Biochemical Pharmacology, Vol. 33, No. 22, pp. 3529–3535, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

PHENCYCLIDINE RECEPTORS IN RAT BRAIN CORTEX

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(*Received* 16 *September* 1983; *accepted* 13 *February* 1984)

Abstract—The binding of [^{3}H]phencyclidine (PCP) to receptors in rat brain cortex has been studied. Two receptors have been detected, a high affinity receptor site with a K_D of 23.5 ± 7.4 nM and a low affinity site with a K_D of 7.6 ± 1.8 μM. The binding of [^{3}H]PCP to its receptors was pH and temperature dependent and was destroyed by heat-denaturation. The binding of [^{3}H]PCP was inhibited by compounds which produce PCP-like behavioral effects including dexoxadrol, etoxadrol and ketamine as well as a novel series of benz(f)isoquinolines. The low affinity site was blocked by PCP, etoxadrol and (+)-SKF-10,047 but not morphine or leu-enkephalin, suggesting that it also represents a specific PCP site. Stereoselective displacement of PCP at the high affinity receptor was observed with the isomers of cyclazocine, cyclorphan, SKF-10,047 and dioxadrol (dexoxadrol and levoxadrol). Naloxone, 4,5,6,7-tetrahydroisoxazolo(S,4-C)pyridin-3-ol (THIP) hydrate and haloperidol inhibited binding poorly (K_i > 1 μM), suggesting that these compounds do not interact significantly with the high affinity PCP receptor *in vivo*. The affinity of ligands for the phencyclidine receptor was highly correlated ($r = 0.714$, $P < 0.01$) with their potency to produce catalepsy in pigeons.

Phencyclidine (PCP) was introduced in the late 1950s as a dissociative anesthetic with a large margin of safety in humans [1–3]. Although limited respiratory depression was a major asset with this drug, other side effects including emergence delirium and prolonged excitation made PCP unsuitable for use in humans [4, 5].

Since that time, PCP has become a major drug of abuse. Clinicians have also noted that PCP psychosis resembles schizophrenia [6–8]. The current treatment for PCP psychosis includes the administration of antianxiety agents such as diazepam [9] or neuroleptics such as haloperidol, which have been shown to improve some of the symptoms [10].

The mechanism of action of PCP is not well understood. It inhibits uptake of the monoamines [11] and increases release of dopamine [12]. At the muscarinic cholinergic receptor, PCP may function as an antagonist [13] or agonist [14]. PCP is also a potent inhibitor of butyryl and acetyl cholinesterase [15] and binds to the potassium ion channel of the nicotinic receptor in torpedo electroplax membranes [16, 17]. In rat brain synaptosomes, PCP has been shown to block the K^+-stimulated release of $^{86}Rb^+$ [18].

Despite these known activities, it is not clear which, if any, of these actions are responsible for the behavioral effects of PCP. To focus more closely on the molecular actions of PCP, we have studied the binding characteristics of PCP to rat and pigeon brain membranes.

Controversy has existed in the literature concerning whether a PCP receptor exists. While Zukin and Zukin [19] and Vincent *et al.* [20] reported the presence of a specific receptor, their results have been challenged by Maayani and Weinstein [21], who reported that the earlier data could be explained as artifact. Despite this, Vincent and his colleagues [22, 23], the Zukins and coworkers [24–26], and Hampton *et al.* [27] have been able to further substantiate the existence of the receptor by eliminating some of the high nonspecific binding observed in their original assays. Quirion *et al.* [28], using radioautography of brain slices, reported the presence of a high affinity specific PCP receptor in rat brain cortex. In the experiments reported here, we have examined the binding of [^{3}H]PCP to membranes of rat cortex and whole pigeon brain (minus cerebellum) under a variety of conditions. The data demonstrate the presence of high affinity receptors with greater affinity for [^{3}H]PCP than previously described by others. In addition, low affinity receptor sites have been identified. The biochemical properties of these high affinity receptors are described, and the interactions of a number of other related drugs with this receptor are reported. Taken together, the data support the conclusion that this receptor has physiological relevance to the action of PCP as a psychotomimetic agent.

MATERIALS AND METHODS

Animals. Sprague–Dawley male rats weighing about 250 g were supplied by Harlan Industries (Indianapolis, IN). White Carneaux male pigeons weighing approximately 500 g were supplied by the Palmetto Pigeon Plant (Sumter, SC). Rats were fed *ad lib.* Purina laboratory animal feed products (Ralston Purina, St. Louis, MO). The pigeons were maintained on restricted diets so that their weight was 80% of their *ad lib.* weight.

