0026-895X/87/060772-13$02.00/0

MOLECULAR PHARMACOLOGY, 32:772-784

Structural Determinants of σ Receptor Affinity

BRIAN L. LARGENT,[1] HÅKAN WIKSTRÖM,[2] ANDREW L. GUNDLACH,[3] and SOLOMON H. SNYDER

Departments of Neuroscience, Pharmacology and Molecular Sciences, and Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Received June 22, 1987; Accepted September 29, 1987

SUMMARY

The structural determinants of σ receptor affinity have been evaluated by examining a wide range of compounds related to opioids, neuroleptics, and phenylpiperidine dopaminergic structures for affinity at σ receptor-binding sites labeled with (+)-[^{3}H] 3-PPP. Among opioid compounds, requirements for σ receptor affinity differ strikingly from the determinants of affinity for conventional opiate receptors. σ sites display reverse stereoselectivity to classical opiate receptors. Multi-ringed opiate-related compounds such as morphine and naloxone have negligible affinity for σ sites, with the highest σ receptor affinity apparent for benzomorphans which lack the C ring of opioids. Highest affinity among opioids and other compounds occurs with more lipophilic *N*-substituents. This feature is particularly striking among the 3-PPP derivatives as well as the opioids. The butyrophenone haloperidol is the most potent drug at σ receptors we have detected. Among the series of butyrophenones, receptor affinity is primarily associated with the 4-phenylpiperidine moiety. Conformational calculations for various compounds indicate a fairly wide range of tolerance for distances between the aromatic ring and the amine nitrogen, which may account for the potency at σ receptors of structures of considerable diversity. Among the wide range of structures that bind to σ receptor-binding sites, the common pharmacophore associated with high receptor affinity is a phenylpiperidine with a lipophilic *N*-substituent.

Certain opioids can induce psychosis in humans (1, 2) and associated effects in animals, implying mediation by a unique opiate receptor subtype, designated σ. σ receptors were first defined based on the unique effects, in the chronic spinal dog, of SKF 10,047, the prototypic σ agonist from the benzomorphan structural class of opioids (3). These "σ" effects of SKF 10,047 include autonomic stimulation (e.g., mydriasis, tachycardia, and tachypnea) and "canine delirium," which was likened to the psychotomimetic effects seen in humans with similar drugs, i.e., σ opioids.

Although "σ" psychotic effects are elicited by widely used opiate analgesics, such as pentazocine, certain pharmacological features of these effects now indicate that they are not mediated via a classically defined type of opiate receptor. Most strikingly, the σ behavioral effects in animals are not antagonized by opiate antagonists such as naloxone (4–8). Moreover, the σ-like behavioral effects manifest reversed stereoselectivity from classical opiate receptors (4, 6, 8–11).

As some psychotomimetic opioids interact with receptor sites for the psychotomimetic drug phencyclidine, it has been suggested that σ-like behavioral effects may involve phencyclidine receptors (12). However, receptor binding sites for σ drugs, which are distinct from phencyclidine receptors, have been identified and display a drug specificity consistent with the pharmacological actions of σ drugs (13–19). For instance, σ-binding sites labeled with the prototypic σ drug (+)[^{3}H]SKF 10,047 demonstrate reversed stereoselectivity from classical opiate receptors and are negligibly influenced by non-psychotomimetic opiates such as morphine and naloxone (14, 17, 20).

This work was supported by United States Public Health Service Grants DA-00266 and NS-16375, Research Scientist Award DA-00074 to S. H. S., and a grant from the McKnight Foundation. B. L. L. is a Glaxo Fellow of the Life Sciences Research Foundation. A. L. G. is the recipient of a National Health and Medical Research Council (Australia) C. J. Martin Fellowship. H. W. is the recipient of a Fogarty International Research Fellowship [NIH 1 F05 TW 03628-01 BI-5 (AHR)] and is additionally funded by the Swedish Medical Research Council (MFR), Göteborgs Kungliga Vetenskaps-och Vitterhets-Samhälle, Magnus Bergvalls Stiftelse, Stiftelsen Lars Hiertas Minne, Adlebertska forskningsfonden, Kungliga Vetenskapsakademien, Sverige-Amerika Stiftelsen, and AB Hässle.

[1] Present address: The Howard Hughes Medical Institute, Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

[2] Present address: Organic Chemistry Unit, Department of Pharmacology, University of Göteborg, S-40033, Göteborg, Sweden.

[3] Present address: Department of Pharmacology, The University of Sydney, Sydney, N.S.W., 2006, Australia.

ABBREVIATIONS: SKF 10,047, *N*-allylnormetazocine; B_{max}, maximal number of binding sites; EBDA, equilibrium binding data analysis; IC_{50}, concentration giving 50% inhibition; K_D, equilibrium dissociation constant; MMP2, molecular mechanics program; 3-PPP, 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine; PCP, phencyclidine; EKC, ethylketocyclazocine; HW165, *trans*-7-hydroxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline; MPTP, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; *N*-1-Pr-MPTP, *N*-1-propyl-4-phenyl-1,2,3,6-tetrahydropyridine; NPA, 1-propyl-norapomorphine; OHBQ, (1,2,3,4,4a,5,6,10b)-octahydrobenzo[*f*]quinoline; RU 38796, 3-(3-hydroxyphenyl)-*N*-(1-propyl)-1,2,5,6-tetrahydropyridine; *i*-Pr, isopropyl; *n*-Pr, *n*-propyl; *n*-Bu, *n*-butyl; Bn; benzyl; Pheth, phenethyl.

The striking differences in the structural determinants of σ receptor-binding sites and conventional opiate receptors have raised questions as to the biologic role of σ-receptor-binding sites and the possibility of endogenous ligands active at these sites (21, 22). Identification of drugs lacking opiate structures, but demonstrating even higher affinity for σ receptors than many of the opiate-associated σ drugs, has emphasized the unique conformational requirements of σ sites. The neuroleptic haloperidol has the highest affinity of any known drug for σ receptors and can serve as a ligand to label σ sites with an equilibrium dissociation constant of about 2 nM (18, 23). The phenylpiperidine (+)-[^{3}H]3-PPP, which in intact animals displays dopaminergic actions (24–28), has nM affinity for σ sites and serves as a useful radioligand in labeling these sites (14, 23).

In the present study we have attempted to characterize the structural determinants of σ receptor affinity by labeling σ receptor-binding sites with (+)-[^{3}H]3-PPP and examining structure-affinity relationships of an extensive series of 3-PPP analogs, opioid drugs, and various other compounds.

Materials and Methods

Radioligand binding assays. In membrane-homogenate binding studies, fresh whole brains (male Sprague-Dawley rats, 150–250 g) were homogenized in 25 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25°) and centrifuged at 45,000 × *g* for 10 min at 4°. Pellets were then resuspended in fresh buffer and recentrifuged. This procedure was repeated once more before membranes were finally suspended in an appropriate volume of 50 mM Tris-HCl buffer (pH 8.0 at 25°; incubation buffer) for use in binding assays. This preparation typically results in a tissue homogenate containing approximately 6% protein by original wet weight with the Pierce BCA protein assay reagent (Pierce Chemical Co., Rockford, IL).

In a final assay volume of 0.25 ml, 1–2 nM (+)-[^{3}H]3-PPP was incubated in the presence of various concentrations of unlabeled drug with the equivalent of 7.5 mg of tissue (original wet weight; approximately 450 μg of protein) for 90 min at room temperature. All experiments were performed in the linear concentration ranges for both tissue and radioligand, and with an incubation time appropriate for the attainment of equilibrium. The level of nonspecific binding of (+)-[^{3}H]3-PPP was defined as that in the presence of 1 μM haloperidol and was always less than 15% of total binding. Incubations were terminated by the addition of 2.5 ml of ice-cold 5 mM Tris-HCl buffer (pH 7.7 at 25°; wash buffer) and membranes were collected by filtration under vacuum onto glass fiber filters (Schleicher and Schuell No. 32; pretreated with 0.5% polyethylenimine). Filters were washed with two consecutive 5-ml aliquots of wash buffer. The total time taken for the filtration/washing procedure was less than 10 sec. Radioactivity remaining on the filters was measured by liquid scintillation spectrometry at 60% efficiency. Drug competition binding data were analyzed with an iterative curve-fitting computer program, EBDA (29), to determine drug IC_{50} values.

Conformational analysis by MMP2. The MMP2 molecular mechanics program (30), operated on a Microvax Workstation II computer, was utilized for conformational analysis. Molecular mechanics, or empirical force field, calculations are based on a classical mechanical model of the molecular structure. In principle, a function describing the steric energy of the molecule is calculated. The function is a sum of energy terms representing contributions from bond stretching, bond bending, angle bending, torsion, and Van der Waals interactions, etc. Given a starting geometry of the molecule, the program modifies that geometry to obtain successively lower calculated steric energies. This iterative process reveals the local minimum energy conformation for that starting geometry. Thus, a number of different starting geometries must be analyzed to find all the possible local minimum conformations. The local minimum conformation having the lowest steric energy is called the global energy minimum conformation.

