0026-895X/89/010001-05$02.00/0

MOLECULAR PHARMACOLOGY, 35:1-5

ACCELERATED COMMUNICATION

Effects of Dextromethorphan Site Ligands and Allosteric Modifiers on the Binding of (+)-[^{3}H]3-(-3-Hydroxyphenyl)-*N*-(1-propyl)piperidine

JOSÉ M. MUSACCHIO, MARTINE KLEIN, and JOHN J. PATURZO
Department of Pharmacology, New York University Medical Center New York, New York 10016
Received August 23, 1988; Accepted October 20, 1988

SUMMARY

Equilibrium binding analysis demonstrated that (+)-[^{3}H]3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [(+)-[^{3}H]3-PPP] binds in guinea pig brain homogenates to high and low affinity sites with K_d values of 25 nM and 0.9 μM, respectively. Competition studies with dextromethorphan (DM) site ligands and other drugs against (+)-[^{3}H]3-PPP demonstrated that their K_i values and rank order of potency are identical to those found previously against [^{3}H]DM. Most significant, ropizine produced a concentration-dependent increase in the binding of (+)-[^{3}H]3-PPP, with an inhibitory component at high concentrations, as described previously for [^{3}H]DM. Similarly, phenytoin increased the binding of (+)-[^{3}H]3-PPP in the same fashion as that of [^{3}H]DM. Computer-assisted analysis of equilibrium binding of (+)-[^{3}H]3-PPP in the presence of 10 μM ropizine demonstrated that the binding increase produced is due to a 3-fold increase in the affinity for (+)-[^{3}H]3-PPP. These results, and our previous finding that σ ligands inhibit [^{3}H]DM binding with a rank order of potency similar to that for sites labeled with (+)-[^{3}H]3-PPP or (+)-[^{3}H]SKF10,047 strongly suggest that σ ligands bind to the high affinity DM site. These findings, and the inability of DM and other antitussives to produce psychotomimetic side effects, suggest that the high affinity DM sites can mediate only the nonpsychotomimetic effects of σ ligands. However, further studies are necessary to determine the physiological role and therapeutic potential of the DM high affinity sites.

The initial pharmacological profile of the dopamine analogue (+)-3-PPP suggested that it was an agonist for presynaptic D_2 receptors (1). However, studies on the binding of (+)-[^{3}H]3-PPP indicated that the regional distribution and the rank order of potency of competing drugs were similar to those of (+)-[^{3}H]SKF10,047; therefore, (+)-3-PPP was deemed a σ site marker (2). (+)-[^{3}H]3-PPP binds in rat brain membranes to an apparently single population of binding sites with a K_d of 30 nM, but several competing ligands display pseudo-Hill coefficients of 0.5–0.8, which indicate a multicomponent binding model (2).

We demonstrated recently that the σ ligands (+)-3-PPP, (+)-NANM [or (+)-SKF10,047], and haloperidol bind with high affinity to sites labeled with [^{3}H]DM (3). The rank order of potency of σ ligands, as indicated by their K_i for the DM high affinity sites, is similar to that for sites labeled with (+)-[^{3}H]3-PPP and (+)-[^{3}H]SKF10,047 (2, 4, 5). The (+)-isomers of several benzomorphans have higher affinity for DM sites than the (−)-isomers (3, 6). In addition, *l*-butaclamol competes against [^{3}H]DM binding more effectively than does the *d*-isomer, displaying the same stereospecificity shown for σ sites (3). These findings demonstrated that there are similarities between DM and σ sites and suggested that further exploration of both sites will be necessary to characterize them.

The nonopioid antitussive DM binds to specific high affinity sites in the guinea pig brain (7, 8). The antitussives caramiphen and carbetapentane compete with high affinity with [^{3}H]DM, suggesting that the DM sites mediate their pharmacological effects (6). Even more significant, DM, carbetapentane, and caramiphen protect rats against maximal electroshock seizures and potentiate the anticonvulsant activity of phenytoin (9, 10). In addition, the anticonvulsant drugs phenytoin and ropizine produce an allosteric enhancement of the binding of [^{3}H]DM to the guinea pig brain (6, 11). These findings support the hypothesis that DM sites mediate anticonvulsant activity when occupied by the appropriate ligands. The nature of the DM sites has not been established, but we have shown that they are not related to opioid receptors (7). PCP, ketamine, and dexoxadrol are poor inhibitors of [^{3}H]DM binding, and excitatory amino acids and analogues do not inhibit the binding of [^{3}H]

This work was supported in part by United States Public Health Service Grants DA-02013, MH-29591, and NS-23926. J.M.M. is the recipient of Research Scientist Award MH-17785.

