

# Role of the Aromatic Group in the Inhibition of Phencyclidine Binding and Dopamine Uptake by PCP Analogs

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CHAUDIEU, I., J. VIGNON, M. CHICHEPORTICHE, J.-M. KAMENKA, G. TROUILLER AND R. CHICHEPORTICHE. *Role of the aromatic group in the inhibition of phencyclidine binding and dopamine uptake by PCP analogs.* PHARMACOL BIOCHEM BEHAV 32(3) 699-705, 1989.—Thirty-seven arylcyclohexylamines including phencyclidine (PCP) and derivatives, N[1-(2-thienyl)cyclohexyl]piperidine (TCP) and derivatives and N-[1-(2-benzo(b)thiophenyl)cyclohexyl]piperidine (BTCP) were assessed for their ability to inhibit [<sup>3</sup>H]PCP binding and [<sup>3</sup>H]dopamine ([<sup>3</sup>H]DA) synaptosomal uptake. Their pharmacological property (ataxia) was measured by means of the rotarod test. A very good correlation was observed between the inhibition of [<sup>3</sup>H]PCP binding and the [<sup>3</sup>H]DA uptake only for arylcyclohexylamines bearing an unmodified phenyl group. Conversely the comparison between the inhibition of [<sup>3</sup>H]PCP binding and the activity in the rotarod test shows a good correlation with arylcyclohexylamines having any aromatic group (phenyl, substituted phenyl and thienyl rings). This study outlined a new compound (BTCP) without ataxic effect, which is one of the more potent inhibitors of the [<sup>3</sup>H]DA uptake (IC<sub>50</sub>=8 nM) and which seems very specific since it has a low affinity for [<sup>3</sup>H]PCP receptors (IC<sub>50</sub>=6 μM). These data show that the aromatic group of the compounds leads to molecules that bind differently to the PCP receptor and to the DA uptake complex. They also suggest that the behavioral properties of arylcyclohexylamines revealed by the rotarod test occur essentially as a result of an interaction with the sites labeled with [<sup>3</sup>H]PCP and that TCP is more selective than PCP itself in this recognition.

Phencyclidine (PCP) and derivatives	Thienyl-phencyclidine (TCP) and derivatives
N-[1-(2-benzo(b)thiophenyl)cyclohexyl]piperidine (BTCP)	[ <sup>3</sup> H]PCP binding
[ <sup>3</sup> H]Dopamine synaptosomal uptake	Rotarod

PHENCYCLIDINE (N-(1-phenylcyclohexyl)piperidine, PCP) and analogs exhibit various behavioral dose-dependent effects in man and mammals. Some of them involve the central monoaminergic systems. Stereotyped behavior or ipsilateral rotations induced by PCP in rats with unilateral destruction of the nigro-striatal dopaminergic pathway (5, 12, 29) seem related to the indirect dopamine agonist properties of the drug. Thus, PCP and analogs block the uptake and enhance the release of dopamine (DA) both in vitro and in vivo (2, 4, 13, 15, 22, 23, 33, 34, 38). PCP activates the dopamine D2 receptors of the midbrain (13), or the striatum (37) and decreases the plasma prolactin level (28), a process which is under the DA control (8). However, lesions of the rat striatum suppress PCP-induced stereotyped behaviors but not hyperactivity in rats (14, 29, 30).

Many reports have demonstrated the presence of high affinity binding sites for [<sup>3</sup>H]PCP in CNS membrane preparations (7, 31, 45, 46) or for its more selective analog

[<sup>3</sup>H]TCP (7, 40, 43), tightly linked to the ionic channel of the NMDA sensitive glutamate receptor (1, 19, 27, 35, 36). This binding is well correlated with the pharmacological activities of the PCP-like compounds measured either with the rotarod test (44,45) or a drug discrimination test (31,46). The investigation of [<sup>3</sup>H]PCP or [<sup>3</sup>H]TCP binding in relation to the dopaminergic system has given various results. Some data indicate that the dopaminergic properties of PCP are not exerted through an interaction with the PCP receptors in the striatum (18,21). However, after lesions of the mesolimbic dopaminergic system, the decrease in hyperactivity is well correlated with the decrease in the [<sup>3</sup>H]PCP binding in the nucleus accumbens (14). Moreover, it has been shown that PCP inhibits the binding of [<sup>3</sup>H]cocaine (32) and [<sup>3</sup>H]nomifensine (11), two inhibitors of the dopamine uptake, with affinities similar to its affinity for the PCP receptor in the same experimental conditions.