For this study, starting geometries for MMP2 calculations were either derived from X-ray crystallography data or created by the MODEL program (courtesy of W. C. Still, Columbia University, New York, NY). Input structures of the model compounds *cis*- and *trans*-8-OH-4-Me-OHBQ were chosen to have half-chair/chair conformations, which represent low energy conformations according to previous studies of analogous compounds (31, 32). Solvent interactions were not considered in the calculations performed. As seen in Table 4 (under Results), the intramolecular distances calculated for the X-ray-derived geometry and the minimized geometry are very similar in each case. This argues that solvent effects might be neglected in the calculations of these molecules since the strong crystal lattice forces (Coulombic interactions, hydrogen bonds, and Van der Waals interactions) in the dense X-ray crystal—which should be stronger than the potential solvent effects in aqueous solution—do not appreciably affect the calculated distances.

Materials. (+)-[^{3}H]3-PPP (107.4 Ci/mmol; 1 Ci = 3.7 × 10^{10} Bq) was supplied by New England Nuclear/Dupont (Boston, MA). *R*-(+)-3-PPP and *S*-(−)-3-PPP (compounds **7** and **8**, respectively) were provided by Astra Laboratories (Södertalje, Sweden). The compounds in the phenylpiperidine and the OHBQ series were provided by the Organic Chemistry Unit, Department of Pharmacology, University of Göteborg (Göteborg, Sweden). (±)- (+)-, and (−)-SKF 10,047 were obtained from the National Institute on Drug Abuse, Research Technology Branch (Research Triangle Park, NC). (±)-Pentazocine, (±)-EKC, and (±)-, (+)-, and (−)-cyclazocine were supplied by Sterling-Winthrop Research Institute (Rensselaer, NY). Buspirone was obtained from Bristol-Myers (Evansville, IN). Haloperidol was provided by McNeil Pharmaceutical (Spring House, PA). The enantiomers of apomorphine, propylnorapomorphine, and butaclamol were supplied by Research Biochemicals, Inc. (Wayland, MA). All other reagents were obtained from commercial sources.

Results

Binding of (+)-[^{3}H]3-PPP to Brain Membranes

Equilibrium-saturation analysis of (+)-[^{3}H]3-PPP binding to rat brain membranes and the drug specificity of those binding sites have been extensively described previously (14, 23). Briefly, (+)-[^{3}H]3-PPP labels a single class of high affinity sites exhibiting a K_D of 30 ± 1.5 nM (n = 9) with B_{max} of 520 ± 40 fmol/mg of protein (n = 9) in rat whole brain membranes (23). The drug specificity of (+)-[^{3}H]3-PPP binding to brain membranes is nearly identical to that demonstrated for σ receptor-binding sites labeled with high affinity by (+)-[^{3}H]SKF 10,047 (13, 14, 17, 20), (±)-[^{3}H]SKF 10,047 in the presence of high concentrations of naloxone (33, 34), [^{3}H]haloperidol (18, 23), or 1,3-di(2-[5-^{3}H]tolyl)guanidine (19). For nearly all of the compounds presented, psuedo-Hill plots of drug competition data reveal slopes of essentially unity. The few exceptions include various benzomorphans which have been shown to exhibit pseudo-Hill plots with slopes of less than unity (23), suggesting a complex interaction with the binding site. Yet, kinetic and equilibrium analyses reveal that the influence of benzomorphans upon (+)-[^{3}H]3-PPP binding to σ sites is consistent with a competitive interaction.[4]

Comparisons of Structural Determinants Among Drug Classes

Opioids. Among classical opioid compounds, the more bulky morphine and morphinan analogs have lower affinity for σ sites than the smaller benzomorphan analogs (Fig. 1, Table 1). Of

[4] B. Largent, unpublished observations.

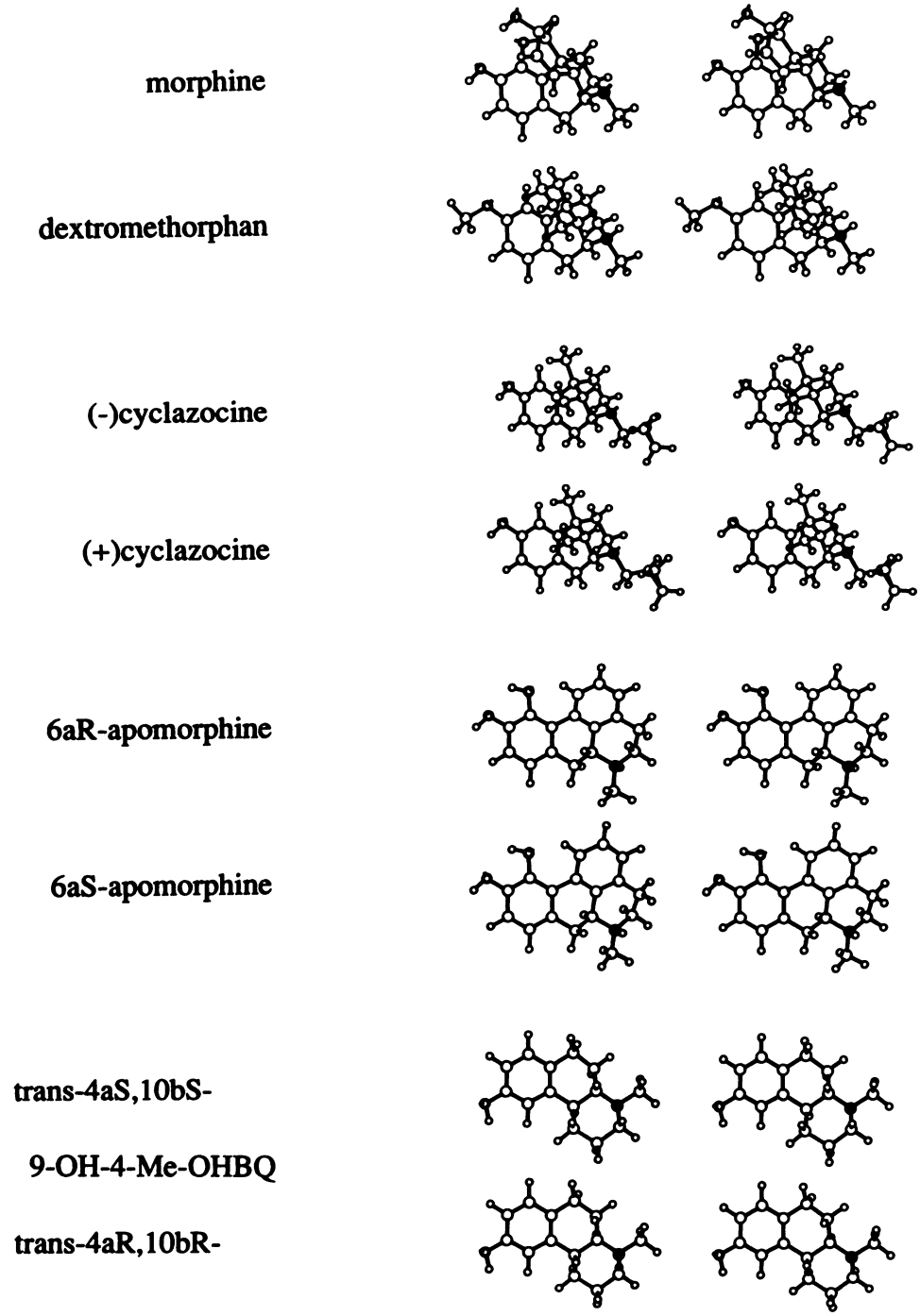


Fig. 1. Structural features determining σ receptor affinity. Emphasis is placed on the importance of proper stereochemistry for σ receptor site affinity. The stereoisomer pairs for cyclazocine, apomorphine, and 9-OH-4-Me-OHBQ are presented together with the lower affinity compound displayed on top. The hindrance on σ site affinity by structural bulkiness in certain compounds is best demonstrated by comparing the structure of morphine (a relatively weak compound) with the benzomorphan cyclazocine (a relatively potent compound). The stereopictures were plotted from the CHEMX graphics system on a Calcomp plotter. The use of stereoviewers is recommended for the best perception in three dimensions. All structures were oriented in the *xy* plane prior to display. Morphine, dextromethorphan, and (−)- and (+)-cyclazocine were subsequently rotated (*y* axis) −20, +20, −30, and +30 degrees, respectively. ●, nitrogen atoms; O and ○, oxygen and carbon atoms, respectively.

perhaps greater importance than relative bulkiness is the lipophilicity of certain portions of the molecule. Removal of the oxy-bridge as well as the hydroxy- and keto-substituent on the C ring results in a greater potency of levallorphan compared with naloxone. The more lipophilic *N*-allyl group of levallorphan may explain its greater potency than levorphanol, which possesses an *N*-methyl substituent.

