0026-895X/88/0650863-17$02.00/0

MOLECULAR PHARMACOLOGY, 34: 863-879

Receptor Site Topographies for Phencyclidine-Like and σ Drugs: Predictions from Quantitative Conformational, Electrostatic Potential, and Radioreceptor Analyses

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Received May 13, 1988; Accepted August 29, 1988

SUMMARY

Computer-assisted molecular modelling techniques and electrostatic analyses of a wide range of phencyclidine (PCP) and σ ligands, in conjunction with radioreceptor studies, were used to determine the topographies of the PCP and σ receptors. The PCP receptor model was defined using key molecules from the arylcyclohexylamine, benzomorphan, bridged benz[*f*]isoquinoline, and dibenzocycloalkenimine drug classses. Hypothetical receptor points (R1, R2) were constructed onto the aromatic ring of each compound to represent hydrophobic interactions with the receptor, along with an additional receptor point (R3) representing a hydrogen bond between the nitrogen atom and the receptor. The superimposition of these key molecules gave the coordinates of the receptor points and nitrogen defining the primary PCP pharmacophore as follows: R1 (0.00, 3.50, 0.00), R2 (0.00, −3.50, 0.00), R3 (6.66, −1.13, 0.00), and N (3.90, −1.46, −0.32). Additional analyses were used to describe secondary binding sites for an additional hydrogen bonding site and two lipophilic clefts. Similarly, the σ receptor model was constructed from ligands of the benzomorphan, octahydrobenzo[*f*]quinoline, phenylpiperidine, and diphenylguanidine drug classes. Coordinates for the primary σ pharmacophore are as follows: R1 (0.00, 3.50, 0.00), R2 (0.00, −3.50, 0.00), R3 (6.09, 2.09, 0.00), and N (4.9, −0.12, −1.25). Secondary binding sites for σ ligands were proposed for the interaction of aromatic ring substituents and large *N*-substituted lipophilic groups with the receptor. The σ receptor model differs from the PCP model in the position of nitrogen atom, direction of the nitrogen lone pair vector, and secondary σ binding sites. This study has thus demonstrated that the differing quantitative structure-activity relationships of PCP and σ ligands allow the definition of discrete receptors. These models may be used in conjunction with rational drug design techniques to design novel PCP and σ ligands of high selectivity and potency.

Shortly after PCP (an arylcyclohexylamine) was introduced into clinical trials as an anaesthetic agent, it was withdrawn due to the regular occurrence of bizarre postoperative side-effects, including vivid dreaming and confusion states (1, 2). PCP possesses desirable features such as minimal cardiorespiratory depression (1); however, recent interest in PCP-like compounds has arisen from animal studies indicating that these drugs act as anticonvulsants (3–6) and anxiolytics (7), and protect against neuronal degeneration caused by ischaemia, anoxia, hypoglycaemia, and endogenous neurotoxins (8, 9). PCP-like drugs act as noncompetitive antagonists at the NMDA subtype of L-glutamate receptor (10–13) by blocking the ion channel of the NMDA receptor-ionophore complex (13–15). The pharmacological activity of PCP-like drugs correlates

Discussions with Drs. A. L. Gundlach and S. H. Synder were greatly appreciated. This work was supported by a Programme Grant from the National Health and Medical Research Council of Australia.