ABBREVIATIONS: (+)-3-PPP, 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine; (+)-NANM, (+)-*N*-allyl-*N*-normetazocine; DM, dextromethorphan; PCP, phencyclidine.

DM (6, 11). These findings indicate that there are no similarities between the PCP/σ sites associated with the *N*-methyl-D-aspartate receptor and the DM sites. In contrast, some of the (+)-isomers of benzomorphans display higher affinities than the corresponding enantiomers for the DM sites (6). Our recent finding that σ ligands inhibit [^{3}H]DM binding with a rank order of potency very similar to that for sites labeled with (+)-[^{3}H]3-PPP or (+)-[^{3}H]SKF10,047 (3) prompted us to explore the possibility that DM site ligands and their allosteric modifiers may affect the binding of (+)-[^{3}H]3-PPP to guinea pig brain.

Methods

The (+)-[^{3}H]3-PPP binding assay and competition experiments were carried out as described previously for the [^{3}H]DM binding assay (12). Briefly, guinea pig cerebellum, midbrain, pons, and medulla oblongata were homogenized in 24 volumes of ice-cold 50 mM sodium phosphate, pH 7.4, using a Brinkmann Polytron tissue disrupter. The binding assay contained 3 nM (+)-[^{3}H]3-PPP (100 Ci/mmol; Du Pont New England Nuclear, Boston, MA), the appropriate concentration of the competing drug, and 50 mM sodium phosphate, pH 7.4. The assay was started by the addition of 100 μl of brain homogenate in a final volume of 1 ml. The samples were incubated at 37° for 20 min, followed by 10 min at 0°, in an ice water bath.

Whatman GF/B glass fiber filters were presoaked in 50 mM sodium phosphate containing 100 mM choline chloride and 0.01% Triton X-100, pH 7.4 (wash buffer). The filters were washed with 5 ml of ice-cold wash buffer and the samples were filtered rapidly under vacuum and rinsed two times with 5 ml of the same buffer, using the multiple cell harvester made by Brandel Biomedical Research and Development (Gaithersburg, MD). All the preceding steps were done in a cold room at 4°. Tritium was determined at a counting efficiency of 52%, and the nonspecific binding was calculated with the LIGAND program (see below). Experiments were carried out with triplicate samples, which varied less than 10%. The number of independent experiments and the number of samples per experiment are indicated in tables and figure legends.

Data were analyzed by the curve-fitting program LIGAND (FORTRAN-77 v. 2.3.10) by Munson and Rodbard (13, 14), which was provided generously by Peter J. Munson. Combined data files from independent experiments were analyzed to determine the best model fit, the K_i and B_{max} values. The equilibrium binding parameters determined for (+)-[^{3}H]3-PPP were used to calculate the K_i values of the drugs tested. Selection of models was based on the root mean square error of each fit, the *F* test, and the randomness of residuals around the fitted curve (14, 15). Protein was determined by the method of Lowry *et al.*, as modified by Peterson (16).

Drugs and reagent sources. The following pharmaceutical companies generously supplied the drugs listed: CIBA Pharmaceutical Co., opipramol; Hoffmann-La Roche Inc., dextromethorphan; Janssen Pharmaceutica, cinnarizine; Searle Research and Development, ropizine (SC-13504); Smith Kline and French Laboratories, caramiphen ethanedisulfonate; and Wallace Laboratories, carbetapentane citrate. (+)-NANM, or (+)-SKF10,047 was provided by the Research Technology Branch of the National Institute on Drug Abuse. Additional drugs and chemicals were purchased from the following companies: Research Biochemicals Inc., (+)-3-PPP; and Sigma, phenytoin (diphenylhydantoin), quinidine. Reagents were purchased from commercial sources and were of the highest purity available.

Results

Equilibrium binding analysis demonstrated that (+)-[^{3}H]3-PPP binds to high and low affinity sites with the equilibrium binding parameters shown in Table 1. The two-site model was significantly better than the one-site, with a $p < 0.003$.