Among the benzomorphans, pentazocine is most potent (IC_{50} = 25 nM; Table 1). Pentazocine's approximately 10-fold higher affinity than SKF 10,047 may be attributable to the *N*-dimethylallyl substituent of pentazocine compared to the *N*-allyl of SKF 10,047. Similarly, the *N*-phenethyl substituent of phenazocine may account for its considerable potency, similar to that of pentazocine. In contrast, the 8-keto substituents of ketocyclazocine and ethylketocyclazocine, conferring greater hydrophilicity, may account for their lower affinity, only about 1% that of pentazocine. The simpler phenylpiperidine opioid structures also retain affinity for σ receptor-binding sites. Of the two phenylpiperidines evaluated, the 6.5-fold greater potency of fentanyl compared to meperidine might reflect the greater lipophilicity of the *N*-phenethyl substituent in fentanyl contrasted to the *N*-methyl of meperidine.

Consistent variations in affinity relate to stereoselectivity. Two benzomorphans, tested in their enantiomeric forms, exhibit moderate stereoselectivity ratios (3.7 and 3.9 for the (+)-isomers of cyclazocine and SKF 10,047, respectively; Table 1).

TABLE 1

Potencies of various compounds for inhibition of (+)-[^{3}H]3-PPP binding to rat whole brain membranes

Rat brain membranes (7.5 mg original wet weight) were incubated with 1–2 nM (+)-[^{3}H]3-PPP for 90 min at room temperature (50 mM Tris-HCl, pH 8.0) in the presence of 10 varying concentrations of drug. Nonspecific binding of (+)-[^{3}H]3-PPP was estimated in the presence of 1 μM haloperidol. Drug IC_{50} values were determined from the data by an iterative computer program, EBDA (29). The IC_{50} values presented are the mean (nM) ± standard error of three to eight determinations. Structures for each of the compounds are provided and drugs are grouped into structural classes for ease of comparison.

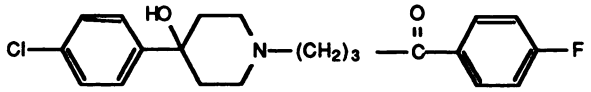
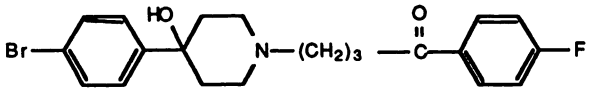
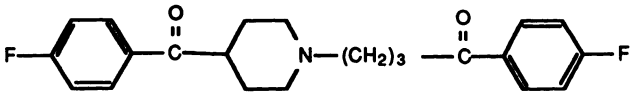
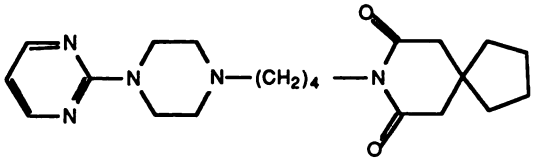
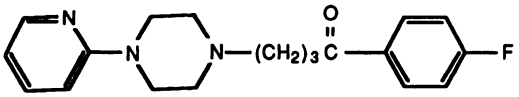
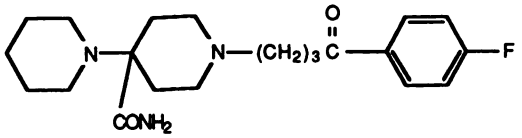
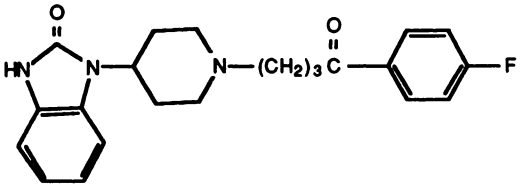
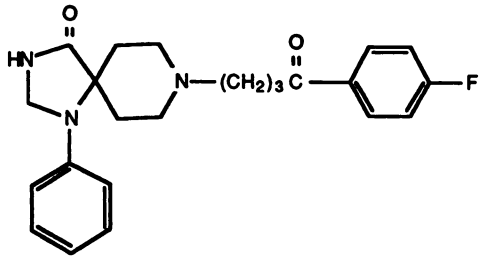
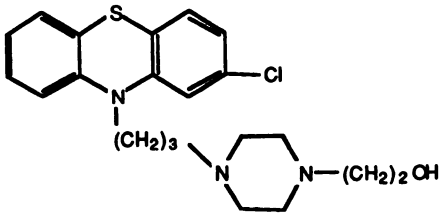
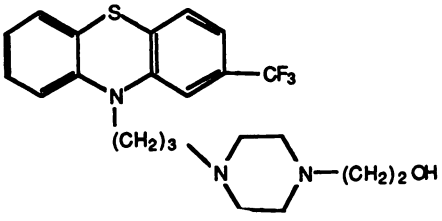
Drug	IC_{50} (nM)	Structure
Butyrophenones and Related Structures		
Haloperidol	3 ± 0.5	
Bromperidol	6 ± 1	
Lenperone	35 ± 7	
Buspirone	129 ± 11	
Azaperone	131 ± 17	
Pipamperone	148 ± 29	
Benperidol	430 ± 70	
Spiperone	695 ± 45	
othiazines and Related Structures		
Perphenazine	26 ± 3	
Fluphenazine	68 ± 4	

TABLE 1—continued

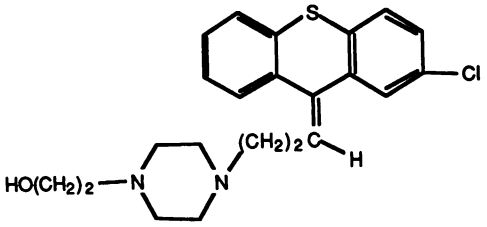
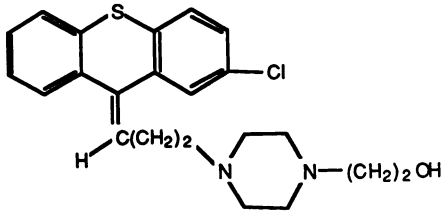
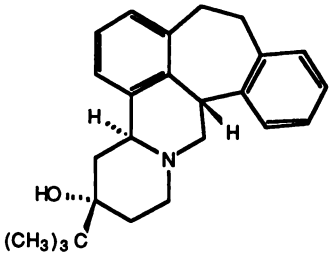
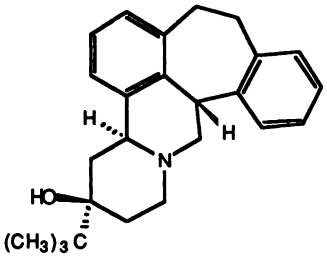
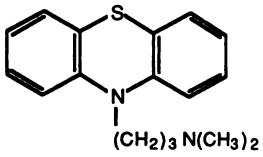
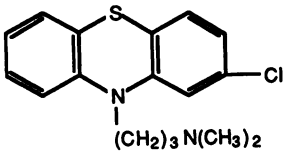
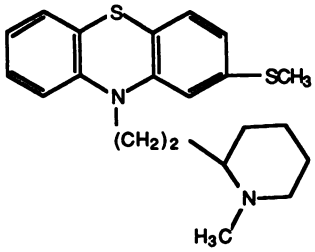
Drug	IC_{50} (nM)	Structure
t-Clopenthixol	145 ± 11	
c-Clopenthixol	152 ± 17	
(−)-Butaclamol	173 ± 21	
(+)-Butaclamol	1940 ± 140	
Chlorpromazine	435 ± 32	
Promazine	440 ± 74	
Thioridazine	820 ± 83	