Materials. [^{3}H]PCP (48.0 Ci/mmole) was obtained from the New England Nuclear Corp. (Boston, MA). Morphine sulfate, unlabeled PCP and the *d*- and *l*-isomers of SKF-10,047, were donated by the

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Research Technical Branch of NIDA (Rockville, MD). Bremazocine was donated by Dietmar Romer from Sandoz (Basel, Switzerland). Cyclazocine was a gift from Sterling-Winthrop (Rensselaer, NY). Cyclorphan was obtained from Warner-Lambert/Parke-Davis (Ann Arbor, MI). Dexoxadrol and etoxadrol were donations of The Upjohn Co. (Kalamazoo, MI). Ketamine·HCl was provided by Warner-Lambert/Parke-Davis. Dextrorphan was a gift from Hoffmann-LaRoche Inc. (Nutley, NJ). Haloperidol was donated by Janssen Pharmaceutica (Beerse, Belgium). THIP [4,5,6,7-tetrahydroisoxazolo (S,4-C)pyridin-3-ol]hydrate was prepared by Robert Archer, Lilly Research Laboratories (Indianapolis, IN). Leucine-enkephalin was purchased from Peninsula Laboratories (San Carlos, CA). Compounds from the isoquinoline series [29] were prepared by D. Zimmerman (Lilly Research Laboratories). The synthesis of the benz(f)isoquinolines and the bridged benz(f)isoquinolines was similar to that reported for other morphine-based analgesics [30]. Polyethylenimine was obtained from the Aldrich Chemical Co., Inc. (Milwaukee, WS). GF/B and GF/C glass fiber filters were purchased from Whatman Inc. (Clifton, NY). PCS liquid scintillation solution was supplied by Amersham (Arlington Heights, IL).

Tissue preparation. Rats were killed by decapitation. Brains were removed and the cortex was dissected free. Pigeons were also decapitated and the whole brain, excluding the cerebellum, was used. Tissue was homogenized in ice-cold 5 mM Tris–HCl, 30 vols pH 7.4 (30 mg/ml), using either a Wheaton glass–Teflon tissue grinder or a Tekmar Tissuemizer (setting 30) for 15–30 sec. The homogenate was centrifuged at 49,000 *g* for 15 min at 4°. The supernatant fraction was discarded and the pellet was washed two times by resuspension in buffer (original volume) followed by centrifugation (49,000 *g*, 15 min). The final pellet was resuspended at 30 mg original tissue weight/ml 5 mM Tris–HCl, pH 7.4.

Binding assays. Suspended membrane preparations (0.1 to 1.3 mg protein/2 ml) were preincubated at 37° for 20 min. Binding was initiated by the addition of [^{3}H]PCP (12–48 Ci/mmole, 1–60 nM) in the presence or absence of unlabeled PCP (100 μM) and was continued for either 20 or 30 min at 37°. The assay was terminated by rapid filtration using an Amicon (FM-II) filtration manifold. Filters (either GF/C or GF/B, 24 mm) were presoaked a minimum of 20 min in 0.05% polyethylenimine in water [27] to reduce specific and nonspecific binding to filters. Following filtration, assay tubes and filters were rinsed with two washes (4 ml) of 5 mM Tris–HCl, pH 7.4. The total filtration time was less than 6 sec. The filters were then transferred to vials containing 10 ml PCS and counted by liquid scintillation spectrometry. Each assay was performed in triplicate.

At all concentrations of [^{3}H]PCP, four measurements of binding were performed: (a) total binding of [^{3}H]PCP to membranes and filters, (b) total binding to membranes and filters in the presence of unlabeled PCP (100 μM), (c) [^{3}H]PCP binding to filters, and (d) [^{3}H]PCP binding to filters in the presence of unlabeled PCP 100 μM). The specific binding to filters (c − d) was subtracted from the total specific binding (a − b) to yield net specific binding of PCP to membranes. This analysis is similar to that described by Vincent *et al.* [22].

Time course of [^{3}H]PCP binding to membranes. To determine an appropriate incubation time, the binding of [^{3}H]PCP to rat cortex membranes was measured as a function of time in five independent preparations. [^{3}H]PCP was added to initiate binding, and samples were removed and immediately filtered at the designated times. Specific binding to membranes was determined as indicated above.

