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BIOCHEMICAL PROPERTIES OF THE BRAIN PHENCYCLIDINE RECEPTOR

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This paper gives a detailed account of techniques which can be used to measure [^{3}H]phencyclidine binding to its receptor. The main properties of the binding component are the following: (i) It is rapidly heat-inactivated at temperatures over 50°C. (ii) It is destroyed by proteases like trypsin, pronase or papain suggesting that it is of a protein nature. The receptor structure is resistant to chymotrypsin. (iii) A good correlation was found between the pharmacological activity of 30 different analogs as measured by the rotarod assay and the affinity of these different molecules for the phencyclidine receptor. (iv) Monovalent and divalent cations antagonize [^{3}H]phencyclidine binding to its receptor. The dissociation constant is 15 mM, the same for Na^+, Li^+, K^+, cholinium or Tris. Na^+ (and other monovalent cations) and phencyclidines bind to distinct sites. The saturation of the Na^+ site by Na^+ modulates the affinity of phencyclidine for its receptor. Divalent cations antagonize [^{3}H]phencyclidine binding in the absence of Na^+. This antagonism is of the non-competitive type. (v) [^{3}H]phencyclidine binding is also antagonized by histrionicotoxin and by local anaesthetics.

Binding techniques Central receptor Ionic strength effect Neuropharmacology

1. Introduction

Phencyclidine, commonly known as PCP, is currently a major drug of abuse in the United States. In recent years, it has received widespread attention because of the violent, homicidal and suicidal behavior of users. Moreover, phencyclidine is one of the most fascinating psychotropic drugs as the psychosis it elicits may provide the best available drug model of schizophrenia (Domino, 1981).

Phencyclidine has been shown to bind to both muscarinic and μ-opiate receptors (Vincent et al., 1978; Fosset et al., 1979, Aronstam et al., 1980) and to block the ionic channel coupled to the nicotinic receptor (Albuquerque et al., 1980). However, probably the most powerful approach for characterizing PCP action stems from recent reports that have identified phencyclidine receptor binding sites in the brain by using tritiated phencyclidine (Vincent et al., 1979; Vincent et al., 1980; Zukin and Zukin, 1979; Quirion et al., 1981). The identification of specific PCP receptors could be of great help in the understanding of how this drug acts and in the search for possible endogeneous phencyclidine-like substances analogous to enkephalins.

The present report extends the earlier characterization of the phencyclidine receptor (Vincent et al., 1979).

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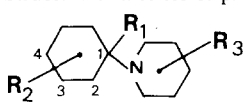
2. Materials and methods

2.1. Materials

Molecules in the phencyclidine series were synthesized as described previously (Vincent et al., 1978). The structure of these drugs is shown in table 1. [^{3}H]Phencyclidine with a radioactivity of 48 or 60 Ci/mmol was obtained from New England Nuclear. The purity of this compound was checked by high-pressure liquid chromatography using a μBondapak C18 column (Waters) with a methanol-water solvent (80/20) containing 5 mM ammonium acetate as mobile phase. Prilocaine and dimethisoquine were obtain from Laboratoire Roger Belon, dibucaine from K and K Laborato-

TABLE 1

Structure of a series of phencyclidine derivatives [a].



Name	R_1	R_2	R_3	Isomer [c]	Abbreviation
N-(1-Phenylcyclohexyl)piperidine or phencyclidine	Ph [b]	H	H	–	Φ
N-(1-[3-Nitrophenyl]cyclohexyl) piperidine	mPhNO_2	H	H	–	mNO_2Φ
N-(1-[4-Nitrophenyl]cyclohexyl) piperidine	pPhNO_2	H	H	–	pNO_2Φ
N-(1-[4-Methoxyphenyl]cyclohexyl) piperidine	pPhOMe	H	H	–	pOMΦ
N-(1-[3-Methoxyphenyl]cyclohexyl) piperidine	mPhOMe	H	H	–	mOMΦ
N-(1-[2-methoxyphenyl]cyclohexyl piperidine	oPhOMe	H	H	–	oOMΦ
N-(1-[3,4-Dimethoxyphenyl] cyclohexyl)piperidine	pmPhdiOMe	H	H	–	mpdOMΦ
N-(1-Phenylcyclohexyl)4-hydroxy-piperidine	Ph	H	4OH	–	Φ4′OH
N-(1-Phenylcyclohexyl)4-methyl-piperidine	Ph	H	4Me	–	Φ4′M
N-(1-Phenylcyclohexyl)3-methyl-piperidine	Ph	H	3Me	–	Φ3′M
N-(1-Phenylcyclohexyl)3-4-des-hydropiperidine	Ph	H	3.4desH	–	Φ3′4′desH
N-(1-[4-Hydroxyphenyl]cyclohexyl piperidine	pPhOH	H	H	–	pOHΦ
N-(1-[3-Hydroxyphenyl]cyclohexyl) piperidine	mPhOH	H	H	–	mOHΦ
N-(1-[2-Hydroxyphenyl]cyclohexyl) piperidine	oPhOH	H	H	–	oOHΦ
N-(1-Phenyl-3-dimethyl-5-methyl) piperidine	Ph	3diMe-5Me	H	cis trans	Φ3dM5Mc Φ3dM5Mt
N-(1-Phenyl-3-methoxycyclohexyl) piperidine	Ph	3OMe	H	cis trans	Φ3OMc Φ3OMt
N-(1-Phenyl-4-methoxycyclohexyl) piperidine	Ph	4OMe	H	cis trans	Φ4OMc Φ4OMt

[a] Other phencyclidine derivatives used in this work have been described elsewhere (Vincent et al., 1979). [b] Ph: Phenyl. [c] Cis or trans isomers are defined by the relative position of R_1 and R_2 in relation to the plane of the cyclohexyl ring.

ries, lidocaine from Astra, procaine, tetracaine, trypsin, chymotrypsin and papain from Sigma, and pronase from Boehringer. Phospholipase A_2 was prepared from bee venom as described by Shipolini et al. (1971). The basic pancreatic trypsin inhibitor (Kunitz) and histrionicotoxin were generous gifts from Choay laboratories and Dr. Daly respectively.

