

Interaction of phencyclidine ("angel dust") with a specific receptor in rat brain membranes

(receptor binding/phencyclidine analogs/psychotomimetics/anesthetics/neuropharmacology)

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ABSTRACT [³H]Phencyclidine binds to synaptic membranes from rat brain in a saturable, reversible, and selective fashion, with a dissociation constant K_d of 0.25 μ M and a maximal binding capacity of 2.4 pmol/mg of membrane protein—i.e., 250 pmol/g of brain. The binding activity is concentrated in synaptosomal fractions, is higher in cerebral cortex and corpus striatum than in other parts of the rat brain, and is not detectable in the spinal cord. Only molecules of the phencyclidine series and ketamine are able to bind to the phencyclidine receptor. [³H]Phencyclidine bound to its receptor is not displaced by the classical neurotransmitters or neuromodulators. There is a good correlation between the apparent affinities of a series of phencyclidine analogs for the phencyclidine receptor and the pharmacological activities of these analogs as measured by the rotarod assay.

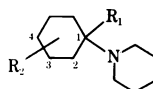
Phencyclidine [*N*-(1-phenylcyclohexyl)piperidine] was introduced in the late 1950s as an intravenous general anesthetic that was nontoxic and nonflammable and produced minimal cardiorespiratory depression (1, 2). These clinical advantages were unfortunately offset by its prolonged duration of action and psychotomimetic effects (3), properties that have contributed to the emergence of phencyclidine as a major drug of abuse in the United States (4, 5). Phencyclidine produces long-lasting psychosis thought to resemble schizophrenia more than does the psychosis produced by any other psychotomimetic (3). Phencyclidine exaggerates psychopathology. Schizophrenics experience severe thought disorders and behavioral problems as long as 1 month after a single injection. Quiet mental patients become catatonic, and reactive ones become overreactive and restless (6, 7). In this paper we investigate the characterization of the phencyclidine receptor in mammalian brain. The data presented here show that a component present in rat brain membranes binds [³H]phencyclidine specifically and with a fairly high affinity. This component may be the physiologically important receptor that mediates the effects of phencyclidine in the central nervous system.

MATERIALS AND METHODS

Materials. Molecules in the phencyclidine series were synthesized as previously described (8). The structures of these drugs are shown in Table 1. [³H]Phencyclidine was prepared on request at New England Nuclear by catalytic reduction at room temperature with tritium gas and purification of the reaction mixture on a silica gel column by high-pressure liquid chromatography. The purity of the product was established by thin-layer chromatography on silica gel in either methanol or 1-butanol/acetic acid/water, 25:4:10 vol/vol/vol. The specific radioactivity of the pure [³H]phencyclidine was 60 Ci/mmol (1 Ci = 3.7×10^{10} becquerels).

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Table 1. Structures of a series of phencyclidine derivatives



Name	R ₁ *	R ₂	Iso-mer†	Abbreviation
<i>N</i> -(1-Phenylcyclohexyl)piperidine or phencyclidine	Ph	H	—	Φ
<i>N</i> -(1-Phenyl-2-methylcyclohexyl)piperidine	Ph	2-Me	<i>Cis</i> <i>Trans</i>	Φ2Mc Φ2Mt
<i>N</i> -(1-Phenyl-3-methylcyclohexyl)piperidine	Ph	3-Me	<i>Cis</i> <i>Trans</i>	Φ3Mc Φ3Mt
<i>N</i> -(1-Phenyl-4-methylcyclohexyl)piperidine	Ph	4-Me	<i>Cis</i> <i>Trans</i>	Φ4Mc Φ4Mt
<i>N</i> -(1-Phenyl-4- <i>tert</i> -butylcyclohexyl)piperidine	Ph	4- <i>t</i> Bu	<i>Cis</i> <i>Trans</i>	Φ4Bc Φ4Bt
<i>N</i> -(1-Phenyl-2-methoxycyclohexyl)piperidine	Ph	2-OMe	<i>Cis</i> <i>Trans</i>	Φ2OMc Φ2OMt
<i>N</i> -(1-Phenyl-4-dimethylcyclohexyl)piperidine	Ph	4-diMe	—	Φ4dM
<i>N</i> -[1-(2-Thienyl)cyclohexyl]-piperidine	Th	H	—	τ
<i>N</i> -[1-(2-Thienyl)-2-methylcyclohexyl]piperidine	Th	2-Me	<i>Cis</i> <i>Trans</i>	τ2Mc τ2Mt
<i>N</i> -[1-(2-Thienyl)-2-methoxycyclohexyl]piperidine	Th	2-OMe	<i>Cis</i> <i>Trans</i>	τ2OMc τ2OMt

* Ph, phenyl; Th, 2-thienyl.