Effect of pH. The effect of pH on the binding of [^{3}H]PCP was measured. Suspensions of rat cortex membranes prepared in 5 mM Tris–HCl, pH 7.4, were titrated using 1.0 to 12.1 N HCl to the indicated pH values. These preparations were preincubated at 37° as described above. The binding of [^{3}H]PCP (2 nM) to membranes and filters was measured at 37° following a 30-min incubation in the presence and absence of unlabeled PCP (100 μM).

Effect of protein concentration and protein denaturation on PCP binding. The relationship between protein concentration and specific binding of PCP was measured at 37° for 30 min using 10 nM [^{3}H]PCP in the presence of increasing protein concentrations of rat cortex membranes. A parallel experiment was done in which the tissue suspension was boiled for 10 min prior to use.

Competitive binding assays. The ability of PCP and other ligands to bind to the PCP receptor of rat cortex was evaluated through competitive binding assays. Assays contained [^{3}H]PCP (2 nM), approximately 500 μg protein/2 ml, and increasing concentrations of ligand. The concentration of 2 nM radiolabel resulted in good sensitivity and reproducibility with low amounts of nonspecific binding. Using a concentration of less than 0.1 K_D also allows the use of the formula: $K_i = IC_{50}/(1 + [L]/K_D)$ to determine K_i values (where $[L]$ is the radioligand concentration [31]). Full displacement was measured using PCP (100 μM).

Studies of the low affinity receptor were carried out using [^{3}H]PCP (2 nM) in the presence of unlabeled PCP (200 nM). Increasing concentrations of etoxadrol, morphine or leu-enkephalin were added as indicated.

Statistics and curve fitting. Tests of significance were performed using Student's *t*-test. The least squares curve fitting program SCAFIT was used to calculate the parameters of the two binding site model [32].

Behavioral assays. Pigeon catalepsy was defined as the loss of the righting reflex without a head-drop response as described by Chen [33]. Briefly, pigeons were injected intramuscularly (breast muscle) with drug at doses from 2.5 to 40 mg/kg. The behavior was observed for up to 2 hr, and the minimum effective dose to produce "cataleptic behavior" in two out of three birds was determined.

RESULTS

The binding of [^{3}H]PCP to rat cortex membranes exhibited two specific saturable sites (Fig. 1A). At low concentrations, the amount of PCP bound appeared to plateau between 80 and 100 nM. At

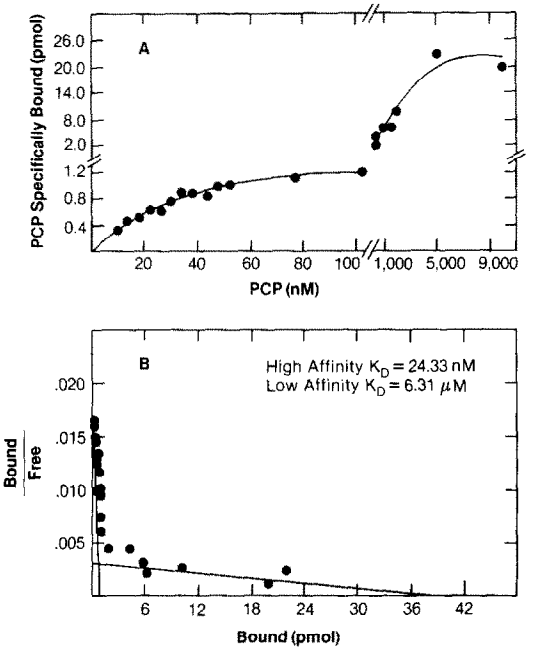


Fig. 1. Specific binding of [^{3}H]PCP to rat cortex membranes. The amount of PCP bound was determined by competitive displacement between [^{3}H]PCP (2 nM) and unlabeled PCP (10 nM–100 μM) as described in Materials and Methods. (A) Two saturable binding sites are obtained. (B) Scatchard plot of the data in A is resolved into two lines yielding high and low affinity dissociation constants.