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ABBREVIATIONS: PCP, phencyclidine; L-AP4, L-amino-4-phosphonobutyric acid; D-AP5, D-2-amino-5-phosphonopentanoic acid; DL-AP7, DL-2-amino-7-phosphonoheptanoic acid; CNS, central nervous system; DPG, 1,3-diphenylguanidine; DTG, 1,3-di(2-tolyl)guanidine; IC_{50}, concentration giving 50% inhibition; K_G, dissociation constant for single binding component; K_H, K_L, dissociation constants for high and low affinity binding components; LY 154045, 3-(cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-4a,10b-propanobenz[*f*]isoquinolin-9-ol; LY 156007, 3-(cyclopropylmethyl)-1,2,3,4,4a,5,6,10b-octahydro-10b-methyl-benz[*f*]isoquinolin-9-ol; (−)2-MDP, (−)-2-methyl-3,3-diphenyl-3-propanolamine; MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzo[*a,d*]cyclohepten-5,10-imine; m-NH_2-PCP, *N*-(1-[3-aminophenyl]cyclohexyl)piperidine; NMDA, *N*-methyl-D-aspartate; OHBQ, 1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline; *m*-OH-PCP, *N*-(1-[3-hydroxyphenyl]cyclohexyl)piperidine; PCA, 1-phenylcyclohexylamine; Pr, propyl; PPP, (+)-3-phenyl-*N*-(1-propyl)piperidine; 3-PPP, 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine; QSAR, quantitative structure-activity relationships; R_H, R_L, receptor concentrations for high and low affinity components; RMS, root mean square; RU 38796, 3-(3-hydroxyphenyl)-*N*-(1-propyl)-1,2,5,6-tetrahydropyridine; SAR, structure-activity relationships; SKF 10,047, *N*-allylnormetazocine; τ, torsion angle; TCP, 1-[1-(2-thienyl)cyclohexyl]piperidine; Bu, butyl.

better with their noncompetitive NMDA antagonist action (12, 16–18) than with other effects. In addition, PCP receptors have been co-localized with NMDA receptors in autoradiographic analyses (19–21).

The possible clinical applications of PCP-like molecules mean that their SAR assume considerable importance. Modifications to the backbone of the arylcyclohexylamine structure have provided considerable data about the SAR of this class of centrally acting psychotomimetic agents (22–30). Few of these SAR studies have diverged greatly from an analysis of the arylcyclohexylamine structure, although Cone and co-workers (22) developed a receptor model for PCP-like drugs consisting of a drug receptor surface with multiple subsites. Recently, our laboratory employed computer graphic techniques to superimpose four PCP-like drugs from different structural classes (31). From these preliminary analyses we described a receptor model for the PCP binding site consisting of a primary pharmacophore and secondary binding site locations (31).

σ Opiates possess some pharmacological properties in common with PCP-like drugs. Martin and co-workers (32) used a chronic spinal dog preparation to examine the actions of several opiate analgesics and described at least three distinct opioid receptors (μ, κ, and σ) based on the actions of morphine, ketocyclazocine, and *N*-allylnormetazocine [(±) SKF 10,047], respectively. (±)SKF 10,047 induced a canine dilerium as well as autonomic stimulation producing mydriasis, tachypnea, and tachycardia. Opioids classified as having σ actions have also been shown to be psychotomimetic in humans (33, 34). In drug discrimination studies, animals do not distinguish between PCP-like drugs and σ opiates (35). In addition, many of the psychotomimetic σ opiates displaced [^{3}H]PCP from its binding site (23, 36). These observations led Zukin and Zukin (37) to propose that σ-like behavioral effects may be exerted through the PCP receptor and they coined the terminology σ/PCP to describe this common site. Recent radioreceptor and autoradiographic studies have, however, clearly delineated a high affinity (+)-[^{3}H]SKF 10,047/σ site from the PCP binding site (31, 38–40). The term 'opiate' now seems inappropriate, inasmuch as the stereochemistry of the benzomorphans differs from that of classical opiates and their actions are not blocked by the opioid antagonists, such as naloxone or naltrexone (35). The recommended nomenclature for this binding site has been standardized and it is now referred to as the σ-binding site (41).

Until recently few SAR studies on σ activity had been conducted. Largent and co-workers (40) surveyed an extensive list of compounds to analyze the structural determinants of σ receptor affinity. More recently, two SAR studies have analyzed the activity of a series of OHBQ and 3-phenylpiperidines at the σ receptor (42, 43). These studies found a wide degree of tolerance for both the stereochemical and topographic/molecular demands of σ ligands (42, 43). Our laboratory recently investigated several σ ligands that were structurally unrelated, using computer graphic techniques (31), and described a receptor model consisting of a primary pharmacophore for σ activity and secondary binding site locations (31). We now present full analyses of the QSAR and radioreceptor studies, which allow us to describe distinct receptor models for σ- and PCP-like drugs.