All these results may suggest that a portion of the sites

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labeled with [<sup>3</sup>H]PCP is related to the dopamine uptake system. In agreement with this conclusion, previous results (41) have shown that for seven PCP derivatives there is a good correlation between their binding properties to [<sup>3</sup>H]PCP sites and their ability to inhibit [<sup>3</sup>H]DA synaptosomal uptake. In this study we have extended this structure-activity relationships investigation to thirty-seven arylcyclohexylamines including PCP and TCP derivatives and a new compound, N-[1-(2-benzo(b)thiophenyl)cyclohexyl]piperidine (BTCP). The two in vitro activities of all the compounds are compared with their in vivo activity (ataxia) measured by the rotarod test and it is shown that BTCP exhibits a very high potency in the inhibition of [<sup>3</sup>H]DA synaptosomal uptake.

#### METHOD

##### Chemicals

[<sup>3</sup>H]PCP (N-1-phenylcyclohexyl)3,4-[<sup>3</sup>H]piperidine, 52 Ci/mmol) was obtained from the "Service des Molécules Marquées" (Commissariat à l'Energie Atomique, Saclay, France). [<sup>3</sup>H]Dopamine (47 Ci/mmol) was obtained from Amersham. Unlabeled PCP and TCP derivatives (Table 1) were synthesized as previously described (6, 16, 17, 24, 25, 40). The new compounds 24, 25, 26 and 27 (m.p. of the HCl forms are respectively 197, 160, 208 and 210°C) were prepared like compounds 4 and 5 (16). Compounds 37 (BTCP, m.p.=80°C for the free base and m.p.=194°C for the HCl form) and 17 (m.p.=57°C and 206°C) were prepared like PCP (16). The monoamine oxidase inhibitor pargyline was from Sigma.

##### [<sup>3</sup>H]DA Uptake

This was performed as described by Vignon and Lazdunski (41) with slight modifications. Freshly prepared synaptosomes (whole rat brain minus the cerebellum) were first incubated at 30°C for 15 min at a final concentration of 0.3 to 0.4 mg protein/ml, in 300 μl of the following incubation medium: NaCl 140 mM, KCl 5 mM, CaCl<sub>2</sub> 2.8 mM, MgSO<sub>4</sub> 1.3 mM, Tris-HCl 10 mM, pH 7.4 plus 0.2 mM pargyline in the absence or presence of various concentrations of the tested derivative. The uptake was started by the addition of 10 nM [<sup>3</sup>H]DA and was stopped 15 min later by filtration of 2×100 μl aliquots on GF/B filters (Whatman). Filters were counted in a scintillation spectrophotometer SL 30 (Inter-technique) in 4 ml of ACS (Amersham).

The lower plateau obtained at high concentrations of PCP (100 μM) was taken as the unspecific uptake. It was the same as that measured at 4°C and represented 5% to 10% of the uptake at 30°C. Results are expressed as IC<sub>50</sub> which are the concentrations of compounds which prevent 50% of the specific uptake. IC<sub>50</sub> measured according to this procedure are similar to those obtained after an uptake measured for two minutes.

##### [<sup>3</sup>H]PCP Binding

[<sup>3</sup>H]PCP binding was performed in competition experiments as already described (44) on homogenates of whole rat brain minus brainstem and cerebellum. Briefly homogenates (0.6–0.8 mg of protein per ml) were incubated in a 50 mM Tris-HCl pH 7.7 buffer in the presence of 2 nM [<sup>3</sup>H]PCP and increasing concentrations of unlabeled derivatives at 25°C for 30 min. Bound [<sup>3</sup>H]PCP was separated from free by rapid filtration on GF/B glass fiber filters pretreated with 0.05% polyethyleneimine to prevent unspecific binding to the fil-

ters. The bound radioactivity was determined by counting the filters in minivials in 3 ml of ACS. The nonspecific binding was determined in the presence of 100 μM unlabeled PCP and the displaceable binding to the filters alone was measured in parallel experiments. Results are expressed as K<sub>0.5</sub> which are the concentrations of unlabeled derivatives which prevent 50% of the maximal specific binding determined in the absence of unlabeled derivative.