2.2. *Tissue preparation*

Adult male Sprague-Dawley rats (180–250 g) were killed by decapitation. The brains minus cerebella were rapidly removed and homogenized at 0°C in 30 vol of a 50 mM Tris-HCl buffer, pH 7.7, with a Brinkmann Polytron (setting 6) for 20 s. The homogenate was centrifuged at $49000 \times g$ 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 a 5 or 50 mM Tris-HCl buffer at pH 7.7 and used without further purification for binding experiments.

2.3. *Binding assays*

2.3.1. *Direct binding experiments*

A fixed quantity of homogenate (4.5 mg of membrane protein in a total vol of 5 ml of a 5 or 50 mM Tris-HCl buffer at pH 7.7) was incubated in the presence of increasing concentrations of [^{3}H]phencyclidine (0.3 Ci/mmol, obtained by dilution of labeled phencyclidine with unlabeled phencyclidine). The total concentration of [^{3}H]-phencyclidine actually available in solution was determined by measuring the radioactivity of a 100 μl aliquot of incubation medium. After completion of the association reaction (10 min at 20°C), duplicate aliquots (2 ml) were filtered under reduced pressure on GF/B glass fiber filters (Whatman) that retain the homogenate. The filters were rinsed promptly twice with 5 ml of incubation buffer. The entire filtration cycle took less than 20 s and the total time of contact between the washing buffer and the filter was less than 4 s. Bound [^{3}H]phencyclidine was then measured by counting the radioactivity trapped by the filter. This was done by liquid scintillation spectrometry (Packard Tri-Carb B 2450) in 8 ml of biofluor (NEN) at a counting efficiency of 45–50%. To measure the quantity of [^{3}H]phencyclidine specifically bound to the homogenate, 4 different incubations were run in parallel at each concentration of labeled phencyclidine.

Exp. A: the homogenate was incubated only with [^{3}H]phencyclidine. The bound radioactivity (dpm_A) is a measure of the total quantity of [^{3}H]phencyclidine bound to the homogenate and to the filter.

Exp. B: same as in A except that the incubation medium also contained a large excess (10^{-4} M) of unlabeled phencyclidine. The radioactivity measured (dpm_B) gives the quantity of [^{3}H]phencyclidine bound to the homogenate and to the filter and which could not be displaced by a large excess of unlabeled phencyclidine.

Exp. C: same as in A except that the homogenate was replaced by an equal volume of the buffer used to suspend the homogenate. The bound radioactivity (dpm_C) represents the total quantity of [^{3}H]phencyclidine bound to the filter alone.

Exp. D: same as in B except that homogenate was replaced by buffer. The radioactivity measured (dpm_D) gives the quantity of labeled phencyclidine bound to the filter alone which could not be displaced by 10^{-4} M unlabeled phencyclidine. The quantity of [^{3}H]phencyclidine specifically bound to the homogenate alone could then be calculated easily from the expression:

$$\Delta = (dpm_A - dpm_B) - (dpm_C - dpm_D)$$

2.3.2. *Competition experiments*

In these experiments, both the quantity of homogenate (5 mg of membrane protein in 5 ml of the Tris-HCl buffer) and the concentration of labeled phencyclidine (1 nM, 60 or 48 Ci/mmol) remained constant. Increasing concentrations of unlabeled phencyclidine or its derivatives were added to the incubation medium and the displacement of bound [^{3}H]phencyclidine by unlabeled phencyclidines was followed by filtration. The binding to the filter alone was estimated in a parallel experiment in which the homogenate was replaced in the incubation medium by an equal vol of the Tris-HCl buffer; here again the specific

displacement of [^{3}H]phencyclidine bound to the homogenate by unlabeled phencyclidine could be calculated from the expression

$$\Delta = (dpm_A - dpm_B) - (dpm_C - dpm_D)$$

where dpm_A is the radioactivity bound when homogenate was present and dpm_C is the radioactivity bound when homogenate was replaced by the buffer. Values of dpm_B and dpm_D are values obtained for dpm_A and dpm_C respectively in the presence of 10^{-4} M unlabeled phencyclidine.

2.3.3. Relationship between membrane concentration and specific binding

Experiments A, B, C and D were run in parallel with a fixed concentration of [^{3}H]phencyclidine (1 nM, 60 or 48 Ci/mmol) and various concentrations of homogenate. Specific binding was estimated as in *2.3.1.*

2.4. Rotarod test

This test, involving the ability of mice to remain on a rotating rod, was carried out as described by Mousseron et al. (1968).