Materials and Methods

Radioligand binding. Male Sprague Dawley rats (200–300 g) were used for radioligand binding experiments and membranes were prepared employing a method similar to that of Largent and co-workers (38). Briefly, whole brain (minus cerebellum) was 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°. The resultant pellet was resuspended in 25 volumes of fresh buffer and centrifuged at 45,000 × *g* for 10 min at 4°. This procedure was repeated once and the final pellet was suspended in the appropriate volume of ice-cold incubation buffer (5 mM Tris·HCl buffer, pH 8.0 at 25°, for [^{3}H]TCP binding and 50 mM Tris·HCl buffer, pH 8.0 at 25°, for (*R*)-(+)-[^{3}H]3-PPP binding).

[^{3}H]TCP and (*R*)-(+)-[^{3}H]3-PPP binding experiments were performed at 25°, using a final volume of 250 μl, which consisted of 100 μl of tissue homogenate containing approximately 7.5 mg of tissue (original wet weight, 370–440 μg of protein), 100 μl of various concentrations of test compounds, and 50 μl of 2–5 nM [^{3}H]TCP or 3 nM (*R*)-(+)-[^{3}H]3-PPP. Protein was determined by the method of Lowry *et al.* (44). Incubations were terminated at 30 or 90 min for [^{3}H]TCP and (*R*)-(+)-[^{3}H]3-PPP binding experiments, respectively, by the addition of 2.5 ml of ice-cold incubation buffer. Membranes were collected by filtration under vacuum onto glass fiber filters pretreated with 0.5% polyethylenimine and washed with two 5-ml aliquots of ice-cold buffer. Nonspecific binding for [^{3}H]TCP and (*R*)-(+)-[^{3}H]3-PPP was defined with 5 μM MK-801 and 2.5 μM haloperidol, respectively. Binding had reached equilibrium under the conditions employed. Bound radioactivity was estimated by liquid scintillation counting.

Data analysis. Binding data from individual displacement studies were analyzed by iterative curve fitting (45) to provide estimates of drug affinity and slope factor (analogous to the Hill coefficient). More detailed analyses were performed using the iterative, nonlinear curve fitting programme LIGAND (46), in which displacement data were analyzed according to a model for the binding of the competing drug to one or two binding sites. A two-site model was retained to which the data fitted significantly better than to a one-site binding model. Estimates of binding parameters for each drug examined were obtained initially by analysis of individual experiments. Final parameters were obtained by simultaneously examining the data curves from multiple experiments, which has been demonstrated to increase statistical reliability (47). These analyses provided estimates of K_G (dissociation constant for single binding component); K_H and K_L (dissociation constants for high and low affinity components); and R_H and R_L (receptor concentration for high and low affinity components). Approximate standard error estimates generated along with these parameters gave an indication of the reliability of the parameters.

Conformational analysis. 1. Molecular choice of compounds for the PCP and σ sites was based on the availability of atomic coordinates and structural diversity. The potency of each compound was determined from radioligand binding studies (present study and Refs. 23–26, 39, and 40) or from behavioral experiments (27, 28). Compounds listed as inactive or having a very low potency were chosen to aid in the location of secondary binding sites. The strategy used was to locate all possible low energy conformations available to each molecule, determine potential energies as appropriate, and then superimpose chosen conformers to define the coordinates of each receptor site.

The primary pharmacophore for the PCP receptor model was defined using MK-801, (+)SKF 10,047, *m*-OH-PCP, and LY 154045 the affinities of which for the [^{3}H]TCP binding site ranged from 6 to 1000 nM. Five molecules [haloperidol, (+)-SKF 10,047 (*N*-invert), DTG, (*R*)-(+)-[^{3}H]3-PPP, and (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ] were used to define the primary pharmacophore of the σ binding site; relative affinities [except (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ] were 12–1500 nM.