##### Rotarod Test

The rotarod test is a device for the measurement of motor function in mice. In this classical procedure, untrained mice are used in a 30-minute (or more) test which measures the ability of mice to stay on a 2.5 cm diameter wooden rod rotating at 15 rpm. Rotarod performance is measured by the percentage of animals which drop off during the test. Three to four groups of 10 animals (male Swiss mice) trained in the test before use were constituted to test only one drug. Drug, dissolved in 0.9% saline solution, was administered SC (0.1 ml/10 g body weight) one minute before the start of the test session. Each animal of each group received the same dose of drug and the ten animals of each group were tested simultaneously during 30 minutes (or more for some drugs) after injection. The maximal injected dose was 150 mg/kg. Three to four doses were used to determine the ED<sub>50</sub> of each drug. Each animal of the group was used only one time.

The ED<sub>50</sub>±SEM (mg/kg) is the dose required to make 50% of the mice fall off the rotarod during a test. The data were computed and analyzed statistically by the rapid method of Litchfield and Wilcoxon (26).

##### Statistical Analysis

The data were initially examined by an analysis of variance using the Pearson's product-moment correlation and subsequently with the 2-tailed Student's *t*-test for paired comparisons. Linear regression lines and the correlation coefficients (*r*) were calculated according to the method of least squares using a computed method. In all statistical evaluations, *p*<0.05 was used as the criterion for the statistical significance.

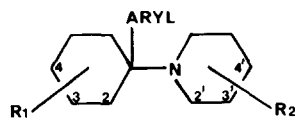
#### RESULTS

Thirty-seven arylcyclohexylamines were tested for: 1) their pharmacological activities in the rotarod test (ED<sub>50</sub>), 2) their abilities to inhibit [<sup>3</sup>H]PCP binding (K<sub>0.5</sub>) and [<sup>3</sup>H]DA uptake (IC<sub>50</sub>). The results are summarized in Table 1. Among these compounds there are PCP (1) and PCP derivatives substituted either on the cyclohexyl group (2 to 17) or on the phenyl and cyclohexyl groups (18 to 27). There are also TCP (28) and TCP derivatives (29 to 36) with a thienyl ring and a new arylcyclohexylamine, BTCP (37) with a benzothio-phenyl ring.

For the 26 compounds (Table 1) which have a measurable ataxic effect (ED<sub>50</sub>≤150 mg/kg) the comparison between their potency to prevent [<sup>3</sup>H]PCP binding and to induce ataxia, results in a good fit to a straight line (*r*=.84, *p*<0.001). However, the slope of this line is low (slope=0.46) and does not agree with that previously reported (44) (slope=0.66).

Figure 1 shows that for 22 compounds, including PCP and PCP derivatives substituted on the cyclohexyl, the piperidine or the phenyl group and TCP derivatives, there is a very good correlation (*r*=.88, *p*<0.001) with a slope of 0.62