3. Results

3.1. The specific binding of [^{3}H]phencyclidine to receptor sites in brain membranes

We have recently demonstrated that [^{3}H]phencyclidine binds selectively and reversibly to synaptic membranes from rat brain (Vincent et al., 1979, 1980). Similar results have been obtained by others (Zukin and Zukin, 1979; Quirion et al., 1981). In the course of these studies, we have observed that [^{3}H]phencyclidine had a tendency to adsorb to the GF/B glass fiber filters used to separate the bound from the free labeled phencyclidine. Moreover, unlabeled phencyclidine and its derivatives were partly able to displace [^{3}H]phencyclidine bound to the filter. Therefore care should be exerted in binding experiments with the tritiated ligand. Fig. 1 gives a detailed description of the experimental techniques used to measure the specific binding of [^{3}H]phencyclidine to its brain receptor. It can be seen that in direct binding experiments (upper inset, fig. 1, left) the binding to the filters that is displaceable by unlabeled phencyclidine is always much lower than the specific binding to rat brain homogenate. At 1.5 μM [^{3}H]phencyclidine, i.e. at a concentration of labeled phencyclidine 6 times higher than K_D, the displaceable binding to the filter ($dpm_C - dpm_D$) represents about 30% of the specific binding to the homogenate

$$(\Delta = (dpm_A - dpm_B) - (dpm_C - dpm_D)).$$

The main difficulty is the high value of the non-specific binding to the homogenate. At 1.5 μM, the non-specific binding to the homogenate ($dpm_B - dpm_D = 7500$ dpm) represents 75% of the total binding to the homogenate ($dpm_A - dpm_C = 10000$ dpm). The specific binding of [^{3}H]phencyclidine to the homogenate, Δ, is a saturable process characterized by the dissociation constant $K_D = 0.25$ μM and maximal binding capacity $B_{max} = 2.4$ pmol/mg of membrane protein (fig. 1, left). Moreover, it is shown in the lower inset of fig. 1, left, that at a fixed concentration (1 nM) of labeled phencyclidine, the specific binding to the homogenate is proportional to the concentration of homogenate between 0 and 1 mg of membrane protein per ml.

Results obtained in competition experiments show that here again the part of the binding to the filter which was sensitive to unlabeled phencyclidine, although not negligible, was always much smaller than the specific binding to the homogenate (inset of fig. 1, right) and was similar for each of the unlabeled phencyclidine derivatives. The binding to the filter which was displaceable can easily be estimated and subtracted from the total displaceable binding to obtain the specific binding to the homogenate, Δ. As already discussed (6) the $K_{0.5}$ value, i.e. the concentration of unlabeled phencyclidine that is able to induce 50% displacement of the labeled phencyclidine bound to its brain receptor, represents the dissociation constant K_D of the complex formed between the homogenate and the unlabeled phencyclidine.

Results shown in fig. 1 concerning the binding of labeled phencyclidine to filters may vary ($\pm 50\%$) depending on the batch of [^{3}H]phencyclidine ob-

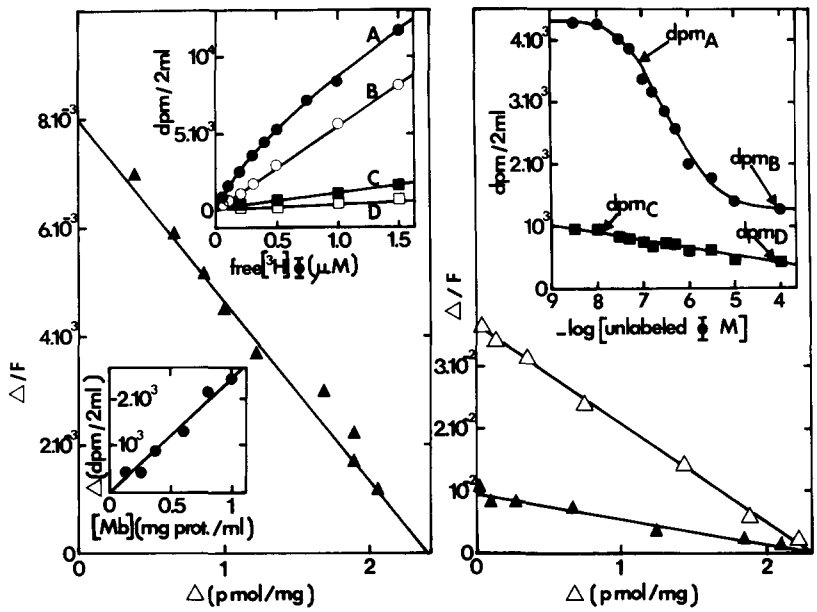


Fig. 1. Binding of [^{3}H]phencyclidine to rat brain homogenate. *Left,* direct binding experiment. *Upper inset,* A (●) [^{3}H]phencyclidine (0.3 Ci/mmol) was incubated at different concentrations with rat brain homogenate (0.9 mg of protein per ml) in 5 ml of a 50 mM Tris-HCl buffer, pH 7.7, at 20°C. After a 10 min incubation, bound [^{3}H]phencyclidine was separated from free [^{3}H]phencyclidine by filtration as described in Materials and methods. B (○), same as in A except that the incubation medium also contained 10^{-4} M phencyclidine. C (■), same as in A except that the homogenate was replaced by an equal volume of 50 mM Tris-HCl buffer. D (□), same as in B except that the homogenate was replaced by buffer. The results of experiments A, B, C and D are expressed as dpm remaining on the filter which received a 2 ml aliquot of incubation medium. *Main figure* (▲), Scatchard plot for the specific binding was calculated from results shown in the upper inset by the expression $\Delta = (dpm_A - dpm_B) - (dpm_C - dpm_D)$ and expressed either as mol/l (ordinate) or as pmol bound per mg of membrane protein (abscissa). The free [^{3}H]phencyclidine concentration, F, was expressed as mol/l. *Lower inset,* [^{3}H]phencyclidine (1 nM, 60 Ci/mmol) was incubated in 50 mM Tris-HCl buffer at pH 7.7, 20°C, with different concentrations of homogenate. The specific binding to the homogenate, Δ, was determined as described above and expressed as dpm bound to the homogenate per 2 ml aliquot of incubation medium. *Right,* competition for binding to the phencyclidine receptor between [^{3}H]phencyclidine and unlabeled phencyclidine. *Inset*: [^{3}H]phencyclidine (1 nM, 60 Ci/mmol) was incubated for 10 min at 25°C with 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 phencyclidine. Bound [^{3}H]phencyclidine was separated from free [^{3}H]phencyclidine by filtration and the radioactivity remaining on the filter was counted (● dpm_A). In a parallel experiment (■ dpm_C) the homogenate was replaced by an equal volume of 50 mM tris-HCl buffer. Values of dpm_B and dpm_D are values obtained for dpm_A and dpm_C respectively in the presence of 10^{-4} M unlabeled phencyclidine. Results are expressed as dpm bound per 2 ml aliquot. *Main figure*: (▲), Scatchard plot for the specific binding of phencyclidine to the homogenate in 50 mM Tris-HCl buffer. The specific binding was calculated from results shown in the inset by the expression $\Delta = (dpm_A - dpm_B) - (dpm_C - dpm_D)$ and expressed either as mol/l (ordinate) or as pmol bound per mg of membrane protein (abscissa). The free phencyclidine concentration, F, was expressed as mol/l. (△), Scatchard plot for the specific binding of phencyclidine to the homogenate in a 5 mM Tris-HCl buffer (detailed results not shown). The data for specific binding to the homogenate are the means of four to six experiments which varied by less than 10%. Φ is an abbreviation for phencyclidine.

