<sup>3</sup>H]TCP binding measured in the absence, and in the presence, of the test compound (D). The plots for PCP, TBA and dexoxadrol were similar to those shown for (+)NANM.

Table 5. Comparison of inhibitor binding constants

Compound	Displacement of [ <sup>3</sup> H]PCP		$K_{app}$ vs I		Schild plot	
	IC <sub>50</sub> (mM)		$K_i$ (mM)		$K_B$ (mM)	Slope
PCP	$64 \times 10^{-6}$		$44 \times 10^{-6}$		$52 \times 10^{-6}$	-0.96
(+)NANM	$550 \times 10^{-6}$		$376 \times 10^{-6}$		$353 \times 10^{-6}$	-0.96
Dexoxadrol	$45 \times 10^{-6}$		$30 \times 10^{-6}$		$20 \times 10^{-6}$	-1.02
TBA	1.6		0.8		0.6	-0.92
4-AP	2.6		Non-linear		1.5	-1.47

For the test compounds listed in column 1, the table shows: (i) column 2, IC<sub>50</sub> values determined from competitive displacement assays of [<sup>3</sup>H]PCP binding; (ii) column 3,  $K_i$  values determined as the negative of the x-intercept from the  $K_{app}$  vs inhibitor (I) plot when the plots are linear; and (iii) columns 4 and 5,  $K_B$  and slope values, respectively, calculated from Schild plots.

Schild plot with a slope of  $-0.96$  (Fig. 3B) which confirms the competitive nature of the inhibition. Although not illustrated here, PCP, TBA and dexoxadrol all yielded plots similar to those shown for (+)NANM.

The plot of  $K_{app}$  versus concentration of 4-AP was concave upward (Fig. 3C). This suggests that the inhibition of [<sup>3</sup>H]TCP binding by 4-AP requires the binding of more than one molecule of 4-AP. The Schild plot for 4-AP (Fig. 3D) was linear, with a slope of  $-1.47$ . This can be interpreted as indicating that 2 molecules of 4-AP are required to displace 1 molecule of [<sup>3</sup>H]TCP from its membrane receptor [43]. From these data, it is apparent that 4-AP is not a simple competitive inhibitor at the PCP receptor.

The data for the five test compounds are summarized in Table 5. The affinity of each test compound, estimated from both the  $K_{app}$  vs [I] plot and from the Schild plot, was similar to the affinity determined from competition binding assay with [<sup>3</sup>H]PCP. The slopes of the Schild plots for all the test compounds, with the exception of 4-AP, were about  $-1.0$ .

## DISCUSSION

*Effect of temperature on binding to the PCP receptor.* This report describes ligand binding studies that characterize the PCP receptor of rat brain synaptic membranes and identify this receptor as part of, or linked to, a K<sup>+</sup> channel.

Binding to the PCP receptor was temperature dependent: increasing temperature increased the apparent affinities of: (a) PCP and its analogs (Table 1), (b) "sigma" ligands and dioxolanes (Table 4), and (c) the TAA compounds (compare Table 2 and Ref. 4) for the receptor. Some of this increase in affinities could be attributed to a decrease in ionic strength [4, 5] because the buffer concentration was reduced from 50 to 20 mM in most experiments when the assay temperature was increased from 0° to 37°. However, when assayed at constant ionic strength, increasing the assay temperature from 0° to 37° doubled the affinities of both PCP and TCP (data not shown). Preliminary results (unpublished) suggest that temperature increases the association rates of these compounds.

In contrast, the affinities of the AP analogs were reduced when the assay temperature was increased and the ionic strength was decreased (compare Table 2 and Ref. 44). The significance of these effects is unknown. However, it is noteworthy that the compounds whose affinities increased with increasing temperature and decreasing ionic strength, namely PCP, (+)NANM, dexoxadrol and TBA, were competitive inhibitors at the high affinity PCP receptor, whereas 4-AP, whose affinity decreased, was an allosteric inhibitor.

*Influence of ion channel effectors on [<sup>3</sup>H]PCP binding.* Of the various agents that were tested (Table 2), only those agents that block K<sup>+</sup> channels, and those that inhibit [<sup>3</sup>H]PCP binding to the nicotinic ACh receptor of *Torpedo* electroplax, also displaced [<sup>3</sup>H]PCP from brain synaptic membranes. This raises the possibility that the brain high affinity PCP receptor may be part of a K<sup>+</sup> channel, and/or part of a *Torpedo*-like ACh receptor in brain. The

binding data (Table 2), *per se*, do not allow a distinction between these two possibilities. K<sup>+</sup> channels and the nicotinic receptor-gated channel of *Torpedo* may have structural similarities, because PCP [10], histrionicotoxin and its analogs [36, 45], and TEA [46, 47] all block both types of channels. Amantadine may also block both types of channels [34].