2. Torsion angles τ(ABCD) varied in this study have been labeled in Figs. 1 and 2 and are defined as the clockwise rotation of atom A required to eclipse atom D while looking along the B—C bond from atom B to atom C (48). Classical potential energy calculations were

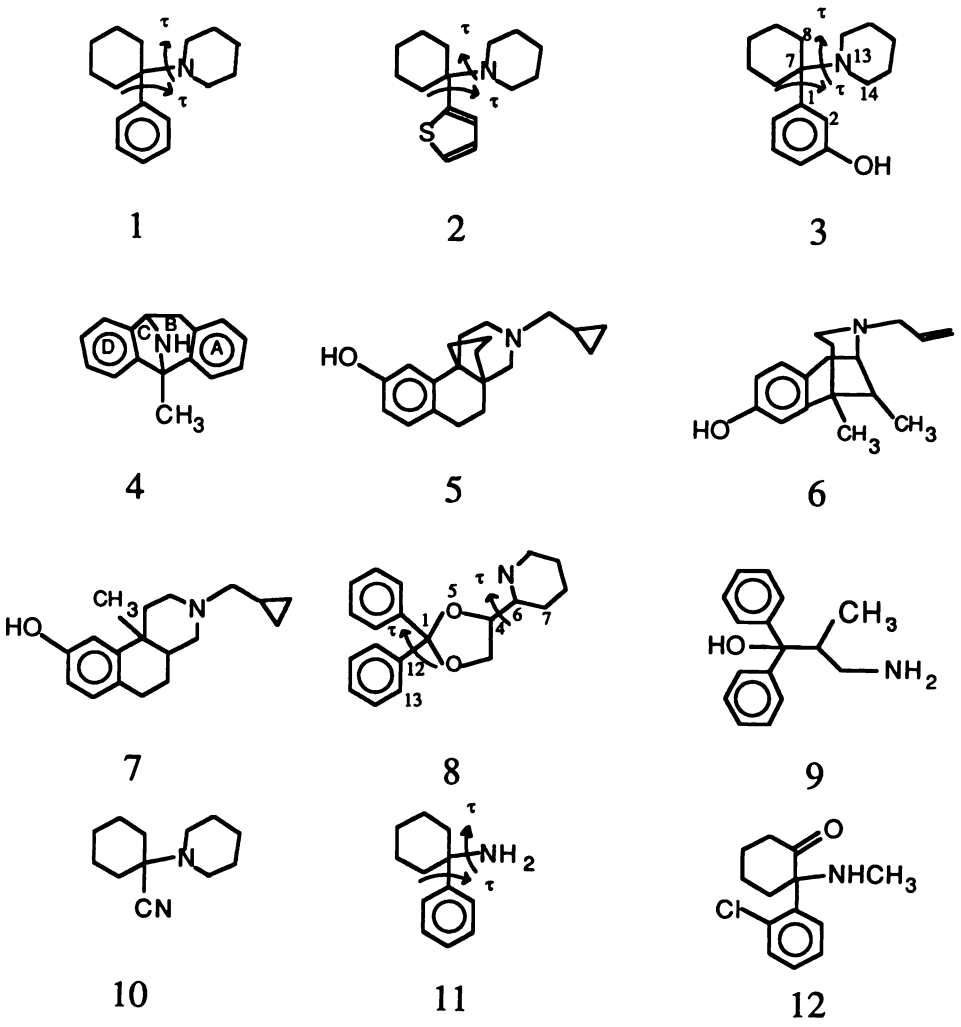

Fig. 1. Structures of some PCP-ligands examined in this study. PCP (**1**), TCP (**2**), *m*-OH-PCP (**3**), MK-801 (**4**), LY 154045 (**5**), (+)-SKF 10,047 (**6**), LY 156007 (**7**), dexoxadrol (**8**), 2-MDP (**9**), 1-piperidinocyclohexanecarbonitrile (**10**), PCA (**11**), and ketamine (**12**). Torsion angles used throughout this study have been indicated (τ). *m*-OH-PCP uses the numbering system employed for the crystal structure of PCP (62).

used to find all low energy conformations for each molecule by varying up to two torsion angles labeled in Figs. 1 and 2 at a time.