tained from New England Nuclear and also on the batch of GF/B glass fiber filters obtained from Whatman. However it should be noted that the specific binding to the homogenate, Δ, was always constant (±10%) under identical experimental conditions. It was also found that increasing from 2 to 4 the number of times the filter was washed with 5 ml of incubation buffer left the value of Δ unchanged. Similarly, increasing the time of contact between the washing buffer and the filter from 4 s to 1 min did not change the cpm values measured in experiments A, B, C or D.

3.2. *The stability of the phencyclidine receptor*

The phencyclidine receptor in rat brain membranes is moderately stable. Storing freshly prepared homogenates at 4°C for 24 h produced a 20–40% decrease of the [^{3}H]phencyclidine binding properties. Conversely, after freezing with liquid nitrogen, membrane preparations could be kept frozen at −70°C during a two-month period without loss or modification of the [^{3}H]phencyclidine binding properties.

Fig. 2 shows the kinetics of heat inactivation of the phencyclidine receptor at different temperatures. The binding activity was lost very rapidly at temperatures over 50°C.

Fig. 3 indicates that the phencyclidine receptor is susceptible to proteases. It was completely degraded by prolonged exposure to trypsin, pronase or papain; it appeared to be resistant to chymotrypsin action. The time-course of proteolytic degradation in the presence of trypsin is indicated in the left part of fig. 3. As expected, preincubation of trypsin with the pancreatic tryp-

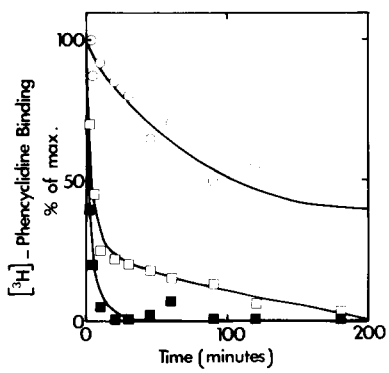


Fig. 2. Thermal denaturation of the phencyclidine receptor. The freshly prepared rat brain homogenate was incubated at a final concentration of 10 mg of protein per ml in 50 mM Tris-HCl buffer, pH 7.7 at 50°C (○), 60°C (□) or 70°C (■). Aliquots were taken at various times and cooled at 0°C for 10 min. Each aliquot was then incubated with [^{3}H]phencyclidine (1 nM, 48 Ci/mmol) in 5 mM Tris-HCl buffer at 25°C for 10 min. The specific binding of [^{3}H]phencyclidine to the homogenate was determined as described in Materials and methods and expressed as percent of maximal [^{3}H]phencyclidine binding. 100% values were obtained from the homogenate that had been incubated during the same times at 0°C instead of the indicated temperatures. Each point is the mean of at least three experiments which varied less than 10%.