higher concentrations, a second specific site was also detectable. The data were plotted in a Scatchard plot (Fig. 1B) and the best fit to the curve (determined by computer modeling) was observed to be a two-site model. Dissociation constants of 24.3 nM and 6.3 μM reflected high and low affinity receptors for PCP on these membranes. The determination of specifically bound PCP was calculated by subtracting from the total amount of PCP bound the amount non-specifically bound to both tissues and filters, as well as a small amount of [^{3}H]PCP which bound specifically to filters [23]. Soaking the filters in 0.05% polyethylenimine reduced this binding by 95%; however, it could not be eliminated completely at high concentrations of PCP. Average values of the dissociation constant from six separate preparations yielded a value of 23.5 ± 7.4 nM for the high affinity site and 7.6 ± 1.8 μM for the low affinity site. Similar studies carried out in pigeon brain (whole brain minus cerebellum) yielded dissociation constants of 40.2 ± 7.5 nM and 5 ± 5 μM (N = 3) for the high and low sites respectively. The maximum numbers of binding sites for the rat cortex and pigeon brain high affinity sites were 2.8 ± 0.9 and 2.5 ± 0.7 pmoles/mg protein respectively. Greater numbers of receptors were found for low affinity sites: 95.8 pmoles/mg in the rat and 125.8 pmoles/mg in the pigeon. PCP bound rapidly to its rat cortex receptor (Fig. 2). At 37°, binding was over 70% complete within 5 min. There was a small but significant increase in binding over the next 15 min.

Effect of pH. The pH dependency of the binding of [^{3}H]PCP to rat cortex membranes was studied over a pH range of 5.0 to 9.5 (Fig. 3). Binding to filters was independent of pH. Nonspecific binding to both filters and membranes was low relative to the total binding except at pH values greater than 8.5. The net specific binding obtained at all pH values studied is shown in Fig. 3. Maximal binding was obtained between pH values of 7.5 and 9.0. Specific binding decreased rapidly at pH values outside this range, due in part to an increase in nonspecific interaction of [^{3}H]PCP with membranes at pH values greater than 8.5.

Binding as a function of protein concentration. The effect of protein concentration on specific binding of [^{3}H]PCP was evaluated over a concentration range of 0.05 to 0.7 mg protein/ml. Total and nonspecific binding (Fig. 4A) increased linearly up to about 0.4 mg protein/ml. At a higher protein concentration, the increase in binding was smaller. Thus, net specific binding (Fig. 4B) was a linear function

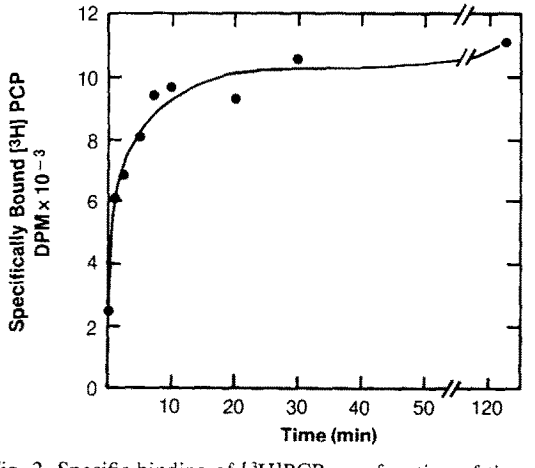


Fig. 2. Specific binding of [^{3}H]PCP as a function of time. Specific binding of [^{3}H]PCP was measured at 37° at the indicated time.

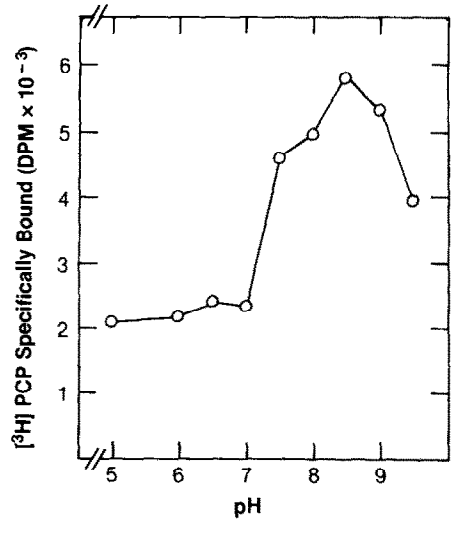


Fig. 3. Specific binding of [^{3}H]PCP as a function of pH.