TABLE 1—continued

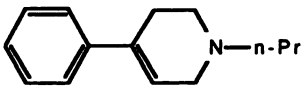
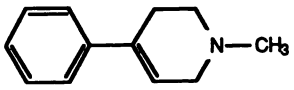
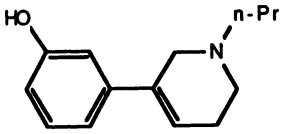
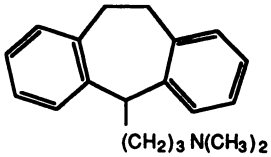
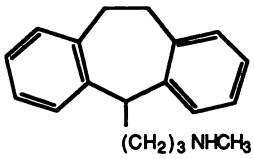
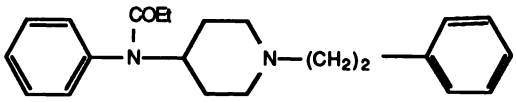
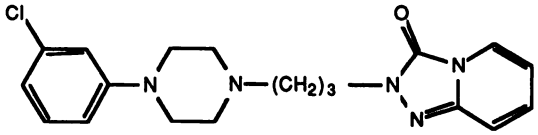
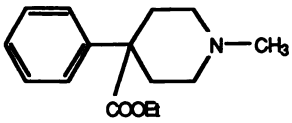
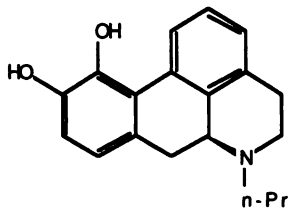
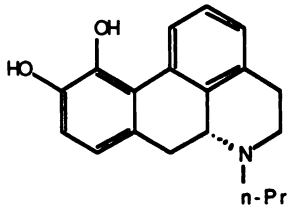
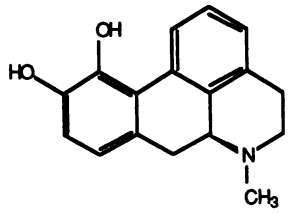
Drug	IC_{50} (nM)	Structure
Tricyclics, Phenylpiperidines, and Other Heterocyclic Compounds		
N-1-Pr-PTP	15 ± 3	
MPTP	2860 ± 350	
RU 38796	41 ± 4	
Imipramine	345 ± 70	
Desipramine	2470 ± 70	
Fentanyl	354 ± 52	
Trazadone	850 ± 21	
Meperidine	2290 ± 430	
Morphines and Related Structures		
S-(+)-NPA	4190 ± 730	
R-(−)-NPA	35,000 ± 3900	
S-(+)-Apomorphine	15,000 ± 1600	

TABLE 1—continued

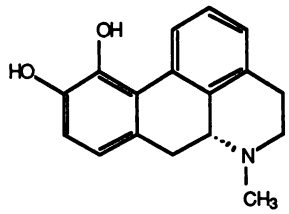
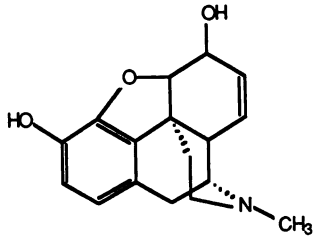
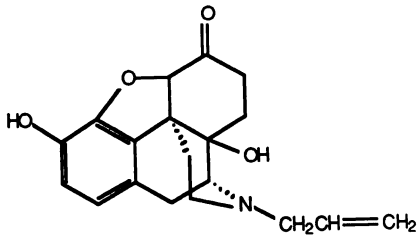
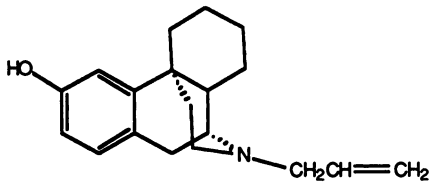
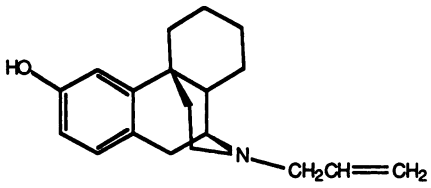
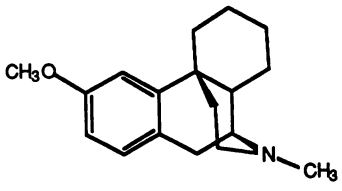
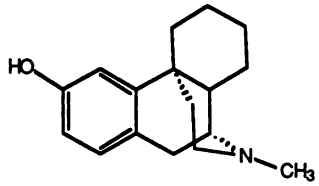
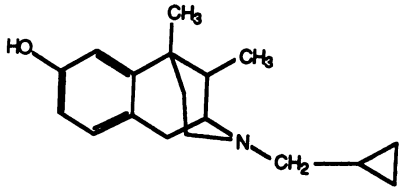
Drug	IC_{50} (nM)	Structure
R-(−)-Apomorphine	110,000 ± 15,000	
Morphine	>100,000	
Naloxone	>100,000	
Morphinans		
Dextrallorphan	163 ± 22	
Levallorphan	1890 ± 140	
Dextromethorphan	810 ± 72	
Levorphanol	>10,000	
Benzomorphans		
(±)-Pentazocine	25 ± 2	

TABLE 1—continued

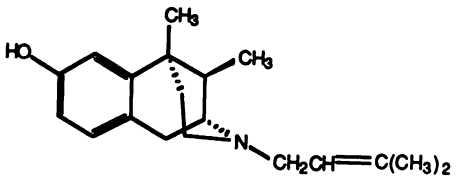
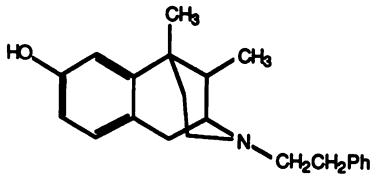
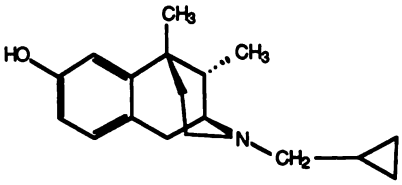
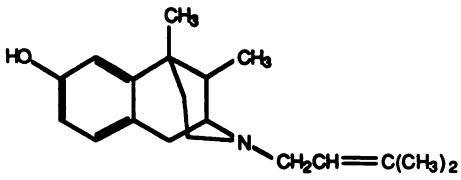
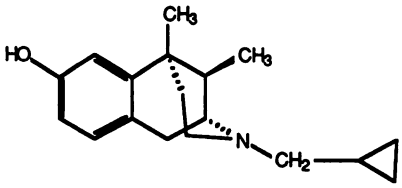
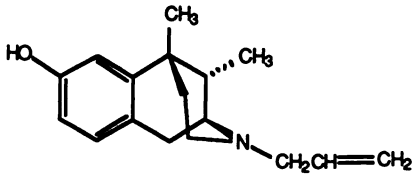
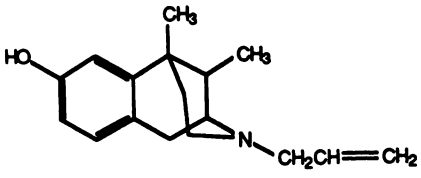
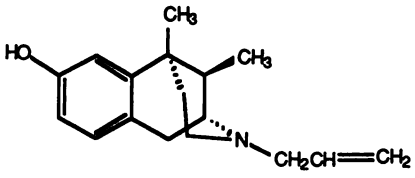
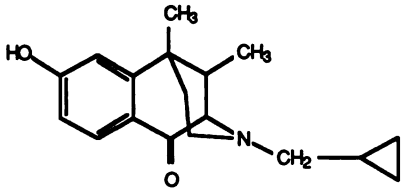
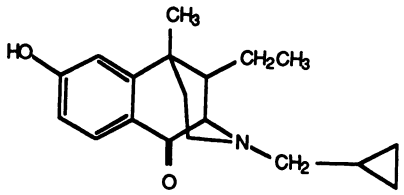
Drug	IC_{50} (nM)	Structure
(−)-Pentazocine	31 ± 6	
(±)-Phenazocine	46 ± 10	
(+)-Cyclazocine	118 ± 14	
(±)-Cyclazocine	102 ± 6	
(−)-Cyclazocine	432 ± 68	
(+)-SKF 10,047	365 ± 33	
(±)-SKF 10,047	395 ± 14	
(−)-SKF 10,047	1440 ± 110	
(±)-Ketocyclazocine	2990 ± 981	

TABLE 1—continued

Drug	IC_{50} (nM)	Structure
(±)-EKC	4840 ± 814	

The absolute configuration of the chiral carbon bound to the nitrogen of the more potent (+)-enantiomers of these benzomorphans is *S*-, representing the same relative stereochemistry as the more active *trans*-4a*R*,10b*R*-OHBQs (discussed below, see Fig. 1). In contrast, morphine and naloxone, which are relatively weak at σ-binding sites, both have the *R* absolute configuration at the chiral carbon which carries the nitrogen. Apomorphine has the 6a*R* absolute conformation and is very weak (IC_{50} = 110 μM; Table 1), whereas the chemically synthesized 6a*S*-enantiomer of apomorphine [*S*-(+)-apomorphine] displays an 8-fold higher affinity for σ-binding sites (IC_{50} = 15 μM), presumably reflecting its common stereochemical configuration with the preferred (+)-benzomorphans. This pattern of stereoselectivity is also observed with the enantiomers of NPA, with *S*-(+)-NPA being, again, approximately 8 times more potent than *R*-(−)-NPA. These stereochemical considerations are further substantiated by the morphinan enantiomers, dextrallorphan and levallorphan, with dextrallorphan having the same relative stereochemical conformation as (+)-benzomorphans and exhibiting roughly 10-fold higher σ site affinity than levallorphan. The non-preferential stereochemistry of morphine and naloxone probably serves as a general explanation for the low σ site affinity of these, as well as other, classical opioid compounds.