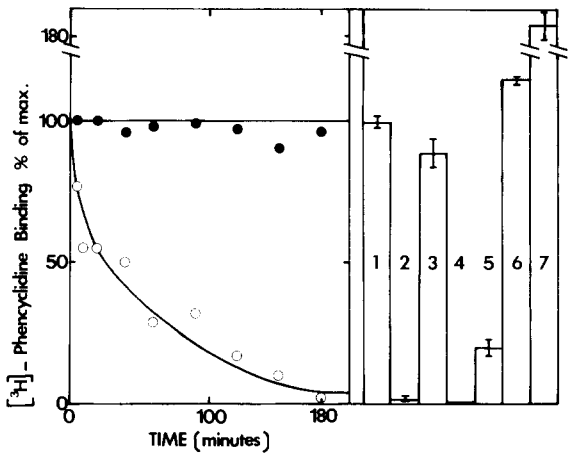


Fig. 3. Effect of enzymes on the phencyclidine receptor. *Left,* kinetics of degradation of the phencyclidine receptor by trypsin. Rat brain homogenate was incubated at a concentration of 10 mg of protein per ml with trypsin (0.01 mg/ml) in 50 mM Tris-HCl buffer, pH 7.7, at 25°C. Aliquots were taken at various times and the enzymatic reaction was stopped by addition of a 2.5 molar excess of basic pancreatic trypsin inhibitor (Kunitz). Trypsin activity was no longer detectable in the incubation medium 1 min after addition of the inhibitor. The specific binding of [^{3}H]phencyclidine (1 nM, 48 Ci/mmol) to each aliquot of trypsin-treated homogenate (○) was determined as described in Materials and methods. In a control experiment (●) the homogenate was incubated during the same time with the trypsin-pancreatic trypsin inhibitor complex (1 : 2.5 mol/mol) instead of trypsin. Results are expressed as percent of initial specific binding. 100% values were determined on aliquots of homogenate that had been incubated for the same time in the absence of trypsin. *Right,* the homogenate (10 mg protein/ml) was incubated in 50 mM Tris-HCl buffer, pH 7.7, at 25°C for 1 h, alone (1) or in the presence of 0.05 mg/ml trypsin (2), 0.05 mg/ml chymotrypsin (3), 0.05 mg/ml pronase (4), 0.01 mg/ml papain (5), 0.01 (6) or 0.1 (7) mg/ml phospholipase A_2. Enzymatic reactions involving trypsin or chymotrypsin were stopped by addition of a 2.5 molar excess of basic pancreatic trypsin inhibitor. The action of pronase, papain or phospholipase A_2 was stopped by 10 fold dilution of the incubation medium with 50 mM Tris-HCl buffer pH 7.7 at 0°C followed by centrifugation at 40000 × *g* for 5 min; both dilution and centrifugation steps were carried out three times. The specific binding of [^{3}H]phencyclidine (1 nM, 48 Ci/mmol) to each enzyme-treated homogenate was determined as described in Materials and methods. Results are expressed as percentages (± S.E. represented by vertical bars) of maximal specific binding. The 100% value was determined on aliquots of homogenate that had been incubated for 1 h under the same experimental conditions but in the absence of enzyme.

sin inhibitor (Kunitz inhibitor) prevents the degradation of the receptor structure. Phospholipase treatment did not destroy the [^{3}H]phencyclidine binding activity. These results strongly suggest that the phencyclidine receptor, as already observed for many other pharmacological receptors, is of a protein nature.

3.3. Correlation between the brain receptor affinity and the pharmacological activity of 30 phencyclidine derivatives

Thirteen derivatives had been tested previously (Vincent et al., 1979). Table 2 gives the binding parameters measured by competition with [^{3}H]-phencyclidine for 20 new phencyclidine derivatives. The pharmacological activity of the different analogues is also given as ED_{50} measured by the rotarod assay. The correlation between the pharmacological (ED_{50}) and biochemical (K_D) parameters is illustrated in fig. 4. The correlation coefficient is $r = 0.883$ ($P < 0.001$). It is probable that the correlation would also be satisfactory with another biological assay, the rat discriminative stimulus test, that has been used by others to measure the pharmacological activity of phencyclidine derivatives in vivo. A good correlation between binding activity to the [^{3}H]PCP binding site and both the rotarod assay and the rat discriminative stimulus test has now been found in two different laboratories (Zukin and Zukin, 1979; Quirion et al., 1981) for a series of phencyclidine derivatives. The results shown in the main panel are based on dissociation constants obtained in 50 mM tris-HCl buffer. The inset of fig. 4 shows that dissociation constants obtained at a lower salt concentration (5 mM Tris-HCl) are also closely correlated to ED_{50} values obtained in the rotarod test.

Some derivatives have an affinity for the [^{3}H]phencyclidine binding site (K_D) that is definitely not well correlated to their activity in the rotarod test (ED_{50}). These derivatives are 11, 19, 24, 27, 29 and 30 i.e., 6 of the 30 derivatives tested. By removing these 6 derivatives from fig. 4 and calculating the correlation between ED_{50} and K_D using only the 24 remaining phencyclidine derivatives i.e., 80% of the total number of molecules tested, one increases the correlation coefficient very significantly to $r = 0.955$ ($P < 0.001$). The correlation can then be considered to be very satisfactory. Among the six derivatives that do not fit the correlation, two have an affinity better than that expected from the ED_{50} value (19 and 27) and the four others are in the opposite situation. For the compounds like 19 that have less biological activity than expected, one could propose as possible

TABLE 2

Comparison between the binding properties to the brain receptor and the activity in the rotarod test for phencyclidine and twenty new phencyclidine derivatives.

Phencyclidine	No.	Binding		Rotarod ED_{50} [c] mg/kg
		$K_{0.5}$ [a] μM	n_H [b]	
Φ	1	0.25	1.0	4
$mNO_2\Phi$	2	11.5	1.2	110
$pNO_2\Phi$	–	25	1.1	>150
pOMΦ	3	1.2	1.0	20
mOMΦ	4	0.09	1.0	4.8
oOMΦ	5	0.5	1.2	26
mpdOMΦ	6	9.5	1.0	115
Φ4′OH	7	2.2	1.2	42
Φ4′M	8	0.4	1.2	20
Φ3′M	9	0.16	1.0	7.1
Φ3′4′desH	10	0.08	1.1	2.1
pOHΦ	11	20	1.0	28
mOHΦ	12	0.03	0.9	2.2
oOHΦ	13	0.75	1.0	18
Φ3dM5Mc	14	6.3	1.1	94
Φ3dM5Mt	–	16	0.9	>150
Φ3OMc	15	1.1	1.2	10
Φ3OMt	16	3	1.1	39
Φ4OMc	17	4	0.9	49
Φ4OMt	18	4.2	1.0	112

[a] $K_{0.5}$ is the concentration of unlabeled ligand that induced 50% dissociation of the labeled phencyclidine. $K_{0.5}$ values were determined in competition experiments similar to those described in the right part of fig. 1 (see also Vincent et al., 1979).

[b] n_H is the Hill coefficient. Values of $K_{0.5}$ and n_H are computed values obtained by fitting the data of competition experiments to the Hill equation with a Wang 2200 calculator according to Atkins (1973).

[c] ED_{50} (mean effective dose) is the quantity of product that induced the fall of half of the mice when they were placed on the rotarod system (Mousseron et al., 1968).