The results of several competition experiments are presented in Table 2, in which it can be observed that DM and (+)-SKF10,047 displace (+)-[^{3}H]3-PPP from two different binding sites. Interestingly, caramiphen, carbetapentane, and the other compounds listed inhibit (+)-[^{3}H]3-PPP binding with a single K_i value for both sites. This is illustrated in Fig. 1; (+)-SKF10,047 has a shallow slope indicative of binding to more than one site with different affinities, whereas that of carbetapentane is steeper. These data are consistent with calculations that showed that all competing drugs with pseudo-Hill coefficient significantly below 1 fit better a two-site model, whereas those with coefficients close to 1 fit a one-site model (data not shown).

Ropizine produced a concentration-dependent increase in the binding of (+)-[^{3}H]3-PPP, which was biphasic with an inhibitory component at higher concentrations, as illustrated in Fig. 2. Interestingly, the increase of (+)-[^{3}H]3-PPP binding produced by ropizine is almost identical to that previously found for [^{3}H]DM (12). Similarly, phenytoin also increased the binding of (+)-[^{3}H]3-PPP in the same fashion as it increased that of [^{3}H]DM (Fig. 2). Computer-assisted analysis of equilibrium binding of (+)-[^{3}H]3-PPP in the presence of 10 μM ropizine demonstrated that the binding increase produced is due to an increase in the affinity for (+)-[^{3}H]3-PPP (Table 1).

Discussion

Equilibrium binding analysis demonstrated that (+)-[^{3}H]3-PPP binds to high and low affinity sites in the guinea pig brain. This is comparable to the binding to rat brain membranes, in which (+)-[^{3}H]3-PPP binds to an apparently single population of sites with a K_d of 30 nM (2), but with several competing ligands that display pseudo-Hill coefficients of 0.5–0.8, indicative of a multicomponent binding model (2, 17).

Competition of DM ligands for sites labeled with (+)-[^{3}H]3-PPP. DM and DM ligands compete for sites labeled with (+)-[^{3}H]3-PPP with K_i values for the high affinity binding similar or identical to those displayed against [^{3}H]DM. In addition, their rank order of potency is identical to that displayed against [^{3}H]DM, and the B_{max} for the high affinity (+)-[^{3}H]3-PPP binding is similar to that of 1.5 ± 10%CV (pmol/mg of protein) found for [^{3}H]DM (12). These findings indicate that [^{3}H]DM and (+)-[^{3}H]3-PPP bind to a common high affinity site, from which they can be displaced by the same set of ligands. In contrast, the DM and (+)-3-PPP low affinity sites are different, as indicated by the disparity of the K_i values of the competing ligands.

The only competing ligand tested that had significantly different K_i values for (+)-[^{3}H]3-PPP and for [^{3}H]DM was quinidine. Work in progress, simultaneously analyzing 14 saturation, dilution, and competition experiments, indicates that quinidine binds with low affinity to the "DM-(+)-3-PPP" common high affinity site and with much higher affinity to the DM low affinity site (results not shown). This explains why quinidine competition is described better with a low affinity one-site model against (+)-[^{3}H]3-PPP and with a high and low affinity two-site model against [^{3}H]DM.

Allosteric enhancement of (+)-[^{3}H]3-PPP binding by ropizine and phenytoin. The allosteric enhancement of the binding of (+)-[^{3}H]3-PPP produced by ropizine and phenytoin illustrates one of the most striking similarities between the binding of [^{3}H]DM and that of (+)-[^{3}H]3-PPP. The effects

TABLE 1

Equilibrium binding analysis of (+)-[^{3}H]3-PPP; effects of ropizine

The binding of (+)-[^{3}H]3-PPP was analyzed under control conditions and in the presence of 10 μM ropizine. Each experiment consisted of 15–18 independent samples analyzed in triplicate. Combined files of three controls and two experiments in the presence of ropizine were analyzed simultaneously, to calculate the equilibrium binding parameters for (+)-[^{3}H]3-PPP, with the LIGAND-PC program, and the statistically best fits are reported. The value of B_{max1} was fixed to that obtained under control conditions; particularly important, the 3-fold increase in affinity produced by ropizine was of identical magnitude when all the parameters were free floating. The nonspecific binding is expressed as the ratio of nonspecifically bound/free ligand (*NB/F*). The percentage coefficient of variation is indicated in parenthesis under each value. $K_{\sigma 1}$ and $K_{\sigma 2}$ are the K_σ values for the high and low affinity sites.