3. Potential energies were calculated at intervals of 15° for each variable with refinements at 1° intervals. These calculations pairwise sum the van der Waals interactions between nonbonded atoms (49). The parameterization employed was developed by Giglio (50) based on hydrocarbon and amide structures. This method, however, holds molecular geometries rigid, ignores electrostatic charges, and tends to overestimate molecular barriers. As a consequence, quantitative energy differences obtained between conformations should not strictly be used to calculate relative conformer populations. However, the qualtitative nature of the results allow the rapid determination of probable molecular configurations. Thereupon, all thermally accessible conformations within 5 kcal/mol of the global minimum conformation were accepted. Potential energy contour maps were generated using the programme CONES, which employs a modified version of the subroutine KONTOR (51). The potential energy maps are contoured with a 2.5 kcal/mol difference between adjacent lines. Only the first 20 lines above the global minimum are shown.

4. Electrostatic potential energies were generated using the program CNDO/INDO (52, 53). This program uses atomic electron densities to calculate electrostatic potentials by utilizing the averaged spherical atomic orbital approximation with complete neglect of differential overlap. The results obtained reproduce important features of *ab initio* calculations (see Ref. 54). Electrostatic potential maps were plotted using the program ELCPOT [a program within the MORPHEUS computer graphics system developed at the Victoria College of Pharmacy, Ltd., Parkville, Australia (55)], which uses the sperical orbital approximations of Giessner-Prettre and Pullman (53).

5. Molecular display, manipulation, and superimposition were carried out using the computer graphics system MORPHEUS (55). Molecular geometries were obtained from X-ray crystal data or from crystal data of related compounds onto which extra groups were constructed using standard bond lengths and angles (56, 57). The molecular mechanics program MM2 (58) was employed to optimize the geometry of LY 154045.

The majority of CNS active drugs share the common features of an aromatic ring and a N atom. On the basis of these and other observations, Lloyd and Andrews (59) advanced the idea of a common structural model for all CNS active drugs. This model used the aromatic ring and N moieties as primary binding groups. We have adopted the methods of Lloyd and Andrews (59) to define the primary pharmacophores for the PCP and σ binding sites by mapping the topographic arrangements of the phenyl ring, N atom, and N lone pair vector. To achieve this, dummy atoms or receptor points R1 and R2 were built 3.5 Å above and below the center of a phenyl ring as origin (centroid) to represent hydrophobic bonding to a receptor (Fig. 3) (60). A point R3 was placed 2.8 Å tetrahedrally from N atoms to represent an interaction between a protonated N atom and its binding site (59, 61). All possible low energy conformers were compared to find common features and receptor points for ligands at the PCP and σ sites. Dreiding molecular models were used initially to identify common features between drug classes. Receptor models were refined using a computerized technique that allowed the simultaneous minimization of both energy and geometric fit between chosen features (55).