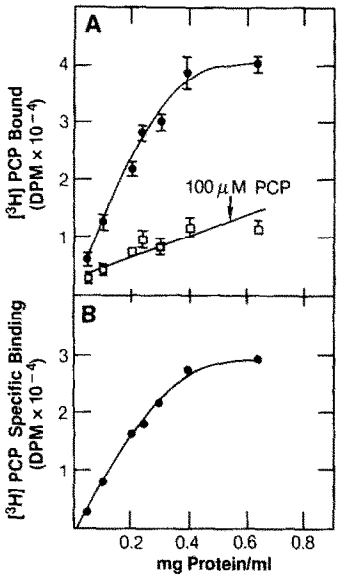


Fig. 4. Binding of [^{3}H]PCP as a function of protein concentration. (A) [^{3}H]PCP (10 nM) was incubated with rat cortex membranes (37°, 30 min) in the absence (●) and presence (□) of unlabeled PCP (100 μM). (B) Specific binding as a function of protein concentration was calculated as the difference between the two curves in panel A. All values were corrected for the interaction of [^{3}H]-PCP with filters.

of protein concentration at least to concentrations of 0.4 mg protein/ml or less.

The effect of denaturation on PCP binding was investigated. A membrane suspension from rat cortex was divided in halves. One half was boiled for 10 min while the other was maintained on ice. The binding of [^{3}H]PCP (10 nM) was then evaluated at 37°. Denaturation by boiling reduced specific binding of [^{3}H]PCP (Fig. 5) by greater than 80%.

Competitive binding assays. The activity of several

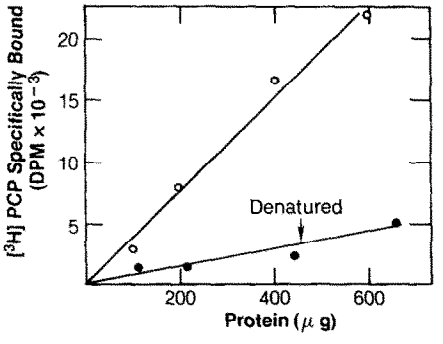


Fig. 5. Effect of denaturation on specific binding of [^{3}H]-PCP. Membranes from rat brain cortex were prepared in 5 mM Tris-HCl, pH 7.4. Half the preparation was boiled for 10 min while the other half was maintained at 4°. Binding was conducted at 37° for 30 min in the presence of [^{3}H]-PCP (10 nM). Total protein per 2 ml is indicated.

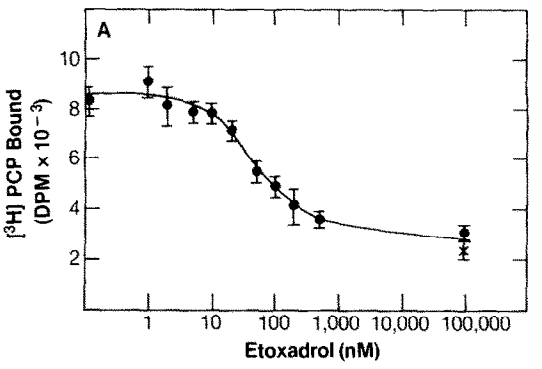


Fig. 6. Displacement of [^{3}H]PCP by etoxadrol. [^{3}H]PCP (2 nM) was incubated with rat cortex membranes and increasing concentrations of etoxadrol (1 nM–100 μM). The amount of [^{3}H]PCP bound in the presence of etoxadrol (●) or 100 μM PCP (×) is shown.

ligands to competitively displace [^{3}H]PCP was evaluated. Using a procedure similar to that for the binding assay, [^{3}H]PCP was incubated (20 min at 37°) in the presence of increasing concentrations of unlabeled ligand (1 nM–100μM, Fig. 6). Controls (no tissue) were performed to evaluate nonspecific binding to the filters. The amount of [^{3}H]PCP bound when full displacement was achieved was measured in the presence of excess (100 μM) unlabeled PCP. The IC_{50} value was determined using a linear regression of the percent displacement as a function of the concentration of the unlabeled ligand in a log probit analysis. K_i values were calculated as $IC_{50}/(1 + [L]/K_D)$ [31]. Typical results are illustrated using etoxadrol (Fig. 6). The K_i of etoxadrol at the high affinity site was 75.8 nM. When the displacement of [^{3}H]PCP by unlabeled PCP was measured over the full dose range of 1 nM–100 μM, a biphasic displacement curve was obtained (data not shown). The presence of low affinity sites with $K_i >$ 1 μM was investigated further by evaluating the specificity of the low affinity site. The displacement of [^{3}H]PCP (2 nM [^{3}H]PCP + 200 nM unlabeled PCP) by etoxadrol, morphine and leu-enkephalin was measured (Fig. 7). Etoxadrol yielded a K_i of