Condition	$K_{\sigma 1}$	$K_{\sigma 2}$	B_{max1}	B_{max2}	Nonspecific binding (NB/F)
	nM	μM	pmol/mg of protein		
Control	25.4 (16)	0.93 (141)	1.22 (17)	2.11 (83)	0.0037
Ropizine, 10 μM	9.5 (8)	1.26 (88)	1.22	2.12 (26)	0.0037

TABLE 2

Effect of DM site ligands and other drugs on the binding of (+)-[^{3}H]3-PPP to guinea pig brain homogenate: comparison with the potencies of the same drugs against [^{3}H]DM binding

Combined files of different experiments with the same drug were analyzed simultaneously. The equilibrium binding parameters for (+)-[^{3}H]3-PPP were input into the LIGAND-PC program to calculate the K_i values of the drugs tested. The statistically best fits are reported; for one-site models, only one K_i value is shown. Each experiment included 16–18 samples in triplicate, and the number of experiments is indicated under *n*. %CV, percentage coefficient of variation.

Drug	(+)-[^{3}H]3-PPP					[^{3}H]DM[a]				
	n	K_{i1}	%CV	K_{i2}	%CV	*n*	K_{i1}	%CV	K_{i2}	%CV
		nM		μM			nM		μM	
(+)-3-PPP	3	25	16	0.93	141	2[b]	27	58	3.0	94
DM	2	37	46	0.78	83	9	57	17	24.0	522
(+)-SKF10,047	2	40	29	0.9	116	2[b]	44	70	3.3	51
Caramiphen	2	9.4	15			2	9.5	43	0.7	194
Carbetapentane	2	11.0	7			2	11.3	23		
Opipramol	2	0.92	12			4	0.41	107	0.16	49
Cinnarizine	2	28.9	8			3	22.0	64	0.40	45
Quinidine	5	20,050	8			3	1,090	48	225	36

[a] The K_i values against [^{3}H]DM are from Klein and Musacchio.[1]
[b] Data from Klein *et al.* (3).

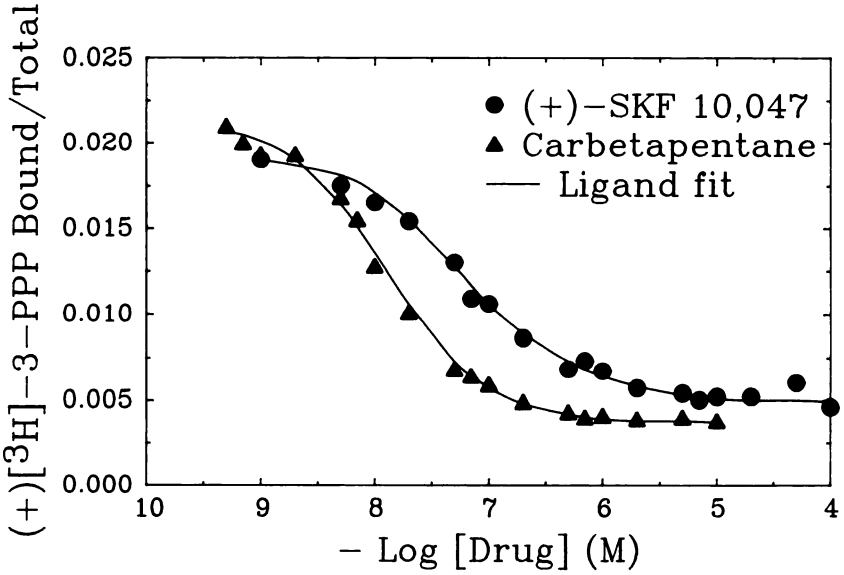

Fig. 1. Effect of (+)-SKF10,047 and carbetapentane on (+)-[^{3}H]3-PPP binding. Guinea pig brain homogenate was incubated with 3 nM (+)-[^{3}H]3-PPP, as described in Methods, in the presence of increasing concentrations of (+)-SKF10,047 or carbetapentane, as indicated on the *abscissa*. The results illustrated are those of one experiment replicated once; the K_i values for both experiments are shown in Table 1. The *filled symbols* correspond to the actual experimental points, which were determined in triplicate. The *continuous lines* are the LIGAND fits. *Ordinate*, ratio of bound over total (+)-[^{3}H]3-PPP.