TABLE 1  
STRUCTURES AND ACTIVITIES OF THE ARYLCYCLOHEXYLAMINES TESTED



No.	Aryl*	R1†	R2	<sup>3</sup> H]PCP		<sup>3</sup> H]DA	Rotarod ED <sub>50</sub> mg/kg
				K <sub>0.5</sub> (μM)	nH	IC <sub>50</sub> (μM)	
1	Ph	H	H	0.25	1.0	0.50	4 ± 1
2	Ph	4-tButyl-cis	H	100	0.7	130	150
3	Ph	4-tButyl-trans	H	104	0.7	10.6	150
4	Ph	4-Methyl-cis	H	0.5	0.8	0.6	>150
5	Ph	4-Methyl-trans	H	0.13	0.9	0.5	5.5 ± 1.3
6	Ph	3-Methyl-cis	H	0.6	0.9	2.2	22 ± 3
7	Ph	3-Methyl-trans	H	0.6	0.9	1.0	>150
8	Ph	2-Methyl-cis	H	0.12	0.8	0.25	7.4 ± 1.4
9	Ph	2-Methyl-trans	H	1.6	1.0	5.0	125 ± 35
10	Ph	2-Methoxy-trans	H	5.0	1.0	2.9	>150
11	Ph	2-Methoxy-cis	H	0.83	1.0	19.9	20 ± 3
12	Ph	4,4-diMethyl	H	5.0	1.0	10	93 ± 14
13	Ph	2-(CH <sub>2</sub> ) <sub>3</sub> -6	H	4.7	0.63	6	>150
14	Ph	3,3-diMethyl	H	6.3	1.1	25	94 ± 14
15	Ph	5-Methyl-cis	H	10	0.9	79.5	>150
16	Ph	H	4'-Hydroxyl	2.2	1.2	5.3	42 ± 17
17	Ph	H	3'-Methyl	0.16	1.0	0.79	7.14 ± 2.7
18	m-Nitro-Ph	H	H	11.5	1.2	1.26	110
19	p-Hydroxy-Ph	H	H	20	1.0	6.17	28 ± 6
20	m-Hydroxy-Ph	H	H	0.03	0.9	1.36	2.2 ± 0.4
21	o-Hydroxy-Ph	H	H	0.75	1.0	51.9	18 ± 4
22	m-Methoxy-Ph	H	H	0.09	1.0	0.49	4.8 ± 1
23	p,m-diMethoxy-Ph	H	H	9.5	1.0	3.89	115 ± 22
24	p-Methoxy-Ph	4-Methyl-cis	H	4.8	1.06	0.18	>150
25	p-Methoxy-Ph	4-Methyl-trans	H	2.2	0.73	0.79	66 ± 10
26	m-Methoxy-Ph	4-Methyl-cis	H	0.44	0.65	0.32	>150
27	m-Methoxy-Ph	4-Methyl-trans	H	0.058	0.60	1.04	23.8 ± 2
28	2-Th	H	H	0.026	1.4	1.6	2 ± 0.25
29	2-Th	2-Methyl-cis	H	0.04	1.0	7.9	3.5 ± 0.4
30	2-Th	2-Methyl-trans	H	1.0	1.0	20	>150
31	2-Th	4-tButyl-trans	H	100	0.95	6.3	56.5 ± 12.3
32	2-Th	4-tButyl-cis	H	370	0.43	58	—
33	2-Th	4-Methyl-cis	H	1.0	0.89	2.2	150
34	2-Th	4-Methyl-trans	H	0.14	0.97	—	19 ± 6
35	2-Th	4-Hydroxy-trans	H	8.0	1.02	25	>150
36	2-Th	H	3',4' desH	0.017	0.91	4.7	4 ± 0.5
37	2-BzTh	H	H	6.8	1.0	0.008	>150

\*Ph=Phenyl, Th=Thienyl, BzTh=Benzothiophenyl.

†Cis/trans refers to the relative positions of piperidine and the substituent R1.

close to that expected. Excluded from this new correlation are the compounds 2, 3, 19 and 31 which show better potencies in the rotarod test than those predicted by their poor affinities for the PCP receptor. Three of them (2, 3 and 31) are PCP or TCP derivatives bearing a bulky tertibutyl group on the C<sub>4</sub> position of the cyclohexyl ring. These three compounds also exhibit Hill numbers nH significantly lesser than 1 (Table 1) which indicates that these drugs may interact

either with an anticooperativity or with multiple sites labelled with [<sup>3</sup>H]PCP. Among the 22 compounds of the correlation five of them, two PCP derivatives (9 and 27) and three TCP derivatives (33, 34 and 36), have pharmacological properties less effective than those predicted by their good affinities for the PCP binding sites (Fig. 1). Table 1 shows also that many compounds (PCP or TCP derivatives) are without effect in the rotarod test (ED<sub>50</sub>>150 mg/kg) although they