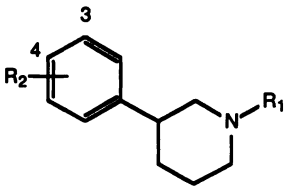
Analogs of 3-PPP. A number of structural analogs of 3-PPP, many resolved to individual enantiomers, were tested for their potencies in inhibiting (+)-[^{3}H]3-PPP binding to rat brain membranes (Table 2). Some of these compounds have been previously tested *in vivo* for apparent pre- and postsynaptic dopaminergic activity (35, 36). Generally, the *R*-enantiomers are more potent than the *S*-analogs as inhibitors of (+)-[^{3}H]3-PPP binding. A prominent trend from this series of resolved phenylpiperidines is that compounds with larger *N*-substituents (R_1) exhibit higher affinity for σ receptor-binding sites. Larger *N*-substituents provide for greater lipophilicity in these compounds (37) in an analogous fashion to the opioid compounds. This pattern is valid in both the *R*- and *S*-series of enantiomers. Interestingly, in the *R*-series this trend is broken by compound **9**, with an *i*-Pr *N*-substituent, which drops 8-fold in affinity as compared to its *n*-Pr analog, while the corresponding *S*-enantiomer (compound **10**) drops only a factor of 1.3 in an analogous comparison. This might indicate differences in sensitivity to steric bulk for the two different *N*-alkyl directions for these enantiomeric series.

For the R_2 function in the phenylpiperidine series, a 4-OH substituent (compound **15**) is less optimal for σ site affinity than the corresponding 3-OH substituent (compounds **7** and **8**). Interestingly, a hydrogen bond donor function (i.e., phenolic hydroxyl), which seems to be necessary for the dopamine-like agonist effects of these analogs, is not a prerequisite for σ site affinity as indicated by the relatively high affinities exhibited

TABLE 2

Potencies of compounds in a series of phenylpiperidines for inhibition of (+)-[^{3}H]3-PPP binding to rat whole brain membranes

The IC_{50} values presented are the mean (nM) ± standard error of three to eight determinations. Details of experimental procedures are presented in the legend to Table 1 and under Materials and Methods. The R_1 group represents the *N*-substituent of the piperidine, while the R_2 group denotes the substituent on the phenyl moiety for the given structure. Abbreviations of substitutions: H, hydrogen; Me, methyl; Et, ethyl. Others are defined in the text footnote.



Compound number	R_1	R_2	IC_{50} R-Enantiomer	IC_{50} S-Enantiomer	S/R
			nM		
1, 2	H	3-OH	4570 ± 190	15,600 ± 2300	3.4
3, 4	Me	3-OH	373 ± 66	1910 ± 350	5.1
5, 6	Et	3-OH	111 ± 12	1030 ± 66	9.3
7, 8	*n*-Pr	3-OH	32 ± 2	165 ± 18	5.2
9, 10	*i*-Pr	3-OH	247 ± 9	208 ± 12	0.8
11, 12	*n*-Bu	3-OH	9 ± 0.8	31 ± 5	3.4
13, 14	Pheth	3-OH	8 ± 1.8	16 ± 3	2.0
15	*n*-Pr	4-OH	276 ± 5[a]		
16	*n*-Pr	3-CF	317 ± 2[a]		
17	*n*-Pr	3-F	49 ± 11[a]		

[a] Compounds **15–17** are racemic mixtures.

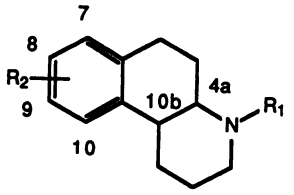
by the 3-F and 3-CF_3 (R_2) analogs, compounds **17** and **16**, respectively. Furthermore, an electronegative aromatic substitution is not necessary as evidenced by the high affinity of the *N*-1-Pr analog (*N*-1-Pr-PTP) of MPTP (Table 1).

***cis*- and *trans*-OHBQs.** The OHBQs comprise a series of semirigid analogs having in their framework the 3-phenylpiperidine moiety, which seems to be the prominent pharmacophore at σ-binding sites for these compounds. Both *trans*- and *cis*-isomers were tested against (+)-[^{3}H]3-PPP binding (Table 3). The *trans*-OHBQs generally have higher affinity for (+)-[^{3}H]3-PPP-binding sites than their *cis* counterparts. The relative *trans*/*cis* affinity ratios are (for the resolved compounds the value for the enantiomer with the higher affinity was chosen): 6.3, 4.1, 4.0, 6.0, and 3.1 for compounds **18/38** (7-OH-OHBQ), **23/40** (7-OH-4-*n*-Pr-OHBQ), **27/41** (8-OH-4-*n*-Pr-OHBQ), **28/42** (9-OH-OHBQ), and **30/43** (9-OH-4-*n*-Pr-OHBQ), respectively. When comparing compounds with the common *n*-Pr *N*-substitution, the 8-position for the OH-substituent seems optimal for highest affinity, followed in order by positions 9, 7, and 10. This pattern is valid for both the *trans*- and *cis*- series. Interestingly, position 8 in the OHBQs is equivalent to the hydroxyl position in the benzomorphans.

TABLE 3

Potencies of compounds in a series of octahydrobenzo[*f*] quinolines for inhibition of (+)-[^{3}H]3-PPP binding to rat whole brain membranes

The IC_{50} values presented are the mean (nM) ± standard error of three to eight determinations. Details of experimental procedures are presented in the legend to Table 1 and under Materials and Methods. The R_1 group represents the *N*-substituent. The series of compounds is divided into groups of *trans*- and *cis*-conformations. The absolute configurations of resolved isomers are indicated.



Compound number	R_1	R_2	Absolute configuration	IC_{50}
				nM
trans-Compounds				
18	H	7-OH	4a*R*,10b*R*	2370 ± 310
19	H	7-OH	4a*S*,10b*S*	3610 ± 290
20	Me	7-OH	racemic	610 ± 30
21	Et	7-OH	racemic	236 ± 25
22	*n*-Pr	7-OH	racemic	135 ± 20
23	*n*-Pr	7-OH	4a*R*,10b*R*	48 ± 8
24	*n*-Pr	7-OH	4a*S*,10b*S*	483 ± 67
25	*n*-Bu	7-OH	racemic	23 ± 4
26	Pheth	7-OH	racemic	65 ± 8
27	*n*-Pr	8-OH	racemic	10 ± 1.6
28	H	9-OH	racemic	1940 ± 380
29	*n*-Pr	9-OH	racemic	19 ± 1
30	*n*-Pr	9-OH	4a*R*,10b*R*	21 ± 2
31	*n*-Pr	9-OH	4a*S*,10b*S*	520 ± 35
32	*n*-Bu	9-OMe	racemic	5 ± 0.5
33	*n*-Bu	9-OH	racemic	10 ± 0.7
34	Bz	9-OMe	racemic	5 ± 0.6
35	Bz	9-OH	racemic	10 ± 0.9
36	Pheth	9-OMe	racemic	14 ± 0.4
37	Pheth	9-OH	racemic	27 ± 1.7
cis-Compounds				
38	H	7-OH	racemic	14,900 ± 2400
39	*n*-Pr	7-OH	4a*R*,10b*S*	416 ± 48
40	*n*-Pr	7-OH	4a*S*,10b*R*	197 ± 13
41	*n*-Pr	8-OH	racemic	40 ± 2
42	H	9-OH	racemic	11,600 ± 1350
43	*n*-Pr	9-OH	racemic	66 ± 4
44	*n*-Bu	9-OH	racemic	35 ± 2
45	*n*-Pr	10-OH	racemic	1930 ± 280

Additionally, substitution of an OMe— for the OH—, for each of the available compounds tested (**32–33; 34–35; 36–37**) results in approximately a 2-fold increase in affinity, again indicating that the ability of substituents of the aromatic ring to be hydrogen bond donors is not essential for high σ site affinity.

There is a stereoselectivity trend within the *trans*- series with preference for 4a*R*,10b*R*- over 4a*S*,10b*S*-enantiomers. The relative affinity ratios are 1.5, 10, and 25 for compounds **18/19** (7-OH-OHBQ), **23/24** (7-OH-4-*n*-Pr-OHBQ), and **30/31** (9-OH-4-*n*-Pr-OHBQ), respectively, with the more potent compounds exhibiting greater stereoselectivity. Among the *cis* compounds, the enantiomers of *cis*-7-OH-4-*n*-Pr-OHBQ were tested (compounds **39** and **40**) with the 4a*S*,10b*R*-enantiomer exhibiting a modest 2-fold stereoselectivity at σ sites. For this example, the stereochemistry at carbon 4a is opposite to that of the more active enantiomers for the *trans*-OHBQs. However, the data available are too limited for an extended comparison between enantiomers of *cis*- and *trans*-OHBQs.