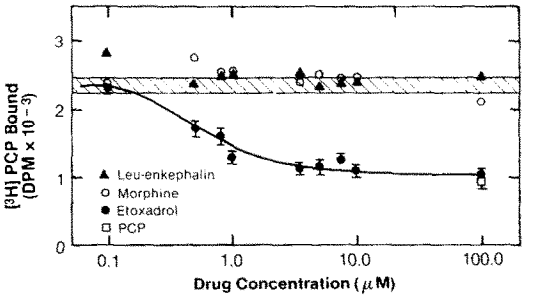


Fig. 7. Displacement of [^{3}H]PCP from the low affinity receptor. Rat cortex membranes were incubated with [^{3}H]-PCP (2 nM) in the presence of 200 nM unlabeled PCP to saturate high affinity receptors (▨). Increasing concentrations of etoxadrol, morphine and leu-enkephalin were added as indicated (100 nM–100 μM).

Table 1. Potency of compounds to inhibit binding of [^{3}H]-PCP and the minimum effective doses (MED) to produce catalepsy in the pigeon

Compound	IC_{50}* (nM)	MED† (mg/kg)
1. PCP	27.3	5
mOH PCP	7.4	
2. Dexoxadrol	20.6	10
3. Etoxadrol	75.7	5
4. (−)-Cyclazocine	82.0	2.5
5. (+)-Cyclazocine	325	10
6. (+)-SKF-10,047	108	20
7. (−)-SKF-10,047	211	20
8. (−)-Cyclorphan	208	20
(+)-Cyclorphan	1,360	NA‡
9. Dextrorphan	281	40
Bremazocine	370	
10. Ketamine	405	20
Naloxone	3,230	NA
Levoxadrol	6,100	
THIP	57,000	NA
Haloperidol	113,000	NA
11. LY156370	123	5
12. LY156007	353	10
13. LY136626	992	40
14. LY163773	232	10
15. LY163774	643	40
16. LY163661	591	40
17. LY154045	37	5
LY154005	6,360	>40

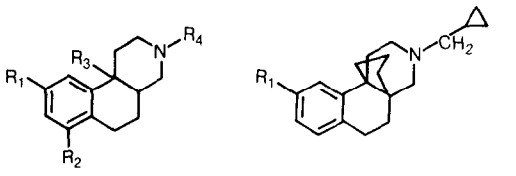
* Competitive binding assays were performed using [^{3}H]-PCP (2 nM) and increasing concentrations of unlabeled ligand. Maximal displacement was obtained using 100 μM PCP. The percent displacement at each concentration was calculated. The IC_{50} values were determined from log probit analysis, and K_i values were calculated from the equation $K_i = IC_{50}/(1 + [L]/K_D)$ [31].

† The minimum effective dose to produce the characteristic catalepsy described by Chen [33].

‡ NA = not active.

523 nM, while neither leu-enkephalin nor morphine significantly reduced binding. In a similar assay (+)-SKF-10,047 yielded a K_i of 2.42 μM. These results suggest that the low affinity site is a specific site at which PCP-like ligands, but not nonspecific drugs, interact.

Table 1 summarizes the results obtained with compounds known either to produce PCP-like effects *in vivo*, or to have activities at other brain receptors. Compounds which produce PCP-like effects *in vivo* bound to the high affinity PCP receptor. The dioxolanes, dexoxadrol and etoxadrol, were highly potent in displacing [^{3}H]PCP. The isomers of cyclazocine and cyclorphan showed a stereoselective preference for the receptor with the (−)-isomer being almost four to six times more potent than the (+)-isomer. The isomers of SKF-10,047 were somewhat less potent and displayed an opposite stereoselective preference, with the (+)-isomer being nearly twice as potent as the (−)-isomer. Ketamine, a second generation dissociative anesthetic, was roughly 10-fold less potent than PCP. Bremazocine, a putative kappa opioid ligand [34], had an intermediate affinity for the PCP receptor. Haloperidol, which is used clinically in the treatment of PCP-psychosis [10],



I. Benz-f-isoquinolines II. Bridged benz-f-isoquinolines

Series	Compound	R_1	R_2	R_3	R_4
I	LY 156370	OH	H	CH_3	CH_2—CH = CH_2
	LY 156007	OH	H	CH_3	CH_2—◁
	LY 136626	OH	H	CH_3	CH_3
	LY 154005	OH	H	$CH_2CH_2CH_3$	CH_2—◁
	LY 163773	H	OH	CH_3	CH_2—CH = CH_2
	LY 163661	H	OH	CH_3	CH_3
	LY 163774	H	OH	CH_3	CH_2—◁
II	LY 154045	OH	—	—	—