† *Cis* or *trans* isomers are defined by the relative position of R₁ and R₂ in relation to the plane of the cyclohexyl ring.

Tissue Preparation. Adult male Sprague-Dawley rats (200–250 g) were killed by decapitation. The brain was rapidly removed and homogenized in 30 vol of a 50 mM Tris-HCl buffer, pH 7.7, with a Brinkmann Polytron (setting 6) for 20 sec. The homogenate was centrifuged at 49,000 × *g* for 15 min and the resulting pellet was resuspended in 30 vol of the same Tris buffer. The homogenization and centrifugation steps were carried out twice. The final pellet was resuspended in 90 vol of Tris buffer and used without further purification for binding experiments.

Binding Assay. Aliquots (4.5 ml) of the freshly prepared homogenate were incubated at 25°C for 10 min in the presence of [³H]phencyclidine at various concentrations and with or without other unlabeled drugs to analyze for a possible competition with [³H]phencyclidine binding to its receptor. Duplicate 2-ml samples were then filtered under reduced pressure through Whatman glass fiber circles (GF/B). Filters were rinsed promptly twice with 5 ml of the Tris-HCl buffer already described. The entire filtration cycle consumed less than 20 sec for each sample. Filters were then shaken with 10 ml of Di-

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milume (Packard) for 1 hr and radioactivity retained by the filters was determined at a counting efficiency of 42–45% in Packard 3390 or 2450 liquid scintillation spectrometers. Protein was determined by the method of Hartree (9) with bovine serum albumin as a standard.

Rotarod Test. This test, involving ability of mice to remain on a rotating rod, was carried out as described (10).

RESULTS

Specific Binding of [³H]Phencyclidine to Rat Brain Homogenate. Fig. 1 shows the results of binding experiments in which increasing concentrations of [³H]phencyclidine were added to a fixed quantity of rat brain homogenate, either in the presence (nonspecific binding) or in the absence (total binding) of a large excess (100 μ M) of unlabeled phencyclidine. The specific binding, which was obtained by subtracting nonspecific binding from total binding, was saturable (Fig. 1). A Scatchard plot of the data is presented (Fig. 1 *inset*). The dissociation constant of the [³H]phencyclidine–brain receptor complex was 0.25 μ M and the maximal binding capacity was 2.4 pmol/mg of protein. Linearity of the Scatchard plot demonstrated that [³H]phencyclidine bound to a single class of noninteracting binding sites.

[³H]Phencyclidine associated rapidly with its brain receptor. Even at the lowest temperature tested (0°C), specific binding was maximum after 1 min of incubation and remained stable for hours. In the same way, addition of an excess (100 μ M) of unlabeled phencyclidine to the incubation medium produced an almost instantaneous dissociation (less than 1 min) of the [³H]phencyclidine specifically bound to the brain homogenate. It is therefore difficult to give precise values of the kinetic parameters that characterize the binding of [³H]phencyclidine

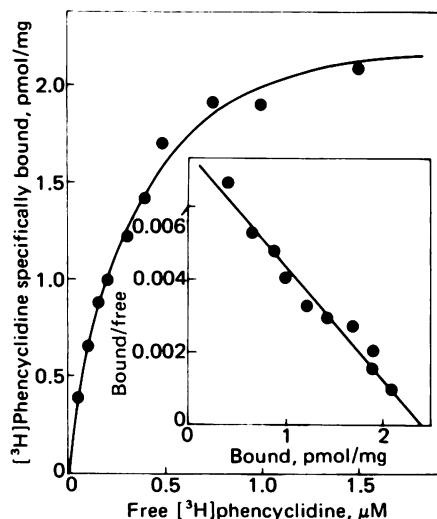


FIG. 1. Binding of [³H]phencyclidine to rat brain homogenate. [³H]Phencyclidine (0.3 Ci/mmol, obtained by dilution of [³H]phencyclidine at 60 Ci/mmol by unlabeled phencyclidine) was incubated at different concentrations with rat brain homogenate (0.9 mg of protein per ml) in 5 ml of a 50 mM Tris-HCl buffer, pH 7.7, at 20°C. After an incubation time of 10 min, bound [³H]phencyclidine was separated from free [³H]phencyclidine by filtration. The specific binding is the difference between [³H]phencyclidine bound to rat brain homogenate in the absence (total binding) and in the presence (nonspecific binding) of 100 μ M unlabeled phencyclidine. As usual (11–15) the nonspecific binding was found to be proportional to the concentration of labeled phencyclidine. The ratio of specific to nonspecific binding is 1 at 0.25 μ M [³H]phencyclidine. (*Main Figure*) Specific [³H]phencyclidine binding as a function of free [³H]phencyclidine concentration. (*Inset*) Scatchard plot of the data.