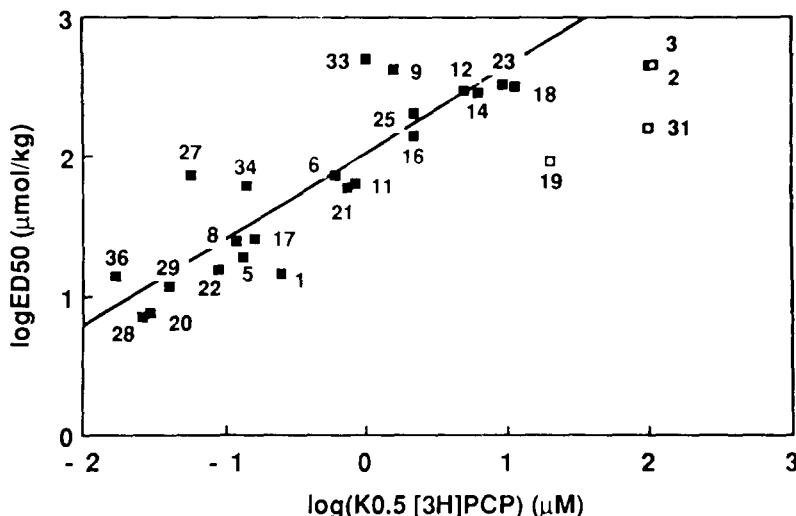


FIG. 1. Correlation between  $K_{0.5}$  values (in  $\mu\text{M}$ ) for the inhibition of  $[^3\text{H}]\text{PCP}$  binding and  $\text{ED}_{50}$  in the rotarod test.  $\text{ED}_{50}$  are expressed in  $\mu\text{mol/kg}$  and were calculated from  $\text{ED}_{50}$  in  $\text{mg/kg}$  (Table 1) of compound in the HCl form. The linear regression was calculated taking into account 22 compounds (■). The equation of the straight line is:  $y = 2.030 + 0.621x$  ( $r = .88$ ,  $p < 0.001$ ). Empty squares (□) refer to compounds which do not follow the correlation.

have good affinities for the PCP receptor (4, 7, 26, 30, Table 1). Some of these compounds could be potential PCP antagonists and others should gain their pharmacological activity by an interaction with another component other than PCP receptors. All of these results support the hypothesis that PCP analogs bind to PCP receptors to produce PCP like ataxic behavior.

The results presented in Table 1 show that all the PCP and TCP derivatives, with affinities for the PCP receptor ranging from  $0.017 \mu\text{M}$  (36) to  $370 \mu\text{M}$  (32), are able to inhibit the  $[^3\text{H}]\text{DA}$  synaptosomal uptake with  $\text{IC}_{50}$  ranging from  $0.18 \mu\text{M}$  (24) to  $130 \mu\text{M}$  (2). At first sight the inhibition of the  $[^3\text{H}]\text{DA}$  uptake by the 37 compounds tested was not related to the ability of the compounds to inhibit the  $[^3\text{H}]\text{PCP}$  binding on nervous membranes. Some compounds, like 36, which have very good affinities for the PCP receptor, have  $\text{IC}_{50}$  in the  $[^3\text{H}]\text{DA}$  uptake inhibition less good than some compounds for which the affinities for the PCP receptor are bad (37 or 24).

However, a careful examination of the data led us to separate the compounds into two groups. The first group (1 to 17) contains molecules with substituted cyclohexyl or piperidine rings and an unmodified phenyl ring. Figure 2A shows that for 14 compounds of this group the inhibition of the  $[^3\text{H}]\text{DA}$  synaptosomal uptake is very well correlated with the inhibition of the  $[^3\text{H}]\text{PCP}$  binding to nervous membranes according to the equation:  $\log(\text{IC}_{50} [^3\text{H}]\text{DA}) = 0.323 + 0.964 \log(K_{0.5} [^3\text{H}]\text{PCP})$  ( $r = .968$ ,  $p < 0.001$ ). The slope of the line is close to 1. The *trans* tert-butyl PCP derivative (3) is out of the correlation; it inhibits  $[^3\text{H}]\text{DA}$  uptake much better than it inhibits  $[^3\text{H}]\text{PCP}$  binding.

For the second group [compounds 18 to 37 (Fig. 2B)] there is no correlation between the two *in vitro* assays ( $r = .208$ ,  $0.50 > p > 0.30$ ). In this group we found arylcyclohexylamines bearing substituted phenyl rings (18 to 27), thienyl (28 to 36) or benzothiophenyl rings (37). Our observations show that: 1) the TCP derivatives were less active in the DA uptake inhibition than the phenyl substituted PCP