Fig. 8. Structures of the benz(f)isoquinolines and bridged benz(f)isoquinoline studied. Eight compounds in this series were evaluated for potency to displace [^{3}H]PCP and minimum effective dose to product catalepsy in pigeons.

displaced poorly and yielded a K_i of 113 μM, while naloxone, an opiate antagonist, yielded a K_i in the micromolar range. The benz(f)isoquinolines and the bridged benz(f)isoquinolines (Fig. 8) are a novel series of compounds found to produce PCP-like agonist effects [29]. Within the benz(f)isoquinolines, the N-allyl derivative, LY156370, has the highest affinity for the receptor while formation of the bridged benz(f)isoquinolines further enhances affinity. However, substitution of a propyl group at position three results in a dramatic loss of affinity for its PCP receptor.

The compounds in Table 1 have been tested for their PCP-like agonist potency in a PCP-type catalepsy test in pigeons. It is known that compounds with PCP-like effects produce a distinct type of catalepsy characterized by a loss in the righting reflex without "head drop" [33]. Also shown in Fig. 9, the

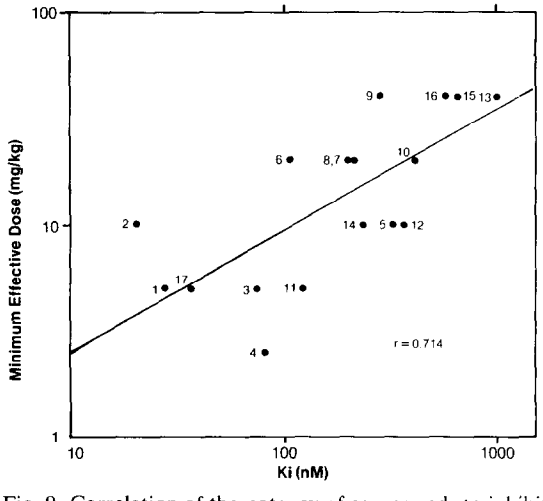


Fig. 9. Correlation of the potency of compounds to inhibit binding of [^{3}H]PCP (log K_i) to rat cortex membranes with the minimum effective dose (log MED) to produce catalepsy in pigeons. The identity, K_i value, and MED value for each of the numbered compounds are in Table 1.

affinities of these ligands for the PCP-receptor and their minimum effective dose to produce catalepsy are highly correlated ($r = 0.714$, $P < 0.01$), suggesting that the receptor we have characterized mediated this behavioral response to these agents.

DISCUSSION

The binding isotherm of [^{3}H]PCP to rat cortex membranes showed two saturable receptor sites with dissociation constants of 24.3 nM and 6.3 μM. Other workers have reported the presence of a high affinity receptor site for phencyclidine; however, the dissociation constants reported are larger than we observed. For example, Zukin and Zukin [19] and Vincent *et al.* [20] reported K_D values of 150 and 250 nM respectively. These studies were carried out at high salt concentrations (50 mM Tris–HCl) in comparison to our studies in low ionic strength buffer (5 mM). Ionic strength has been shown to inversely affect PCP binding with increasing salt concentrations resulting in less bound PCP [23]. In particular, the affinity of PCP for its receptor in brain [23] has been reported to decrease from 63 nM at 5 mM Tris to 250 nM at 50 mM Tris or by a factor of five in guinea pig ileum [24]. The high affinity value for PCP observed in our assay is likely, therefore, a result of the low ionic strength buffer which was employed. Correlations of the ED_{50} of PCP-like drugs in the rotorod test with their potency to inhibit [^{3}H]PCP binding in 5 mM Tris have been shown to be as good or better than correlations obtained in 50 mM buffer [23].

More recent work by Gintzler *et al.* [24] demonstrated high affinity ($K_D = 36$ nM) and low affinity ($K_D = 2$ μM) receptors in guinea pig longitudinal muscle from measurements taken in low ionic strength buffers. The presence of high (30 nM) and low (80 nM) affinity sites in brain was suggested by Hampton *et al.* [27]; however, these values reflect the stereoselective difference in binding obtained with the enantiomers, dexoxadrol and levoxadrol, and, therefore, cannot be directly compared with our data. More recently, Zukin *et al.* [26] reported a single class of binding sites in whole rat brain homogenates when low salt concentrations were used. In the experiments reported here, the binding of [^{3}H]PCP to neuronal membranes was studied under low ionic strength conditions to enhance the sensitivity of the assay and facilitate correlation of the data with behavioral results.