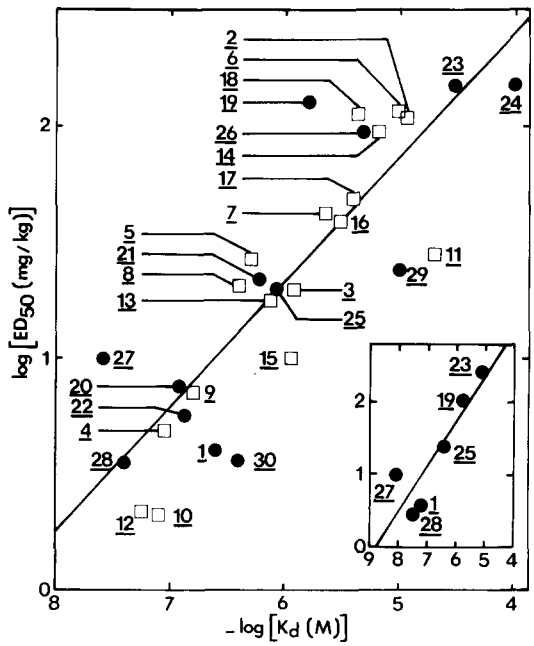


Fig. 4. Correlation between inhibition of [^{3}H]phencyclidine binding and activity in the rotarod test for phencyclidine and its derivatives. $K_{0.5}$ an ED_{50} have been defined in table 2. $K_{0.5}$ values were determined in either 5 mM (*inset*) or 50 mM (*main figure*) Tris-HCl buffer, pH 7.7. Equations of the straight lines in the main figure ($y=-0.536\ x+4.516$) and in the inset ($y=-0.502\ x+4.632$) were obtained by the least-squares method. The correlation coefficients and statistical significances are $r=0.883$ and $P<0.001$ (main figure) and $r=0.904$ and $P<0.001$ (inset) respectively. The statistical calculations were done with a Wang 2200 calculator (program PS.01-2200.01A-OOFI-1-O). Phencyclidine derivatives are designated by their number as given in table 1 (□) or in a previous paper (●; Vincent et al., 1979). 19 = Φ2Mc, 20 = Φ2Mt, 21 = Φ3Mt, 22 = Φ4Mc, 23 = Φ4Bc, 24 = Φ4Bt, 25 = Φ20Mt, 26 = Φ4dM, 27 = τ, 28 = τ2Mt, 29 = τ20Mc, 30 = τ20Mt. The correlation coefficient and statistical significance for the series of the new derivatives tested (□) are $r=0.949$ and $P<0.001$.

explanation that they work as partial agonists of phencyclidine. They would then associate efficiently to the receptor but would only partly trigger subsequent biochemical events that normally lead to the pharmacological effect of phencyclidine.

A more general explanation is that there may be differences in bioavailability or/and of metabolism between the six compounds that do not fit the correlation and all the others for which the correlation is good. Measurements of the pharmacological activity of phencyclidine or phencyclidine derivatives using the rotarod test or the rat discriminative stimulus test are probably too indirect but at present there unfortunately exists no more direct assay.

3.4. Monovalent and divalent cations antagonize [^{3}H]phencyclidine binding to its receptor

A series of monovalent cations including Na^+, Li^+, K^+, cholinium and Tris (used as chloride salts) were tested for their capacity to prevent phencyclidine binding.

All these cations decreased [^{3}H]phencyclidine binding when their concentration was increased. Dose-response curves for the inhibitory effect of all these cations are nearly superimposable (inset of fig. 5). The dissociation constant calculated from the data presented in fig. 5 was 15 mM for all monovalent cations.

The main part of fig. 5 presents Scatchard plots for [^{3}H]phencyclidine binding obtained at 0 and 120 mM Na^+. These data show that increasing the Na^+ concentration did not change the maximal number of phencyclidine binding sites but decreased the affinity of phencyclidine for its receptor. At 0 mM Na^+, K_D for the phencyclidine-receptor complex is 0.063 μM, whereas $K_D = 0.4$ μM at 120 mM Na^+. These results cannot be accounted for by a simple scheme in which both Na^+ and phencyclidine would bind to the same site. In such a case the inhibition would be of the competitive type and saturating concentrations of Na^+ would completely inhibit phencyclidine binding. Since complete inhibition was not observed even at high Na^+ concentrations (see also the lower part of the inset in fig. 5), the simplest interpretation of the data is that Na^+ or other monovalent cations on one hand and phencyclidine on the other bind to distinct sites. Saturation of the Na^+ site (which has a low specificity for monovalent cations) modulates the affinity of phencyclidine for its receptor. Similarly, it has been shown that Na^+ ions modulate the affinity of opiate agonists for the opiate receptor in rat brain (Simantov et al., 1976).