produced by ropizine on (+)-[^{3}H]3-PPP binding are almost identical to those produced on the binding of [^{3}H]DM, including the inhibitory component produced at concentrations higher than 10 μM ropizine (12). We previously demonstrated that the effects of phenytoin and ropizine on the binding of [^{3}H]DM are mediated by a decrease in its dissociation rate that results in a 3- to 4-fold increased binding affinity (6, 12). The magnitude of this increase is comparable to the 2.7-fold increase in (+)-[^{3}H]3-PPP binding affinity found in this investigation. Because it is quite unlikely that both ropizine and phenytoin will produce identical allosteric effects at different DM and (+)-3-PPP sites, these findings strongly support the idea that DM and σ ligands bind to at least one common site. The similarities of some σ and DM binding sites are described further in a previous study (3) in which we demonstrated that σ ligands bind to DM sites with a rank order of potency similar to that for sites labeled with (+)-[^{3}H]3-PPP (2, 18) and (+)-[^{3}H]SKF10,047 (4). Parenthetically, the allosteric enhancement of [^{3}H]DM binding

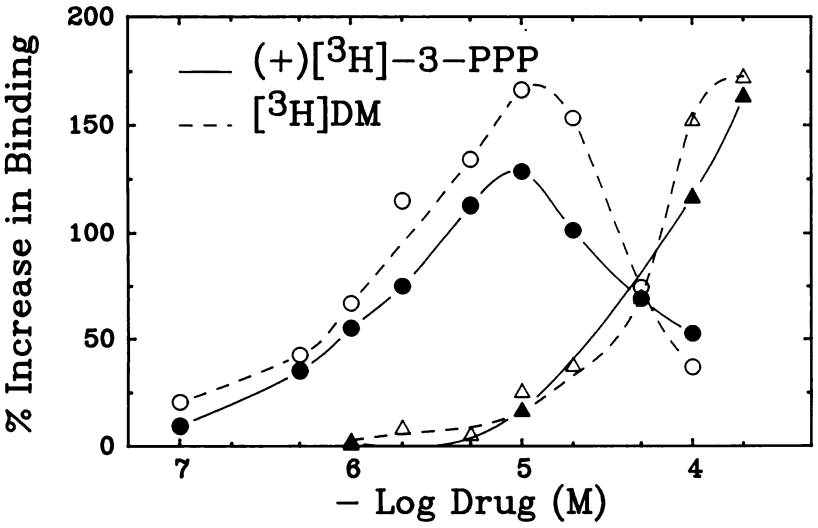

Fig. 2. Effects of ropizine and phenytoin on the specific binding of (+)-[^{3}H]3-PPP. Guinea pig brain homogenates were incubated with 3 nM (+)-[^{3}H]3-PPP (*filled symbols with continuous line*) in the presence of increasing concentrations of ropizine (●) or phenytoin (▲) as indicated on the *abscissa*. The nonspecific binding was determined in the presence of 10 μM (+)-3-PPP. The results of the effects of ropizine are the average of two experiments and those of phenytoin of one experiment replicated once (without the 0.2 mM point). The effects of ropizine (○) and phenytoin (△) on the binding of [^{3}H]DM (*open symbols with dashed line*) from previous publications (12, 24) are included for comparison. The percent increase in (+)-[^{3}H]3-PPP or [^{3}H]DM specific binding is indicated on the *ordinate*.

produced by ropizine cannot be demonstrated either in the rat or in the mouse at pH 7.4.[1]