Table 2. Subcellular distribution of specific [³H]phencyclidine binding in rat brain

Fraction	[³ H]Phencyclidine binding	
	fmol/mg protein	pmol/g brain
Whole homogenate	145 ± 5	14.3
Crude nuclear pellet (P ₁)	110 ± 5	1.9
Crude mitochondrial pellet (P ₂)	155 ± 5	10.0
Crude microsomal pellet (P ₃)	58 ± 3	0.3
Myelin fraction (A)	60 ± 3	0.6
Synaptosomal fraction (B)	180 ± 3	7.5
Mitochondrial fraction (C)	10 ± 2	0.2

Subcellular fractions of rat brain were prepared as described by Whittaker (16). Each fraction was suspended in 5 ml of a 5 mM Tris-HCl buffer, pH 7.4, containing 0.32 M sucrose to a final concentration of about 1 mg of protein per ml and the binding assay was carried out with a [³H]phencyclidine concentration of 15 nM. Nonspecific binding was determined in the presence of 100 μ M unlabeled phencyclidine. Data in the table represent the mean ± SEM of quadruplicate determinations. The experiment was repeated twice.

to its brain receptor. For practical reasons we have chosen to carry out all binding experiments with an incubation time of 10 min at 25°C.

Subcellular Distribution of [³H]Phencyclidine Binding in Rat Brain. Rat brain homogenate was submitted to differential centrifugation as described by Whittaker (16), and the relative [³H]phencyclidine binding activity was determined in various subcellular fractions. The results presented in Table 2 clearly show that the binding was essentially recovered in synaptic fractions P₂ and B. The specific binding found in the crude nuclear pellet P₁ was probably due to a contamination by P₂. The P₂ pellet contained more than 80% of the binding activity originally observed with the whole homogenate. The synaptosomal fraction B contained more than 90% of the binding activity observed in P₂. Hypotonic shock and moderate sonication did not affect the specific binding activity (not shown).

Regional Distribution of [³H]Phencyclidine Binding in Rat Brain. Brains were dissected according to Glowinski and Iversen (17). Tissues were then homogenized and assayed for [³H]phencyclidine binding. Results in Table 3 show that the

Table 3. Regional distribution of specific [³H]phencyclidine binding in the rat central nervous system

Region	Specific [³ H]phencyclidine binding, fmol/mg protein
Cerebral cortex	9.2 ± 0.5
Corpus striatum	7.4 ± 0.6
Thalamus	4.4 ± 0.4
Hippocampus	3.6 ± 0.3
Medulla oblongata–pons	2.0 ± 0.2
Olfactory bulb	1.4 ± 0.3
Hypothalamus	1.3 ± 0.2
Cerebellum	1.0 ± 0.3
Spinal cord	0.1 ± 0.3

Brain regions from eight rats were isolated by the method of Glowinski and Iversen (17). Each region was pooled, weighed, and homogenized in 20 vol of a 5 mM Tris-HCl buffer, pH 7.4, containing 0.32 M sucrose at 0°C. The homogenates were centrifuged at 1000 × g for 10 min. Pellets were discarded and supernatants were used to determine the binding capacity and the protein content of each region. The [³H]phencyclidine concentration in these binding experiments was 1 nM. Values presented in the table are the mean ± SEM of duplicate determinations. The experiment was repeated twice.

Table 4. Comparison between the binding properties to the brain receptor and the activity in the rotarod test for phencyclidines

Phencyclidine	Binding		Rotarod ED ₅₀ , [†] mg/kg
	K _{0.5} ,* μM	n _H [†]	
Φ	0.25	1.0	4.0
Φ2Mc	1.60	1.0	125.0
Φ2Mt	0.12	0.8	7.4
Φ3Mc	0.60	0.9	>150
Φ3Mt	0.60	0.9	22.0
Φ4Mc	0.13	0.9	5.5
Φ4Mt	0.50	0.8	>150
Φ4Bc	30.0	0.7	150.0
Φ4Bt	100.0	0.7	150.0
Φ2OMc	5.0	1.0	>150
Φ2OMt	0.83	1.0	20
Φ4dM	5.0	1.0	93
τ	0.026	1.4	10
τ2Mc	1.0	1.0	>150
τ2Mt	0.040	1.0	3.5
τ2OMc	10.0	1.0	24
τ2OMt	0.40	1.0	3.6
Ketamine	7.0	0.6	—

* K_{0.5} is the concentration of unlabeled ligand that induces 50% dissociation of the labeled phencyclidine.