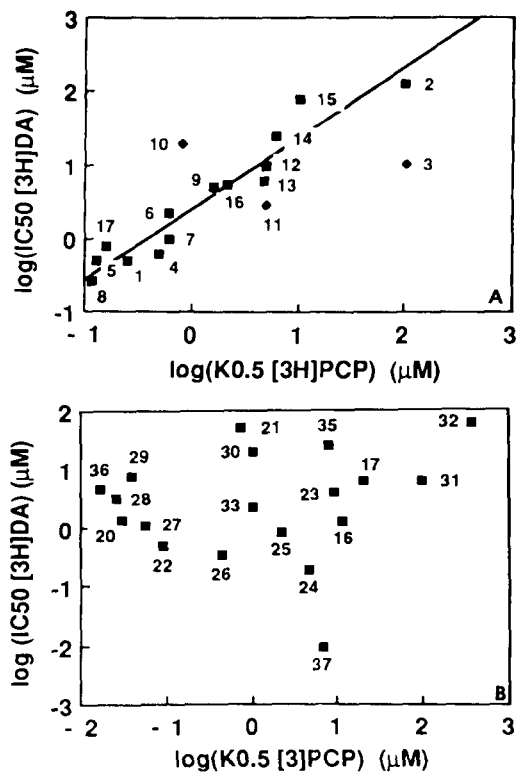


FIG. 2. Correlation between  $\text{IC}_{50}$  values for the inhibition of  $[^3\text{H}]\text{dopamine}$  synaptosomal uptake and  $K_{0.5}$  values for inhibition of  $[^3\text{H}]\text{PCP}$  binding, both expressed in  $\mu\text{M}$ . A shows the correlation obtained with 14 PCP derivatives (■) with an unsubstituted phenyl ring. The equation of the straight line is:  $y = 0.409 + 0.964x$  ( $r = .968$ ). Compounds 3, 10 and 11 (◆) do not follow the correlation. B shows that neither TCP derivatives (28 to 36), nor PCP derivatives with a substituted phenyl ring (18 to 27) or BTCP (37), give a correlation between  $\text{IC}_{50}$  values for the inhibition of  $[^3\text{H}]\text{DA}$  uptake and  $K_{0.5}$  values for the inhibition of  $[^3\text{H}]\text{PCP}$  binding.

derivatives of this group 2; 2) several TCP derivatives (28, 29, 36) which were very potent inhibitors of [<sup>3</sup>H]PCP binding were poor inhibitors of [<sup>3</sup>H]DA uptake, much less potent than PCP derivatives of the first group having a good affinity for the PCP receptor; 3) BTCP (37) is a very potent inhibitor of the [<sup>3</sup>H]DA uptake ( $IC_{50}=8$  nM) while it has a low affinity for the PCP sites ( $K_{0.5}=6$   $\mu$ M) and no ataxic effects ( $ED_{50}>150$  mg/kg, Table 1).

#### DISCUSSION

Our data confirm previous studies which showed a correlation between the potencies of PCP derivatives to inhibit [<sup>3</sup>H]DA uptake and [<sup>3</sup>H]PCP binding (41). However, an important feature arises: this correlation is verified only for PCP analogs with an unmodified phenyl ring. Other aryl groups lead to compounds having various degrees of ability to inhibit [<sup>3</sup>H]DA uptake. The order of effectiveness is:

thienyl < substituted phenyl << benzothiophenyl.

This result demonstrates the significant role of the nature of the aromatic group in the expression of the inhibitory property of the [<sup>3</sup>H]DA synaptosomal uptake. This study evidences a new compound, BTCP, which has a low affinity for the PCP receptor (6  $\mu$ M) and which is likely to inhibit [<sup>3</sup>H]DA uptake with a very high affinity (8 nM). In the [<sup>3</sup>H]DA uptake inhibitory process, BTCP is much more effective than cocaine (32), nomifensine (11) or mazindol (20) and as potent as GBR 12783 (3). Results obtained in our laboratory indicate that [<sup>3</sup>H]BTCP is a powerful molecular probe for the study of the dopamine uptake sites in the CNS (39,42) and that PCP is a competitive inhibitor of this selective tritiated ligand (42) on the dopamine uptake complex.

That PCP and PCP derivatives with an unmodified phenyl group give a good correlation between the inhibition of [<sup>3</sup>H]DA uptake and [<sup>3</sup>H]PCP binding may suggest that dopamine uptake sites and PCP receptors share a common molecular structural feature similarly recognized by the phenyl group of these compounds. In these inhibitory processes the role of cyclohexyl or piperidyl substitutions is to modulate the affinities of the compounds for the two sites.