Divalent cations like Ca^{2+}, Mg^{2+}, Mn^{2+} and

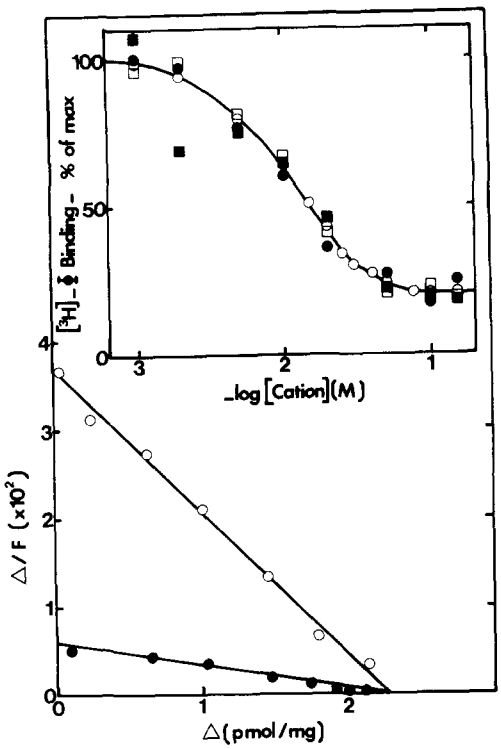


Fig. 5. Effect of monovalent cations on the binding of phencyclidine to its receptor. *Inset,* rat brain homogenate (1 mg protein/ml) was incubated with [^{3}H]phencyclidine (1 nM, 48 Ci/mmol) in 5 mM Tris-HCl buffer, pH 7.7, containing the indicated concentrations of NaCl (○), LiCl (●), KCl (□) or choline chloride (■). After an incubation time of 10 min at 25°C, bound [^{3}H]phencyclidine was separated from free [^{3}H]phencyclidine by filtration and the specific binding to the homogenate was determined as described in Materials and methods. Results are expressed as percent of maximal [^{3}H]phencyclidine binding. The 100% value was determined in the absence of monovalent cation. *Main figure,* competition experiments involving rat brain homogenate (1 mg protein/ml), [^{3}H]phencyclidine (1 nM, 48 Ci/mmol) and different concentrations of unlabeled phencyclidine were carried out in 5 mM Tris-HCl buffer, in the absence (○) or presence (●) of 120 mM NaCl. Results are presented as Scatchard plots. The specific binding to the homogenate, △, and the free phencyclidine concentration, F, were calculated and expressed as described in the legend of fig. 1 (*right*). Data are the mean of three or four experiments which varied by less than 10%. Φ, phencyclidine.

Zn^{2+} also prevented specific [^{3}H]phencyclidine binding when they were used at high enough concentrations (inset of fig. 6) under conditions of low monovalent ion concentrations (5 mM Tris).

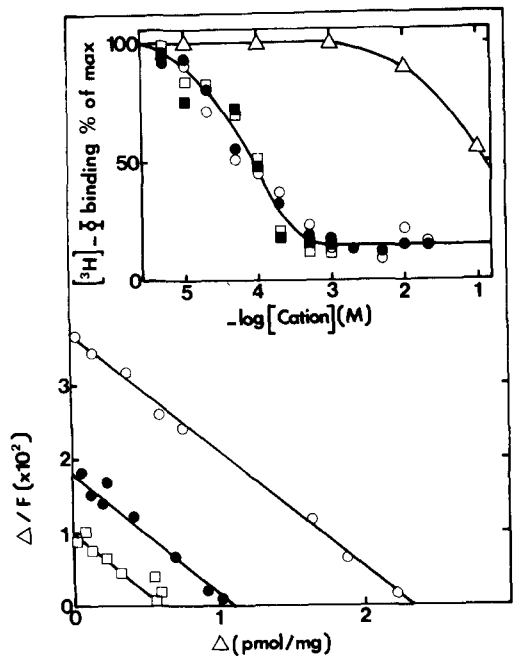


Fig. 6. Effect of divalent cations on the binding of phencyclidine to its receptor. *Inset,* rat brain homogenate (1 mg protein/ml) was incubated with [^{3}H]phencyclidine (1 nM, 48 Ci/mmol) in 5 mM (○, ●, □, ■) or 50 mM (△) Tris-HCl buffer, pH 7.7, containing the indicated concentrations of $CaCl_2$ (○, △), $MgCl_2$ (●), $MnCl_2$ (□) or $ZnCl_2$ (■). After incubation for 10 min at 25°C, bound [^{3}H]phencyclidine was separated from free [^{3}H]phencyclidine by filtration and the specific binding to the homogenate was determined as described in Materials and methods. Results are expressed as percent of maximal [^{3}H]phencyclidine binding. The 100% value was determined in the absence of divalent cation. *Main figure,* competition experiments involving rat brain homogenate (1 mg protein/ml), [^{3}H]phencyclidine (1 nM, 48 Ci/mmol) and different concentrations of unlabeled phencyclidine were carried out in 5 mM Tris-HCl buffer, pH 7.7, containing no (○), 0.1 mM (●) or 0.5 mM (□) $CaCl_2$. Results are presented as Scatchard plots. The specific binding to the homogenate, △, and the free phencyclidine concentration, F, were calculated and expressed as described in the legend of fig. 1 (*right*). Data are the means of at least three experiments which varied less than 10%. Φ, phencyclidine.

Dose-response curves obtained for the different divalent cations are nearly superimposable as for monovalent cations. However the efficiency of divalent cations in antagonizing specific [^{3}H]phencyclidine binding was higher than that of monovalent cations; they were active at much lower concentrations. Scatchard plots for [^{3}H]phencyclidine

binding obtained at different Ca^{2+} concentrations show that the antagonism between divalent cations and phencyclidine is not of the same type as that observed with monovalent cations. Increasing concentrations of Ca^{2+} did not change the dissociation constant of the [^{3}H]phencyclidine-receptor complex but they decreased the maximal number of binding sites. This is typical non-competitive behavior.

At high monovalent ion concentrations when the monovalent cation binding site is nearly saturated (50 mM Tris), the inhibitory effect of divalent cations like Ca^{2+} on [^{3}H]phencyclidine binding disappeared for concentrations between 0 and 1 mM Ca^{2+} (fig. 6, inset (Δ)).

3.5. [^{3}H]Phencyclidine binding was antagonized by histrionicotoxin and by local anaesthetics

Histrionicotoxin, a toxin extracted from the frog Dendrobates Histrionicus, blocks both the ionic channel coupled to the nicotinic receptor (Eldefrawi et al., 1977) and voltage-dependent K^+ channels (Lapa et al., 1975). It has an action similar to that of local anaesthetics. Fig. 7 shows that increasing concentrations of histrionicotoxin prevented [^{3}H]phencyclidine binding to its receptor (inset fig. 7). The dissociation constant measured for the histrionicotoxin-receptor complex from fig. 7 is 5.8 μM. Similarly, local anaesthetics also antagonize phencyclidine binding (fig. 7 and table 3). The most active of all the molecules tested was procaine. The apparent dissociation constants

TABLE 3

Displacement of [^{3}H]phencyclidine bound to the phencyclidine receptor by histrionicotoxin and local anaesthetics.