[†] n_H is the Hill coefficient. Values of K_{0.5} and n_H are computed values obtained as described in the legend of Fig. 2.

[†] ED₅₀ (mean effective dose) is the quantity of product that induces the fall of half of the mice when they are placed on the rotarod system.

specific binding was highest in cerebral cortex and corpus striatum, intermediate in thalamus and hippocampus, and lowest in medulla oblongata-pons, olfactory bulb, hypothala-

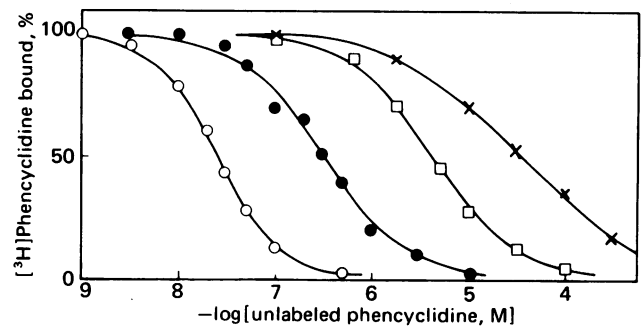


FIG. 2. Competition for specific binding to the phencyclidine receptor between [³H]phencyclidine and unlabeled phencyclidine (●) or phencyclidine analogs τ (○), Φ2Mc (□), and Φ4Bc (×) (see Table 1). [³H]Phencyclidine (1 nM, 60 Ci/mmol) was incubated for 10 min at 25°C with the rat brain homogenate (1 mg of protein per ml) in 5 ml of a 50 mM Tris-HCl buffer, pH 7.7, in the presence of the indicated concentrations of unlabeled phencyclidines. Under these experimental conditions, the ratio of specific to nonspecific binding was 4. Data were fitted to the Hill equation with a Wang 2200 calculator according to Atkins (18). Curves presented in the figure are the theoretical ones that give the best fit with the experimental points.

mus, and cerebellum. No significant binding could be detected in the spinal cord.

Displacement of [³H]Phencyclidine Bound to Rat Brain Homogenate by Unlabeled Molecules of the Phencyclidine Series and by Ketamine. Specific binding of phencyclidines to rat brain membranes can also be demonstrated by competition experiments involving rat brain homogenate, [³H]-phencyclidine, and unlabeled phencyclidines. Fig. 2 shows that phencyclidine derivatives were able to displace [³H]phencyclidine from rat brain homogenate. Results in Fig. 2 were ob-

Table 5. Molecules having no effect on [³H]phencyclidine binding to rat brain

Drug type	Maximal concentration assayed		
	10 μM	100 μM	100 μM
Neurotransmitters and neurotransmitter agonists and antagonists	Pilocarpine	Acetylcholine	Carbamoylcholine
	Atropine	Glycine	Oxotremorine
	Scopolamine	Glutamate	Arecholine
	Strychnine	γ-Aminobutyric acid	Isoproterenol
	Alprenolol	Epinephrine	Propranolol
	Morphine	Norepinephrine	Phentolamine
	Snake neurotoxin I from <i>Naja mossambica mossambica</i>	Serotonin	Nicotine
		Dopamine	Decamethonium
		Histamine	<i>d</i> -Tubocurarine
	Peptides	[Met]Enkephalin	Substance P
[Leu]Enkephalin		Neurotensin	[D-Ala ² ,Met]-Enkephalin
Gastrin		Somatostatin	
Apamin from bee venom			
Amino acids	Proline		
	Leucine		
	Methionine		
	Serine		
	Histidine		
Miscellaneous	Librium		
	Valium		
	Tetrahydrocannabinol		