Pharmacological effects of σ drugs. The behavioral and pharmacological effects produced by σ ligands are quite different from those produced by DM. *Sigma* "agonists" such as SKF10,047, cyclazocine, and pentazocine produce delirium in dogs and dysphoria and hallucinations in humans (19). These behavioral manifestations are comparable to the psychotomimetic effects produced by PCP in humans (20). Even though the behavioral effects of σ agonists and PCP are similar, the PCP and σ sites are distinct, as shown by the different ligand selectivity and the distribution in different brain regions (4, 5). The σ "receptors" are postulated to mediate the psychotomimetic effects induced by various compounds (21). However, the binding site responsible for the psychotomimetic effects of PCP/σ drugs has not been unequivocally identified (20), but the available evidence suggests that they are mediated by the PCP site (22). In addition, the psychotomimetic σ ligands, haloperidol, and several antipsychotic agents bind with nanomolar affinity to the sites labeled with (+)-[^{3}H]SKF10,047 (4) or (+)-[^{3}H]3-PPP (2). Haloperidol and the antipsychotic agents are thought to act as "antagonists" at these sites (21), but there is no conclusive evidence that this is the case. We have previously reported that several antipsychotic and antidepressant agents compete with high affinity against [^{3}H]DM binding (6).

The finding that the binding of (+)-3-PPP is increased by phenytoin and ropizine in the same fashion as that of DM indicates that (+)-3-PPP and DM bind preferentially to the same conformation of the binding site. Likewise, this implies that DM and (+)-3-PPP produce a similar conformational change. Therefore, both ligands should elicit the same pharmacological effects through this common site. We do not know whether DM and (+)-3-PPP are agonists, antagonists, or channel-blocking agents, but the effects of quinidine, potassium, and other ions suggest that these high affinity binding sites may be associated with ion channels.

Pharmacological effects of DM ligands. In contrast to the σ "agonists," the antitussives DM, carbetapentane, and caramiphen are widely used and do not produce psychotomimetic effects in humans. We have previously suggested that DM, caramiphen, and carbetapentane exert their anticonvulsant and antitussive effects by binding to the high affinity DM sites (6). The ability of the anticonvulsant drugs phenytoin and ropizine to increase the binding of [^{3}H]DM indicates that they induce a conformational change that is similar to that produced by DM. This implies that the DM binding sites are located in a macromolecule or macromolecular aggregate with several interacting sites (23). It is not known whether the allosteric interactions of phenytoin and DM are related to the potentiation of their anticonvulsant activity (9).

Conclusion. The similarities of the affinities of DM ligands for sites labeled with [^{3}H]DM and (+)-[^{3}H]3-PPP and the striking analogy of the effects of the allosteric modifiers ropizine and phenytoin on [^{3}H]DM and (+)-[^{3}H]3-PPP binding strongly suggest that DM and (+)-3-PPP bind to at least one common site. This is supported by our previous findings that σ ligands inhibit [^{3}H]DM binding with a rank order of potency similar to that for sites labeled with (+)-[^{3}H]3-PPP or (+)-[^{3}H]SKF10,047 (3). These considerations suggest that the nonpsychotomimetic effects of σ ligands may be mediated through the high affinity DM sites. If this hypothesis is correct, the σ ligands with high affinity for the DM sites should have anticonvulsant and antitussive activity similar to that of DM, caramiphen, and carbetapentane.

[1] M. Klein, and J. M. Musacchio. High affinity dextromethorphan binding sites in guinea pig brain: effect of σ ligands and other agents. Submitted.

References

1. Hjorth, S., A. Carlsson, H. Wikstrom, P. Lindberg, D. Sanchez, U. Hacksell, L.-E. Arvidsson, U. Svensson, and J. L. G. Nilsson. 3-PPP, a new centrally acting dopamine receptor agonist with selectivity for autoreceptors. *Life Sci.* **28**:1225–1238. (1981).
2. Largent, B. L., A. L. Gundlach, and S. H. Snyder. Psychotomimetic opiate receptors labeled and visualized with (+)-[^{3}H]3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine. *Proc. Natl. Acad. Sci. USA* **81**:4983–4987 (1984).
3. Klein, M., J. J. Paturzo, and J. M. Musacchio. The effects of σ ligands on the binding of [^{3}H]dextromethorphan to guinea pig brain. *Neurosci. Lett.*, in press.
4. Tam, S. W., and L. Cook. *Sigma* opiates and certain antipsychotic drugs mutually inhibit (+)-[^{3}H]SKF10,047 and [^{3}H]haloperidol binding in guinea pig brain membranes. *Proc. Natl. Acad. Sci. USA* **81**:5618–5621 (1984).
5. Largent, B. L., A. L. Gundlach, and S. H. Snyder. Pharmacological and autoradiographic discrimination of σ and phenylcyclidine receptor binding sites in brain with (+)-SKF10,047, (+)-[^{3}H]3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine and [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine. *J. Pharmacol. Exp. Ther.* **238**:739–748 (1986).
6. Craviso, G. L., and J. M. Musacchio. High-affinity dextromethorphan binding sites in guinea pig brain. II. Competition experiments. *Mol. Pharmacol.* **23**:629–640 (1983).