Name	$K_{0.5}$ (μM) [a]
Histrionicotoxin	5.8
Dimethisoquine	60
Procaine	52
Tetracaine	270
Dibucaine	230
Prilocaine	742
Lidocaine	1350

[a] $K_{0.5}$ values were obtained as described in table 2.

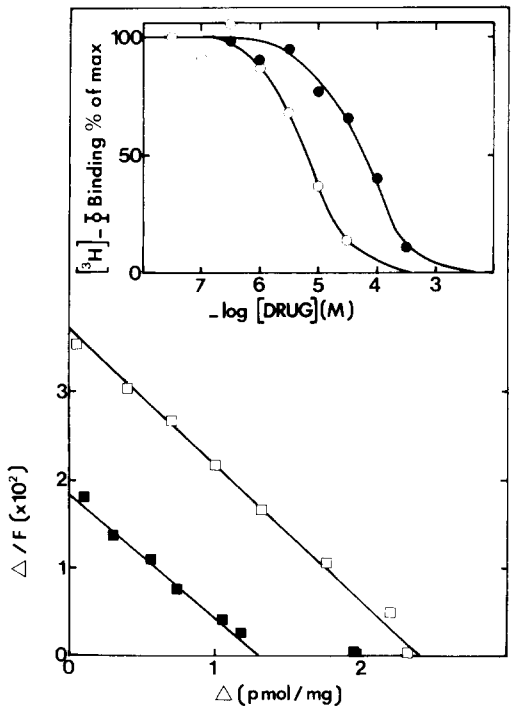


Fig. 7. Effect of histrionicotoxin and procaine on the binding of phencyclidine to its receptor. *Inset,* rat brain homogenate (1 mg protein/ml) was incubated with [^{3}H]phencyclidine (1 nM, 48 Ci/mmol) in a 5 mM Tris-HCl buffer, pH 7.7, in the presence of the indicated concentrations of histrionicotoxin (○) or procaine (●). After incubation for 10 min at 25°C, bound [^{3}H]phencyclidine was separated from free [^{3}H]phencyclidine by filtration and the specific binding to the homogenate was determined as described in Materials and methods. Results are expressed as percent of maximal [^{3}H]phencyclidine binding. The 100% value was determined in the absence of toxin and anaesthetic. *Main figure,* competition experiments with rat brain homogenate (1 mg protein/ml), [^{3}H]phencyclidine (1 nM, 48 Ci/mmol) and different concentrations of unlabeled phencyclidine were carried out in a 5 mM Tris-HCl buffer, pH 7.7, in the absence (□) or presence (■) of 50 μM procaine. Results are presented as Scatchard plots. The specific binding to the homogenate, △, and the free phencyclidine concentration, F, were calculated and expressed as described in the legend of fig. 1 (*right*). Data are the means of three experiments which varied by less than 10%. Φ, phencyclidine.

measured from the antagonism between local anaesthetics and [^{3}H]phencyclidine are given in table 3.

The main part of fig. 7 shows, with procaine as example, that increasing concentrations of local anaesthetics behave as typical non-competitive inhibitors of specific [^{3}H]phencyclidine binding.

4. Discussion

It had already been observed that although phencyclidines bind to muscarinic and μ-opiate receptors (Vincent et al., 1979), their action on these receptors is not compatible with their pharmacological activity (Domino, 1981).

Moreover, a direct demonstration was made that binding of [^{3}H]phencyclidine to its 'true' receptor in the brain is not antagonized by muscarinic or μ- and δ-opiate agonists or antagonists (Vincent et al., 1979).

The brain receptor for [^{3}H]phencyclidine is now better characterized biochemically and a satisfactory correlation has been found between the affinity of 30 different phencyclidine analogs for the phencyclidine receptor and the pharmacological activity of all these molecules. There are considerable differences in binding affinity between phencyclidine analogs of different structures even when these analogs are stereoisomers. The phencyclidine receptor can be heat-inactivated and destroyed by proteolytic enzymes suggesting that it is of a protein nature. There is a receptor site for monovalent cations on the phencyclidine receptor. When this site is saturated by Na^+ under normal physiological conditions, the affinity for phencyclidine is decreased.

The antagonist effect of monovalent cations, local anaesthetics and histrionicotoxin on [^{3}H]-phencyclidine binding may suggest that the molecular target of phencyclidine is the ionic channel coupled to the nicotinic receptor as was already suggested (Eldefrawi et al., 1977; Kloog et al., 1980). It is known that this channel is not very specific for monovalent cations (Watanabe and Narahashi, 1979) and that it is blocked by histrionicotoxin and other local anaesthetics (Lapa et al., 1975, Huang et al., 1978). However at the present time, this interpretation should be considered only as a hypothesis since neither local anaesthetics nor histrionicotoxin are specific for the nicotinic channel (Lapa et al., 1975; Fink, 1980) and since modulation by monovalent cations has also been found for the association of opiates with their receptor (Simantov et al., 1976). It has also been suggested very recently that phencyclidine and the 'σ'-opiates act at the same sites since psychotomimetic benzomorphans classed as 'σ'-opiates' are quite potent displacers in vitro with PCP-like behavioral properties in vivo (Quirion et al., 1981). The possibility still exists that phencyclidine binds to a receptor for which no neurotransmitter or neuromodulator has yet been identified.

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