tained by incubating rat brain homogenate with an [^3H]-phencyclidine concentration (1 nM) much lower than the dissociation constant of the [^3H]phencyclidine-brain receptor complex (0.25 μM , see Fig. 1). Under these experimental conditions, the $K_{0.5}$ values—i.e., the doses of unlabeled drug that are able to induce 50% displacement of the labeled phencyclidine from its association to its brain receptor—represent the dissociation constants of complexes formed between rat brain homogenate and each one of the unlabeled phencyclidine derivatives. $K_{0.5}$ values for phencyclidines varied from 26 nM to 100 μM (Table 4); corresponding Hill coefficients n_H were between 0.7 and 1.4, most derivatives having a Hill coefficient near 1. The $K_{0.5}$ and n_H values obtained for phencyclidine itself by this competition study were identical with those determined by direct binding of [^3H]phencyclidine (Fig. 1). This result shows that tritiated phencyclidine is identical to unlabeled phencyclidine and demonstrates the validity of the competition experiment to measure dissociation constants and Hill coefficients for each one of the unlabeled phencyclidine derivatives.

Specificity of the Interaction of Phencyclidines with Their Receptor in Rat Brain. Table 5 gives a list of various drugs that were unable to displace [^3H]phencyclidine from its brain receptor. This list includes a number of molecules that are currently postulated to be neurotransmitters in the central nervous system. The only drug that possessed some displacing activity was ketamine (Table 4), a drug chemically and pharmacologically similar to phencyclidine (19).

Correlation between the Brain Receptor Affinity and the Pharmacological Activity of Phencyclidines. The rotarod test has been found to give a good estimate of the pharmacological activity of molecules in the phencyclidine series (10, 20). This test is a measure of the disturbance induced by phencyclidines in the forced motor activity of mice. The mean effective dose, ED_{50} , is the dose that induces the fall of half of the animals when they are placed on the rotarod system. The results are presented in Table 4 together with the values of $K_{0.5}$ and n_H

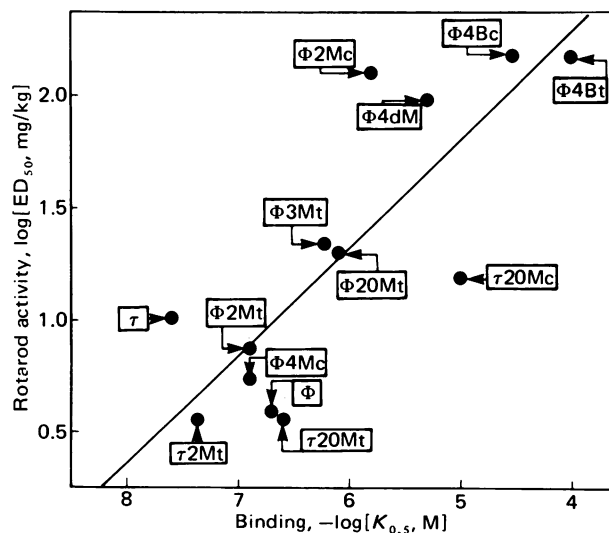


FIG. 3. Correlation between inhibition of [^3H]phencyclidine binding and activity in the rotarod test for phencyclidine and its derivatives. The equation of the straight line in the figure ($y = -0.483x + 4.225$) was obtained by the least-squares method. The correlation coefficient and the statistical significance are $r = 0.843$ and $P < 0.001$, respectively. The statistical results were obtained with a Wang 2200 calculator (program PS.01-2200.01A-00FI-1-0). Phencyclidine derivatives are designated by their abbreviations as given in Table 1.

determined for the association of each phencyclidine derivative with its brain receptor. There exists a clear correlation between pharmacological (ED_{50}) and biochemical ($K_{0.5}$) parameters. This correlation is illustrated in Fig. 3. Phencyclidines that possess the highest affinity for the brain receptor also exhibit the best pharmacological activity. The correlation is highly significant ($r = 0.843$, $P < 0.001$).

Influence of Temperature, pH, and Ions on the Binding of [^3H]Phencyclidine to Its Brain Receptor. The specific binding of [^3H]phencyclidine (1 nM) to rat brain membranes varied less than 10% between 10°C and 37°C and decreased to 55% of maximal value at 0°C. There was a broad pH optimum between pH 6 and 8. The binding decreased to 73% of the maximum at pH 5 and to 64% at pH 9. Ca^{2+} , Na^+ , or K^+ at 10 mM were without effect on the specific [^3H]phencyclidine binding. A 100 mM concentration of these cations decreased the binding to 75% of the maximal value.