This correlation is not verified for PCP derivatives bearing an aryl group different from a phenyl ring. Obviously there is no correlation between the inhibition of the DA uptake and the activity in the rotarod-test for the totality of the molecules tested in this study. This indicates that the rotarod-test reflects mainly the interaction of the molecules with the PCP receptor and not with the dopamine uptake site. This conclusion is based on: 1) there is a good correlation between the binding on the PCP receptor and the activity in the rotarod test; 2) the correlation rotarod activity vs.  $K_{0.5}$ [<sup>3</sup>H]PCP binding includes PCP and TCP derivatives which are out of the correlation  $IC_{50}$  [<sup>3</sup>H]DA uptake vs.  $K_{0.5}$ [<sup>3</sup>H]PCP binding; 3) conversely, compounds which are excluded from the correlation  $IC_{50}$  [<sup>3</sup>H]DA uptake vs. [<sup>3</sup>H]PCP binding belong to the correlation rotarod activity vs. [<sup>3</sup>H]PCP binding. Such a conclusion agrees well with those of other groups (21,34) and with the fact that destruction of the dopaminergic striatal neurons is not followed by a significant decrease in the sites measured either with [<sup>3</sup>H]PCP (34) or its more specific analog [<sup>3</sup>H]TCP (18).

Since PCP is able to inhibit striatal [<sup>3</sup>H]DA synaptosomal uptake and binding to striatal membranes of [<sup>3</sup>H]nomifensine (11) or [<sup>3</sup>H]cocaine (32), two ligands of the DA uptake sites, with half inhibitory concentrations close to that found for the inhibition of [<sup>3</sup>H]PCP binding, we should find a decrease in [<sup>3</sup>H]PCP binding to striatal membranes after destruction of DA neurons. This is not observed because previous experiments of [<sup>3</sup>H]PCP binding to striatal membranes (18,34) obtained from 6-OHDA-treated animals were performed in a Na<sup>+</sup>-free medium which permitted the detection of only the high specific binding sites of [<sup>3</sup>H]PCP (PCP receptors) which are sensitive to the ionic strength (44,45), but not the detection of the DA uptake sites, which on the contrary, are Na<sup>+</sup>-dependent (11,32). However, the decrease of [<sup>3</sup>H]PCP binding on the nucleus accumbens after lesions of the mesolimbic DA systems observed by French *et al.* (14) has also been detected in a Na<sup>+</sup>-free medium (5 mM Tris-HCl, 50 mM sucrose, pH 7.4). This observation can be explained either by a colocalization of the PCP receptors and the DA uptake sites on DA neurons of the nucleus accumbens or, if we consider that the DA uptake sites of the mesolimbic and mesocortical area are recognized by PCP with a better affinity than those of the nigrostriatal regions (9). In this last case the decrease of [<sup>3</sup>H]PCP binding reflecting the DA uptake sites would be more evident after lesions of mesolimbic or mesocortical dopaminergic neurons than after lesions of the nigrostriatal pathway.

Our data also show that among 17 PCP derivatives with an unmodified phenyl group the compound 3 (4-tertiobutyl trans cyclohexyl phenyl piperidine) is out of the correlation  $IC_{50}$  [<sup>3</sup>H]DA uptake vs.  $K_{0.5}$  [<sup>3</sup>H]PCP binding (Fig. 2A). This compound is also one of those which do not follow the very good correlation observed between the inhibition of [<sup>3</sup>H]PCP binding and [<sup>3</sup>H]TCP binding (6). This compound, which possesses a better affinity for the sites labeled with [<sup>3</sup>H]TCP than for those labeled with [<sup>3</sup>H]PCP ( $IC_{50}=2.9$   $\mu$ M and 104  $\mu$ M respectively), has also a better affinity for the DA uptake sites than for the [<sup>3</sup>H]PCP sites (Table 1, Fig. 2A). The abnormal behavior of this PCP derivative could be related to its original properties, observed with T lymphocytes, where it inhibits the intracellular Ca<sup>++</sup> mobilization more efficiently than PCP or TCP (10).

In conclusion, our results demonstrate that the two activities of arylcyclohexylamines (inhibition of DA uptake and of PCP binding) are clearly separated. Modifications of the aryl group change the selectivity of the compounds for the two sites: thus, BTCP is specific for the DA uptake site; TCP is specific for PCP receptors; PCP and its derivatives with an unmodified phenyl ring with close affinities for the two sites are not selective. Thus, it is expected that PCP exerts its pharmacological activity via an action on the DA uptake and on the PCP receptor while the pharmacological activity of TCP is mediated mainly via its interaction with the PCP receptor.

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