7. Craviso, G. L., and J. M. Musacchio. High-affinity binding of the antitussive dextromethorphan to guinea pig brain. *Eur. J. Pharmacol.* **65**:451–453 (1980).
8. Craviso, G. L., and J. M. Musacchio. High-affinity dextromethorphan binding sites in guinea pig brain. I. Initial characterization. *Mol. Pharmacol.* **23**:619–628 (1983).
9. Tortella, F. C., and J. M. Musacchio. Dextromethorphan and carbetapentane: centrally acting non-opioid antitussive agents with novel anticonvulsant properties. *Brain Res.* **383**:314–318 (1986).
10. Tortella, F. C., J. M. Witkin, and J. M. Musacchio. Caramiphen, a non-opioid antitussive with potent anticonvulsant activity in rats. *Eur. J. Pharmacol.* **155**:69–75 (1988).
11. Santiago, L. J., M. Klein, and J. M. Musacchio. Effect of anticonvulsant drugs and other agents on the high-affinity binding of [^{3}H]dextromethorphan to guinea pig brain. *Soc. Neurosci. Abstr.* **13**:1157 (1987).
12. Musacchio, J. M., M. Klein, and L. J. Santiago. High affinity dextromethorphan binding sites in the guinea pig brain: further characterization and allosteric interactions. *J. Pharmacol. Exp. Ther.* **247**:424–431 (1988).
13. Munson, P. J., and D. Rodbard. Computerized analysis of ligand binding data: basic principles and recent developments, in *Computers in Endocrinology*. (M. Rodbard and G. Forti, eds.). Raven Press, New York, 117–145 (1984).
14. Munson, P. J, and D. Rodbard. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107**:220–239 (1980).
15. Munson, P. J. Experimental artifacts and the analysis of ligand binding data: results of a computer simulation. *J. Recep. Res.* **3**:249–259 (1983).
16. Peterson, G. A. A simplification of the protein assay method of lowry *et al.*, which is more generally applicable. *Anal. Biochem.* **107**:220–239 (1977).
17. Itzhak, Y. Pharmacological specificity of some psychotomimetic and antipsychotic agents for the *sigma* and PCP binding sites. *Life Sci.* **42**:745–752 (1988).
18. Largent, B. L., H. Wikstrom, A. L. Gundlach, and S. H. Snyder. Structural determinants of σ receptor affinity. *Mol. Pharmacol.* **32**:772–784 (1987).
19. Martin, W. R. Pharmacology of opioids. *Pharmacol. Rev.* **35**:283–323 (1983).
20. Sonders, M. K., J. F. W. Keena, and E. Weber. Phencyclidine and psychotomimetic σ opiates: recent insights into their biochemical and physiological sites of action. *Trends Neurosci.* **11**:37–40 (1988).
21. Manallack, D. T., P. M. Beart, and A. L. Gundlach. Psychotomimetic σ-opiates and PCP. *Trends Pharmacol. Sci.* **7**:448–451 (1986).
22. Zukin, R. S., and S. R. Zukin. Phencyclidine, σ and NMDA receptors: emerging concepts, in *Sigma and Phencyclidine-like Compounds as Molecular Probes in Biology*, (E. F. Domino and J. M. Kamenka, eds.). NPP Books, Ann Arbor, MI, 407–424 (1988).
23. Musacchio, J. M., M. Klein, and L. J. Santiago. Allosteric modulation of dextromethorphan binding sites. *Neuropharmacology* **26**:997–1001 (1987).
24. Musacchio, J. M., and M. Klein. Dextromethorphan binding sites in the guinea pig brain. *Cell. Mol. Neurobiol.* **8**:149–156 (1988).

Send reprint requests to: José M. Musacchio, Department of Pharmacology, N.Y.U. Medical Center, 550 First Avenue, New York, NY 10016.