Butyrophenones and related compounds. Of all the compounds tested, the butyrophenone haloperidol demonstrates the highest affinity (IC_{50} = 3 nM) for (+)-[^{3}H]3-PPP-binding sites. When comparing the structure of haloperidol to those of other compounds, the lipophilic *N*-alkyl substituent, i.e., the butyrophenone moiety of haloperidol, appears important for the high affinity. However, the 4-phenylpiperidine moiety is probably the primary pharmacophore. Thus, the butyrophenone spiperone, which lacks the 4-phenylpiperidine group, is 200-fold less potent than haloperidol. The high affinity (IC_{50} = 148 nM) of the butyrophenone pipamperone suggests that the aromatic portion of the phenylpiperidine moiety is not absolutely necessary. Pipamperone contains a nonaromatic piperidine ring system instead of the aromatic phenylpiperidine. The only aromatic ring in pipamperone is the phenyl ring of the butyrophenone moiety.

The structural similarity between haloperidol and the neurotoxin MPTP led us to test this compound as well as its *N*-1-Pr analog (Table 1). MPTP is weak (IC_{50} = 2860 nM) at inhibiting (+)-[^{3}H]3-PPP binding, whereas its *N*-1-Pr analog, with a more lipophilic *N*-substituent, is 2000 times more potent (IC_{50} = 15 nM), supporting the generalization that increased lipophilicity of the *N*-alkyl substituent greatly improves affinity for σ receptors.

pH Dependency of σ Site Binding

The effect of pH on the binding affinity of (+)-[^{3}H]3-PPP is also consistent with a role for lipophilicity within the piperidine *N*-alkyl region in determining affinity at σ receptor-binding sites. (+)-[^{3}H]3-PPP binding is enhanced with increasing pH values in the range 7.0–8.9 (Fig. 2). The shape of the pH dependency binding curve suggests that the binding affinity may continue to increase at pH values above 8.9. The macroscopic pK_a values for the phenolic hydrogen and the piperidine nitrogen of 3-PPP are in the range of 9.3 to 9.8.[5] Thus, one may reasonably predict that the effect of incubation pH on (+)-[^{3}H]3-PPP binding is partially mediated through net changes in charge at those substituents in the 3-PPP molecule. Since substitution of an —OMe for the phenolic hydroxyl in OHBQs increases affinity 2-fold to σ sites, it is unlikely that elevated pH enhances binding by creating net negative charge on the phenolic hydroxyl. Conversely, increasing pH removes net positive charge at the piperidine nitrogen, effectively increasing the ring's lipophilicity, which suggests that enhanced affinity at higher pH reflects a more lipophilic, uncharged piperidine.

Conformational Calculations for Phenothiazines and Other Structures

Phenothiazines are not readily compared to the other structures discussed above, due to their flexible side chains. Phenothiazines and thioxanthines with a piperazine side chain have higher affinities than those with an *N*,*N*-dimethyl substitution pattern, which in turn give higher affinities than those with the mono-*N*-methyl substituent, again indicating that more carbon atoms attached to the "piperidine" nitrogen (e.g., higher lipophilicity) results in higher σ site affinity.

Conceivably, when interacting with σ receptor-binding sites,

[5] H. Van de Waterbeemd, Universitee de Lausanne, Switzerland, unpublished observations.

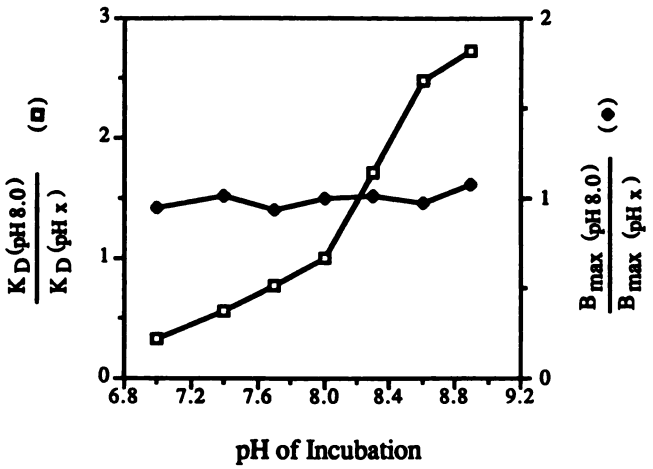


Fig. 2. pH dependency curve for binding of (+)-[^{3}H]3-PPP to σ receptors. The effect of incubation pH on the affinity of (+)-[^{3}H]3-PPP for σ receptor-binding sites is evaluated as the ratios of apparent K_D and B_{max} at a given pH to their corresponding values at pH 8.0. Note the lack of significant variation in B_{max} values, whereas the apparent K_D decreases with increasing incubation pH. Data were obtained from equilibrium-saturation experiments in which rat whole brain membranes were incubated with 1–2 nM (+)-[^{3}H]3-PPP and various concentrations of unlabeled 3-PPP (1–1000 nM). K_D and B_{max} values were derived from the data by computer-assisted analysis utilizing EBDA (29) and are the average of two separate experiments with 12 concentration points (in quadruplicate), with the results varying by less than 20%. In this instance, the values obtained at pH 8.0 were K_D = 47 nM and B_{max} = 1070 fmol/mg of protein.

the aromatic ring and the closest nitrogen for phenothiazines mirror corresponding groups in the more rigid structural classes. To explore the importance of the nitrogen to aromatic ring distance, as well as other intramolecular distances, molecular mechanics calculations were performed for haloperidol, *cis*- and *trans*-clopenthixol, (−)-cyclazocine, and (+)-dexclamol (a very similar structural analog of butaclamol with an isopropyl substituted for the *tert*-butyl group), morphine, dextromethorphan, and apomorphine. The corresponding structures derived from X-ray crystallography data were used for these compounds as input to the molecular mechanics calculations, with the assumption that those structures represent low energy conformations, which are close to the global minima in these calculations. Intramolecular distances for the X-ray structures and for the energy-minimized structures of the molecules investigated can be compared with corresponding data for the model compounds *cis*- and *trans*-8-OH-4-Me-OHBQ (Table 4). Since the *cis*- compound can have two different chair conformations of the piperidine ring, one with *N*-axial and one with *N*-equatorial, both of these conformations are included in the calculations. Since the difference in steric energy between the free amine forms of *cis*-*N*-axial and *cis*-*N*-equatorial is only 0.97 kcal/mol, with the *cis*-*N*-equatorial being the more stable conformation, initially, both conformations should be considered.

The calculated minimized conformations of (−)-cyclazocine, *cis*- and *trans*-clopenthixol, haloperidol, and (+)-dexclamol are close to their respective X-ray structures. The *N*-aromatic ring plane distance is not critical for σ receptor interactions of these compounds, because that distance varies substantially (0.08–2.9 Å) (selecting the shortest distance for *cis*- and *trans*-clopenthixol) within the various structures. The distances *N*-(midpoint of the aromatic ring) and *N*-(polar function) vary between 4.3–6.4 Å and 6.5–8.9 Å, respectively. This large degree of variation among interatomic distances may explain why such a variety of chemical structures exhibit reasonable affinity for σ-binding sites.

TABLE 4

Experimental and calculated data for intramolecular distances within various compounds tested for σ receptor site affinity

Comparisons are made among various selected compounds of intramolecular distances which have been calculated by the MMP2 molecular mechanics program (30) or obtained from X-ray data. Reference to the appropriate drug structures in Tables 1 and 3 will facilitate the identification of the described intramolecular distances being measured.

Compound	Intramolecular distances (Å)		
	N-Aromatic ring plane[a]	*N*-Midpoint aromatic ring plane[b]	*N*-polar function[c]
cis-8-OH-4-Me-OHBQ			
Axial calculated	1.9	4.3	6.6
Equatorial calculated	0.28	5.2	7.9
trans-8-OH-4-Me-OHBQ (calculated)	0.080	5.2	7.9
Morphine			
X-ray	0.59	4.8	7.1
Calculated	0.78	4.8	7.1
Dextromethorphan			
X-ray	1.6	4.7	6.9
Calculated	1.6	4.7	7.0
(−)-Cyclazocine			
X-ray	1.3	4.6	7.1
Calculated	1.6	4.5	7.0
cis-Clopenthixol			
X-ray	2.7/3.8	6.4/7.5	7.3/6.8
Calculated	2.9/4.2	6.3/7.5	7.1/6.7
trans-Clopenthixol			
X-ray	1.9/0.4	5.8/7.6	7.6/8.7
Calculated	2.4/0.6	5.8/7.6	7.5/8.8
Haloperidol[d]			
X-ray	0.7	5.7	8.8
Calculated	1.3	5.7	8.9
(+)-Dexclamol			
X-ray	0.19	5.2	
Calculated	0.54	5.2	
Apomorphine			
X-ray	0.86	5.2	6.5
Calculated	1.1	5.2	6.5

[a] For *cis*- and *trans*-clopenthixol the distances are: (closest *N* to its closest ring)/(most distant *N* to its closest ring) obtained from X-ray data.

[b] For *cis*- and *trans*-clopenthixol the distances are: (closest *N* to midpoint of *cis*-ring/closest *N* to midpoint of *trans*-ring).