DISCUSSION

This study indicates that there exists a specific receptor for phencyclidines in rat brain. The binding is saturable and reversible. The dissociation constant for the complex formed by phencyclidine itself (Φ in Table 4) is 0.25 μM . Modification of the parent phencyclidine molecule can produce derivatives with very different affinities for the brain receptor. Replacement of the phenyl ring in phencyclidine by a 2-thienyl ring gives a derivative, τ , the affinity of which is about one order of magnitude higher than that of phencyclidine itself. On the other hand, introduction of a *tert*-butyl group in position 4 on the cyclohexyl ring ($\Phi 4\text{Bt}$) decreases the affinity for the brain receptor by a factor of 400 (Table 4).

The maximal binding capacity of [^3H]phencyclidine to rat brain is 2.4 pmol/mg of membrane protein—i.e., 250 pmol/g of brain. This stoichiometry of the phencyclidine receptor is high as compared to that of other receptors that have been characterized in rat brain. For example, stoichiometries for the γ -aminobutyric acid (11), benzodiazepine (12), serotonin (13), opiate (14), and muscarinic cholinergic (15) receptors are 10, 18, 20, 30, and 65 pmol/g of brain, respectively.

In a previous study (8), we have shown that molecules of the phencyclidine series are also able to recognize the muscarinic and opiate receptors in rat brain. Therefore, it may appear surprising at first sight that muscarinic as well as opiate agonists and antagonists are unable to displace [^3H]phencyclidine bound to its brain receptor (Table 5). This apparent contradiction is easily resolved after a quantitative analysis of the data. Phencyclidine itself binds to its own brain receptor with an affinity ($K_{0.5} = 0.25 \mu\text{M}$) that is two orders of magnitude higher than its affinity for the muscarinic and opiate receptors [30 μM and 26 μM , respectively (8)]. Moreover, the quantity of the phencyclidine receptor is 3.8 and 8.3 times higher than that of muscarinic and opiate receptors, respectively. Consequently, under our present experimental conditions ([^3H]phencyclidine concentration = 1 nM) it can be calculated that only 0.22% and 0.15% of the bound [^3H]phencyclidine is actually bound to the muscarinic and opiate receptors, respectively, and 99.63% of [^3H]phencyclidine is bound to the specific phencyclidine receptor. It is not surprising that under these conditions muscarinic and opiate drugs do not displace [^3H]phencyclidine binding if they are unable to recognize at low concentration the specific phencyclidine receptor. A similar situation has previously been found for benzodiazepines. These molecules are able to bind to their own receptor in rat brain (12) but also to the glycine receptor (21). Unlabeled benzodiazepines are able to displace labeled benzodiazepines from their complex with the specific receptor (12), but they also displace [^3H]strychnine, a

potent antagonist of the glycine receptor (21). However, neither strychnine nor glycine is able to displace the tritiated benzodiazepine bound to its own specific receptor (12).

The good correlation that exists between the apparent affinities of phencyclidines for the specific phencyclidine receptor described here and the results of the rotarod assay (Fig. 3) strongly suggests that this receptor plays an important role in the central effects of this psychotomimetic molecule. The affinities of phencyclidine and of most of its structural derivatives for receptors in the central nervous system are in the order: specific phencyclidine receptor > muscarinic receptor > opiate receptor. The correlation presented in Fig. 3 is no longer observed when $K_{0.5}$ values for the specific phencyclidine receptor are replaced by $K_{0.5}$ values of the same phencyclidine derivatives for the muscarinic or the opiate receptor found previously (8).

One of the possible interests of the binding assay described above is that it could probably be used as a means of identification and quantification of the drug in clinical overdose cases. The lowest detectable concentration of phencyclidine in human biological materials (e.g., blood or urine) is of the order of 0.3 $\mu\text{g/ml}$ —i.e., about 1 μM —using gas/liquid chromatography with flame ionization detection (22). Competition experiments described in Fig. 2 show that the [^3H]phencyclidine binding assay should permit easy detection of phencyclidine concentrations between 0.1 and 1 μM . Such concentrations would displace between 25 and 80%, respectively, of [^3H]phencyclidine bound to its specific brain receptor. The [^3H]phencyclidine binding assay would have the advantage of being rapid and simple.

The identification of an opiate receptor in rat brain by using tritiated morphine and naloxone has been an important step in the discovery of the existence and of the mode of action of endorphins and enkephalins (23). Similarly, because it does not appear presently that the specific phencyclidine receptor is the receptor of an already well-known neurotransmitter or neuromodulator, it appears worthwhile to start looking for an endogenous molecule in the mammalian brain that has a high affinity for the specific phencyclidine receptor.

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