Two groups [48, 49] recently isolated several polypeptides from brain that exhibit structural homology with the *Torpedo* and skeletal muscle ACh receptors. The apparent molecular weights of the subunits of this (presumed) brain ACh receptor are similar to those of the *Torpedo* and muscle ACh receptors, but different from the two polypeptides we recently covalently labeled with a photoaffinity analog of PCP [44]. Therefore, we suggest that the brain PCP receptor is not part of a neuronal ACh receptor.

*The rat brain PCP receptor is part of a K<sup>+</sup> channel.* Three results help to identify the brain PCP receptor as part of a K<sup>+</sup> channel:

(1) K<sup>+</sup> channel blockers displaced [<sup>3</sup>H]PCP from binding to synaptic membranes (Table 2 and Refs. 44 and 50). The order of potency of the AP molecules, 4-AP = 3,4-diAP > 2-AP > 3-AP, was the same as that reported for their abilities to increase neurotransmitter release [51, 52]; this effect is a consequence of K<sup>+</sup> channel blockade [53]. The order of potency of the TAA ions in displacing [<sup>3</sup>H]PCP, TBA > TEA ≫ TMA, also corresponded to their order of potency for block of K<sup>+</sup> channels in squid axons [54]. Furthermore, the data in Fig. 3 and Table 5 show that TBA is a competitive inhibitor and, thus, shares a common receptor with PCP.

4-AP is an allosteric inhibitor (also see Ref. 50) at the PCP receptor. It is not clear whether the binding of 4-AP sterically inhibits the binding of PCP to its receptor, or whether the binding of 4-AP induces a conformational change at the PCP receptor. It has been reported [50] that the binding of 4-AP increases the dissociation rate of PCP: this is consistent with the latter interpretation. In either case, these data indicate that there is a link between the binding site for 4-AP and the binding site for PCP.

The affinities of the AP analogs increased at reduced pH where a larger fraction of the AP analogs are protonated (Fig. 2). This indicates that the PCP receptor preferentially recognizes the charged species of these molecules. The charged forms of the APs are also preferred for block of the squid giant axon K<sup>+</sup> channel [55]. The relative prevalence of the charged species of the four APs, as determined from their pK<sub>a</sub> values (9.2 for 4-AP and 3,4-diAP, 6.8 for 2-AP, 5.9 for 3-AP) may explain their relative effectiveness in displacing [<sup>3</sup>H]PCP: 4-AP = 3,4-diAP > 2-AP > 3-AP.

(2) PCP and its behaviorally-active analogs selectively block a voltage-gated K<sup>+</sup> channel in rat brain [14]. The order of potency of PCP and its analogs for receptor binding correlated with their order for block of this K<sup>+</sup> channel (Table 3).

(3) "Sigma" ligands and dioxolanes blocked the same brain K<sup>+</sup> channel as does PCP [19, 42]. Table 4 shows that the stereospecificities and the relative potencies of several stereoisomer pairs of "sigma" ligands and dioxolanes for blocking the brain K<sup>+</sup> channel, and for displacing [<sup>3</sup>H]PCP binding, were

similar. Dexoxadrol and (+)NANM were competitive inhibitors at the PCP receptor (Fig. 3, Table 5), indicating that these compounds share a common binding site. The affinities and stereospecificities of (+)NANM and (−)NANM for displacing [<sup>3</sup>H]PCP, and the finding that (+)NANM was a competitive inhibitor at the PCP receptor, are consistent with previous reports [56, 57] that the low affinity NANM receptor is also the PCP receptor.

The binding properties of PCP and its analogs, the "sigma" ligands and the dioxolanes, described here, also correlated with the abilities of these compounds to antagonize the effects of *N*-methyl-D-aspartate (NMDA) [58–60]. However, these effects must represent a functional, rather than a structural, antagonism because NMDA agonists do not displace [<sup>3</sup>H]PCP from its binding sites [8, 58].

In summary, this study provides evidence that the rat brain PCP receptor is associated with a K<sup>+</sup> channel. However, conclusive identification of the PCP receptor as a K<sup>+</sup> channel requires purification of the receptor and demonstration that the purified receptor has K<sup>+</sup> channel activity. The behavioral impairments produced by PCP intoxication may occur, at least in part, through the ability of PCP to bind to this receptor, which, we suggest, results in the block of a voltage-gated, non-inactivating K<sup>+</sup> channel [10, 14]. PCP induces a toxic confusional psychosis with many schizophrenia-like properties [1, 2]. Thus, it seems possible that our observations may provide new clues to the pathophysiology of these behavioral disorders.

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