[c] Polar functions are OH, OH, OMe, OH, S/Cl, Cl, and 11-OH for *cis*- and *trans*-8-OH-4-Me-OHBQ, morphine, dextromethorphan, (−)-cyclazocine, *cis*- and *trans*-clopenthixol, haloperidol, and apomorphine, respectively.

[d] The distances for haloperidol were retrieved from the phenylethlyamine moiety (ring A).

Discussion

This study describes structure-affinity relationships for various groups of compounds at σ receptor-binding sites. These binding sites were labeled with (+)-[^{3}H]3-PPP, which selectively binds to σ sites, in contrast to (+)-[^{3}H]SKF 10,047, which labels both σ and PCP receptor-binding sites with only a 10-fold selectivity for σ sites (13, 14).

Several structural requirements are evident. First, the primary pharmacophore at σ sites seems to be the phenylpiperidine moiety (3- or 4-phenylpiperidine), which is present in most compounds that are potent at σ receptor-binding sites. Second, affinity is markedly influenced by the *N*-alkyl substituents, with more lipophilic substitutions affording greater affinity for

σ receptor-binding sites. Third, compounds of many different structural classes demonstrate substantial affinity for σ sites, suggesting that certain intramolecular distances (e.g., *N* to aromatic ring) are not subject to rigid constraints. Calculated intramolecular distances for several compounds support this conclusion. This "permissive" nature of σ receptors is further evidenced by the lack of strict stereospecificity at the binding sites. Although a consistent stereoselectivity is noted for many isomer pairs, the degree of stereoselectivity is less than that at various other receptor types (38). The importance of a phenylpiperidine with a lipophilic *N*-substituent is apparent from the commonality of this moiety among several structurally diverse compounds with substantial σ site affinity.

Although σ sites were initially defined under an opioid receptor classification as "σ opiate receptors" (3), the failure of opioid antagonists such as naloxone to antagonize "σ" pharmacologic effects indicates that they are not classical opiate receptors (4–8, 14). The structure-affinity relationships of σ sites support their distinction from conventional opioid receptor subtypes. Thus, the stereoselectivity noted at σ receptor-binding sites is generally opposite to that seen at opioid receptors, e.g., the (+)-isomers of benzomorphans have greater affinity than the (−)-isomers at σ sites. The absolute configuration at the chiral carbon atom bound to the nitrogen for the preferred (+)-benzomorphans is opposite to that for classical opioid compounds, such as morphine and naloxone, which lack affinity for σ-binding sites.

(+)-3-PPP and related structures have been extensively characterized for their potential as agonists at presynaptic dopamine receptors (25, 39, 40). In comparing the dopaminergic effects of these compounds to their affinities at σ-binding sites, both similarities and differences are apparent. Almost consistently, larger *N*-alkyl substituents provide greater σ site affinity in the three series of free or embedded 3-phenylpiperidines. The same structural features predict *in vivo* dopamine agonist activity for *S*-phenylpiperidines and the *trans*-4a*S*,10b*S*-7-OH-OHBQs, but such a relationship is less straightforward in the other series of compounds (36, 41).

Focusing on the divergent structural requirements of compounds for dopamine receptors and σ sites, new compounds with better selectivity for σ sites can be designed. Interestingly, among the OHBQs, *cis*- analogs with a large *N*-substituent have considerable σ receptor affinity. By contrast, the *cis*-OHBQs are weak at dopamine receptors compared to the corresponding *trans*- compounds, some of which are very potent dopamine D_2 receptor (pre/postsynaptic) agonists (42). The dopamine D_2 receptor agonists have two defined directions for their *N*-alkyl substituents, one of which is sensitive and the other of which is insensitive to steric bulk. The restricted direction is the one in which aporphines, ergolines, and *trans*-9-OH-OHBQs point their respective *N*-alkyl groups when positioned at the receptor site according to current dopamine receptor models (32, 41). It is known that *N*-*n*-Pr substitution in the restricted direction enhances dopaminergic potency, but *N*-*n*-Bu analogs are much less active or even inactive, presumably from steric hindrance. In contrast, at σ sites, compound **33** (9-OH-4-*n*-Bu-OHBQ), with an *N*-*n*-butyl substituent, has higher affinity than its *n*-Pr analog (compound **29**), and even bulkier groups such as benzyl (Bn) (compound **35**) and phenethyl (Pheth) (compound **37**) can be accommodated by σ-binding sites with very high affinity. Thus, there are several interesting opportunities to design ligands for σ-binding sites which are devoid of pre/postsynaptic dopamine agonist effects. This goal can be achieved by selecting a *cis*-OHBQ with high "σ" affinity, since all *cis*-OHBQs studied so far are poor dopamine receptor agonists (42). Another option is to select one of the non-hydroxylated phenylpiperidines, which are not dopaminergic, among which the optimal choice would seem to be the 3-*F* analog (compound **17**) because of its low dopamine D_2 receptor affinity.[6]

In considering the number of dopaminergic drugs that exhibit affinity for σ-binding sites, such as the phenylpiperidine analogs, OHBQs, and various butyrophenones and phenothiazines, it is tempting to suggest a functional link or an evolutionary relationship between dopaminergic receptors and σ receptor-binding sites. In this context, it is interesting to note that σ compounds (e.g., SKF 10,047 and cyclazocine) influence, by an indirect fashion, dopamine metabolism and neurotransmission (5, 43–46).

Acknowledgments

The authors wish to thank Adele Snowman for her expert technical assistance. Bengt Andersson and Thomas Elebring are gratefully acknowledged for the additional organic synthetic work that was needed to complete this paper, while H. W. was in the laboratory of Dr. Allinger, Department of Chemistry, The University of Georgia, performing molecular mechanics studies. Karin Sabel is gratefully acknowledged for plotting the stereopictures in Fig. 1 with the CHEMX graphics system.

References

1. Haertzen, C. A. Subjective effects of narcotic antagonists cyclazocine and nalorphine on the Addiction Research Center Inventory (ARCI). *Psychopharmacologia* **18**:366–377 (1970).
2. Keats, A. S., and J. Telford. Narcotic antagonists as analgesics. Clinical aspects. *Adv. Chem. Ser.* **45**:170–176 (1964).
3. Martin, W. R., C. G. Eades, J. A. Thompson, R. E. Huppler, and P. E. Gilbert. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmacol. Exp. Ther.* **197**:517–532 (1976).
4. Brady, K. T., R. L. Balster, and E. L. May. Stereoisomers of *N*-allylnormetazocine: phencyclidine-like behavioral effects in squirrel monkeys and rats. *Science (Wash. D. C.)* **215**:178–180 (1982).
5. Iwamoto, E. T. Locomotor activity and antinociception after putative *mu*, *kappa* and *sigma* opioid receptor agonists in the rat: influence of dopaminergic agonists and antagonists. *J. Pharmacol. Exp. Ther.* **217**:451–460 (1981).
6. Katz, J. L., R. D. Spealman, and R. D. Clark. Stereoselective behavioral effects of *N*-allylnormetazocine in pigeons and squirrel monkeys. *J. Pharmacol. Exp. Ther.* **232**:452–461 (1985).
7. Vaupel, D. B. Naltrexone fails to antagonize the σ effects of PCP and SKF 10,047 in the dog. *Eur. J. Pharmacol.* **92**:269–274 (1983).
8. Young, G. A., and N. Khazan. Differential neuropharmacological effects of *mu*, *kappa*, and *sigma* opioid agonists on cortical EEG power spectra in the rat—stereospecificity and naloxone antagonism. *Neuropharmacology* **23**:1161–1165 (1984).
9. Holtzman, S. G. Discriminative stimulus properties of opioids that interact with mu, kappa, and PCP/σ receptors, in *Behavioral Pharmacology: The Current Status* (L. S. Seiden and R. L. Balster, eds). Alan R. Liss, Inc., New York, 131–147 (1985).
10. Slifer, B. L., and R. L. Balster. Reinforcing properties of stereoisomers of the putative σ agonist *N*-allylnormetazocine and cyclazocine in rhesus monkeys. *J. Pharmacol. Exp. Ther.* **225**:522–528 (1983).
11. Steinfels, G. F., S. W. Tam, and L. Cook. (+)-3-(3-Hydroxyphenyl)-*N*-(1-propyl)piperidine [(+)-3-PPP] but not (−)-3-PPP produces (+)-*N*-allylnormetazocine-like (SKF 10,047) discriminative stimuli. *Life Sci.* **29**:2611–2615 (1986).
12. Zukin, R. S., and S. R. Zukin. Multiple opiate receptors: emerging concepts. *Life Sci.* **29**:2681–2690 (1981).
13. Gundlach, A. L., B. L. Largent, and S. H. Snyder. Phencyclidine and σ opiate receptors in brain: biochemical and autoradiographical differentiation. *Eur. J. Pharmacol.* **113**:465–466 (1985).
14. Largent, B. L., A. L. Gundlach, and S. H. Snyder. Pharmacological and autoradiographic discrimination of σ and phencyclidine receptor binding sites in brain with (+)[^{3}H]SKF 10,047, (+)-[^{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).
15. Largent, B. L., A. L. Gundlach, and S. H. Snyder. *Sigma* receptors on NCB-20 hybrid neurotumor cells labeled with (+)[^{3}H]SKF 10,047 and (+)[^{3}H]3-PPP. *Eur. J. Pharmacol.* **124**:183–187 (1986).