Synaptosomes from rat forebrain have been reported recently to have high and low affinity sites for PCP [18]. A functional relationship of both of these sites was suggested by the biphasic effect of PCP to block K^+-stimulated $^{86}Rb^+$ efflux from these synaptosomes [18]. The ability of PCP, (+)-SKF-10,047 and etoxadrol, but not morphine or the leuenkephalin, to displace bound [^{3}H]PCP at the low affinity receptor suggests that this binding is specific and represents a specific PCP binding site.

In the pigeon brain, a high affinity binding site with a larger dissociation constant was detected. This larger value may reflect the average of binding sites over different regional populations of receptors within the whole brain. A low affinity site was also identified.

Binding measurements at 37° were carried out at equilibrium, which was achieved within 20 min, and at protein concentrations of which binding was a linear function.

Specific binding of [^{3}H]PCP was dependent on pH over a broad range (7.5 to 9.0), while nonspecific interactions with filters and tissue were pH independent. Denaturation of tissue by boiling destroyed substantial specific binding activity (80%) while reducing nonspecific interaction only by 40%.

These data all suggest that the interaction of [^{3}H]PCP observed here is with a biologically relevant receptor, a site that is regulated by pH, temperature and the integrity of protein and membrane structure.

Other compounds which have been developed as dissociative anesthetics or analgesics interact with the PCP receptor. For example, etoxadrol has been shown in humans to produce analgesia, surgical anesthesia and amnesia, as well as psychotomimetic effects [35]. Similarly, dexoxadrol has been reported to have both analgesic and psychotomimetic effects in humans [36]. In behavioral tests, ketamine [37, 38], dextrorphan [39] and dexoxadrol [37, 38] have been shown to share discriminative stimulus effects with phencyclidine.

Binding to the PCP receptor was stereoselective as reflected in the relative potencies of the isomers of SKF-10,047 and the 300-fold difference in the potency of dexoxadrol and levoxadrol. The stereoselective preference of the receptor for the (−)-isomers of cyclazocine has also been reported by Zukin [25]. Mu and kappa opioid receptors show a more pronounced stereoselective affinity for the (−)-isomer (5000 for mu receptors and 164 for kappa receptors [25]). Thus, the smaller, 4- to 6-fold difference in potency seen here is indicative of a separate, unique receptor.

The neuroleptic, haloperidol, had a high K_i value of 113 μM. It is unlikely, therefore, that its therapeutic effect in treating PCP psychosis [10] is mediated through the PCP receptor. Similarly, recent reports that THIP may have utility in reducing the severity of PCP-induced behaviors [40] are not explained by the action of this compound at the PCP-receptor site.

Naloxone, a competitive antagonist for opiate receptors which is active at the mu and kappa receptors, had a high K_i value of 3.2 μM. Naloxone has been shown to decrease PCP stereotypy, ataxia and hyperactivity in rats [41]; however, high doses were required (8 mg/kg) and mild reductions in locomotion were seen in naloxone-treated controls. Thus, it is unlikely that naloxone exerts a therapeutic effect mediated by the PCP receptor.

The potency of many compounds to competitively displace [^{3}H]PCP at the rat cortex receptor correlates well with their potency to produce behavioral effects in humans, as well as in animal models. Using the characteristic cataleptic behavior produced in pigeons by phencyclidine [33], we have obtained a good correlation between the binding potency to rat cortex membranes and minimum cataleptic dose in pigeons for a number of phenylcyclohexylamines, dioxolanes, benzomorphans [42], benz(f)- and

bridged benz(f)isoquinolines described here. In addition, it has been shown previously that the potencies of the PCP-like effects as determined by the catalepsy test in pigeons and those determined in the discrimination test in pigeons and monkeys show a very high correlation [29], further indicating the pharmacological relevance of this binding site. The high reliability of this binding assay in predicting pharmacological activity of compounds will greatly facilitate analysis of the structure–activity requirements of the phencyclidine receptor.

In summary, high and low affinity receptor sites for phencyclidine have been identified in rat cerebral cortex and pigeon brain. Binding is dependent on the integrity of the membrane proteins as well as physiological variables such as pH and temperature. Compounds which are potent displacers of [^{3}H]PCP are known to produce PCP-like effects *in vivo*, while compounds with known activity at other receptors do not compete effectively for high affinity sites. This evidence suggests that the phencyclidine receptor described here is correlated with the pharmacological effects of phencyclidine.

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