[6] B. Largent, unpublished observations.

16. Martin, B. R., J. S. Katzen, J. A. Woods, H. L. Tripathi, L. S. Harris, and E. L. May. Stereoisomers of [^{3}H]-*N*-allylnormetazocine bind to different sites in mouse brain. *J. Pharmacol. Exp. Ther.* **231**:539–544 (1984).
17. Tam, S. W. Naloxone-inaccessible *sigma* receptor in rat central nervous system. *Proc. Natl. Acad. Sci. USA* **80**:6703–6707 (1983).
18. Tam, S. W., and L. Cook. σ opiates and certain antipsychotic drugs mutually inhibit (+)-[^{3}H]SKF 10,047 and [^{3}H]haloperidol binding in guinea pig brain membranes. *Proc. Natl. Acad. Sci. USA* **81**:5618–5621 (1984).
19. Weber, E., M. Sonders, M. Quarum, S. McLean, S. Pou, and J. F. W. Keana. 1,3-Di(2-[5-^{3}H]tolyl)guanidine: a selective ligand that labels σ-type receptors for psychotomimetic opiates and antipsychotic drugs. *Proc. Natl. Acad. Sci. USA* **83**:8784–8788 (1986).
20. Tam, S. W. (+)-[^{3}H]SKF 10,047, (+)-[^{3}H]ethylketocyclazocine, *mu*, *kappa*, *delta* and phencyclidine binding sites in guinea pig brain membranes. *Eur. J. Pharmacol.* **109**:33–41 (1985).
21. Sonders, M., B. Cambell, and E. Weber. Identification and partial characterization of an endogenous compound that competitively and reversibly inhibits [^{3}H]-DTG from binding to the haloperidol-sensitive sigma (opioid) receptor. *Soc. Neurosci. Abstr.* **12**:172 (1986).
22. Su, T.-P., A. D. Weissman, and S.-Y. Yeh. Endogenous ligands for σ opioid receptors in the brain ("sigmaphin"): evidence from binding assays. *Life Sci.* **38**:2199–2210 (1986).
23. 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).
24. Arnt, J., K. P. Bogeso, A. V. Christensen, J. Hyttel, J.-J. Larsen, and O. Svendsen. Dopamine receptor agonistic and antagonistic effects of 3-PPP enantiomers. *Psychopharmacology* **81**:199–207 (1983).
25. Hjorth, S., A. Carlsson, H. Wikström, P. Lindberg, D. Sanchez, U. Hacksell, L.-E. Arvidsson, U. Svensson, and J. L. G. Nilsson. 3-PPP, a new centrally acting DA-receptor agonist with selectivity for autoreceptors. *Life Sci.* **28**:1225–1238 (1981).
26. Hjorth, S., A. Carlsson, D. Clark, K. Svensson, H. Wikström, D. Sanchez, P. Lindberg, U. Hacksell, L.-E. Arvidsson, A. Johansson, and J. L. G. Nilsson. Central dopamine receptor agonist and antagonist actions of the enantiomers of 3-PPP. *Psychopharmacology* **81**:89–99 (1983).
27. Markstein, R., and D. Lahaye. *In vitro* effect of the racemic mixture and the (−)enantiomer of *N*-*n*-propyl-3-(3-hydroxyphenyl)-piperidine (3-PPP) on postsynaptic dopamine receptors and on a presynaptic dopamine autoreceptor. *J. Neural Transm.* **58**:43–53 (1983).
28. Mulder, A. H., R. Draper, P. Sminia, A. N. M. Schofflemeer, and J. C. Stoof. Agonist and antagonist effects of 3-PPP enantiomers on functional dopamine autoreceptors and postsynaptic dopamine receptors *in vitro*. *Eur. J. Pharmacol.* **197**:291–297 (1985).
29. McPherson, G. A. A practical computer-based approach to the analysis of radioligand binding experiments. *Comput. Programs Biomed.* **17**:107–114 (1983).
30. Allinger, N. L., and Y. Yuh. Molecular mechanics II. *Quantum Chem. Program Exchange* **13**:395 (1980).
31. Kocjan, D., and D. Hadzi. Conformationally restricted dopamine congeners—a molecular mechanics-based study. *J. Pharm. Pharmacol.* **35**:780–785 (1983).
32. Liljefors, T., and H. Wikström. A molecular mechanics approach to the understanding of presynaptic selectivity for centrally acting dopamine receptor agonists of the phenylpiperidine series. *J. Med. Chem.* **29**:1896–1904 (1986).
33. Su, T.-P. Psychotomimetic opioid binding: specific binding of [3H]SKF-10047 to etorphine-inaccessible sites in guinea pig brain. *Eur. J. Pharmacol.* **75**:81–82 (1981).
34. Su, T.-P. Evidence for σ opioid receptor: binding of [^{3}H]SKF-10047 to etorphine-inaccessible sites in guinea-pig brain. *J. Pharmacol. Exp. Ther.* **223**:284–290 (1982).
35. Hacksell, U., L.-E. Arvidsson, U. Svensson, J. L. G. Nilsson, D. Sanchez, H. Wikström, P. Lindberg, S. Hjorth, and A. Carlsson. 3-Phenylpiperidines. Central dopamine-autoreceptor stimulating activity. *J. Med. Chem.* **24**:1475–1482 (1981).
36. Wikström, H., D. Sanchez, P. Lindberg, U. Hacksell, L.-E. Arvidsson, A. M. Johansson, S.-O. Thorberg, J. L. G. Nilsson, K. Svensson, S. Hjorth, D. Clark, and A. Carlsson. Resolved 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine and its analogs: central dopamine receptor activity. *J. Med. Chem.* **27**:1030–1036 (1984).
37. Van de Waterbeemd, H., N. Tayar, B. Testa, H. Wikström, and B. L. Largent. QSAR and eudismic analyses of the presynaptic dopaminergic activity and dopamine D-2 and sigma receptor affinities of 3-(3-hydroxyphenyl)piperidines and octahydrobenzo[*f*]quinolines. *J. Med. Chem.*, in press.
38. Smith, D. F., ed. *CRC Handbook of Stereoisomers: Drugs in Psychopharmacology* CRC Press, Boca Raton, FL (1984).
39. Clark, D., S. Hjorth, and A. Carlsson. Dopamine receptor agonists: mechanisms underlying autoreceptor selectivity. I. Review of the evidence. *J. Neural Transm.* **62**:1–52 (1985).
40. Clark, D., S. Hjorth, and A. Carlsson. Dopamine receptor agonists: mechanisms underlying autoreceptor selectivity. II. Theoretical considerations. *J. Neural Transm.* **62**:171–207 (1985).
41. Wikström, H., B. Andersson, D. Sanchez, P. Lindberg, L.-E. Arvidsson, A. M. Johansson, J. L. G. Nilsson, K. Svensson, S. Hjorth, and A. Carlsson. Resolved monophenolic 2-aminotetralins and 1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinolines: structural and stereochemical consideration for centrally acting pre- and post-synaptic dopamine receptor agonists. *J. Med. Chem.* **28**:215–225 (1985).
42. H. Wikström, D. Sanchez, P. Lindberg, L.-E. Arvidsson, U. Hacksell, A. Johansson, J. L. G. Nilsson, S. Hjorth, and A. Carlsson. Monophenolic octahydrobenzo[*f*]quinolines: central dopamine- and serotonin-receptor stimulating activity. *J. Med. Chem.* **25**:925–931 (1982).
43. Freeman, A. S., and B. S. Bunney. The effects of phencyclidine and *N*-allylnormetazocine on midbrain dopamine neuronal activity. *Eur. J. Pharmacol.* **104**:287–293 (1984).
44. Haubrich, D. R., and A. B. Pflueger. The autoreceptor control of dopamine synthesis. *Mol. Pharmacol.* **21**:114–120 (1981).
45. Iwamoto, E. T. Comparison of the neuropharmacologic effects of *N*-allylnormetazocine (NAMN) and phencyclidine (PCP). *Pharmacologist* **23**:421 (1981).
46. Wood, P. L., M. Stotland, J. W. Richard, and A. Rackham. Actions of *mu*, *kappa*, *sigma*, and *delta* agonist/antagonist opiates on striatal dopaminergic function. *J. Pharmacol. Exp. Ther.* **215**:697–703 (1980).

Send reprint requests to: Dr. Solomon H. Snyder, Department of Neuroscience, The Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205.