6. In the superimposition of molecules to define the PCP primary pharmacophore, the crystal structure of the highly potent and struc-

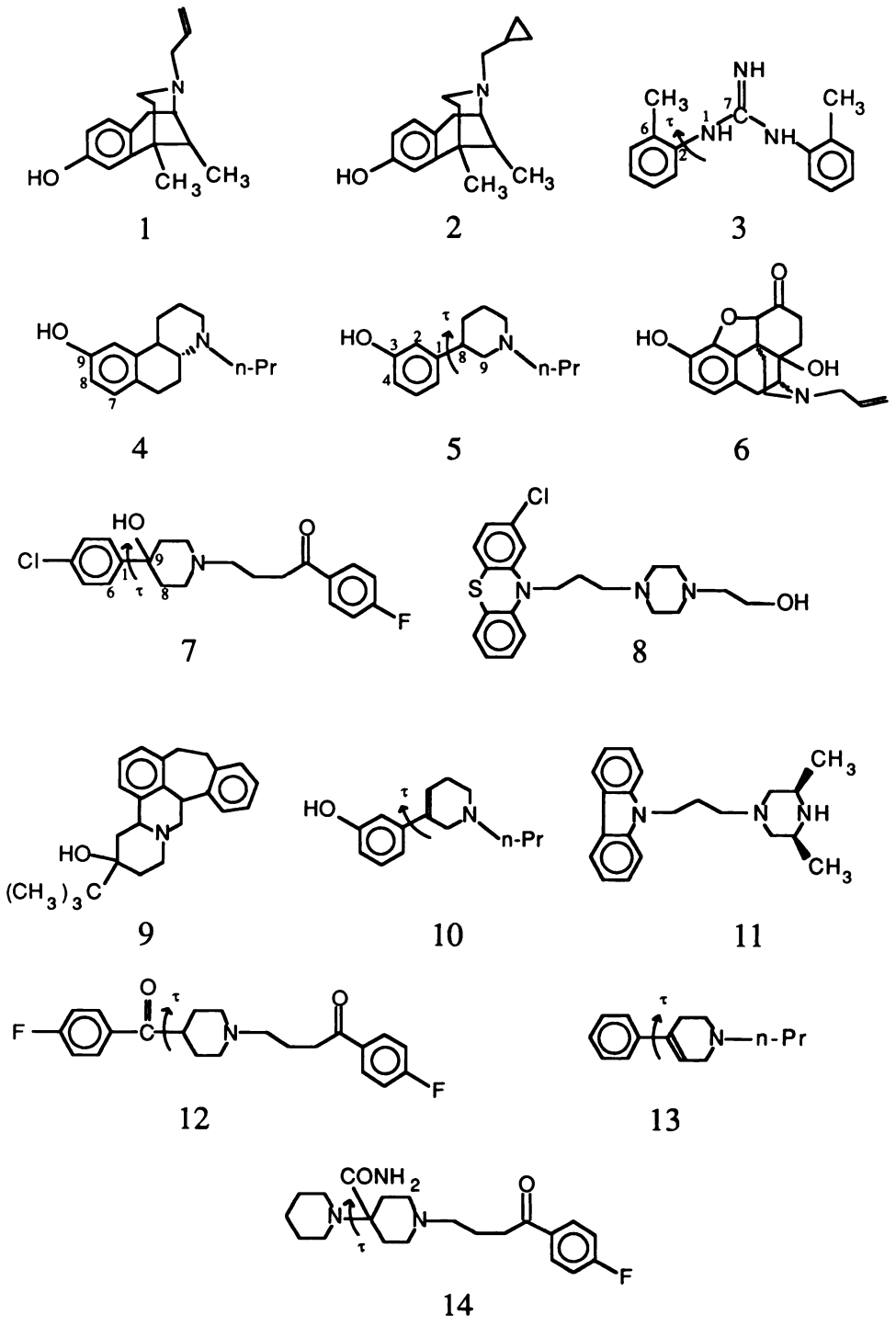

Fig. 2. Structures of some of the drugs used to investigate the σ binding site, (+)-SKF 10,047 (*N*-invert) (**1**), cyclazocine (*N*-invert) (**2**), DTG (**3**), (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ (**4**), (*R*)-(+)3-PPP (**5**), naloxone (**6**), haloperidol (**7**), perphenazine (**8**), butaclamol (**9**), RU 38796 (**10**), rimcazole (**11**), lenperone (**12**), *N*-1-*n*-propyl-4-phenyl-1,2,5,6-tetrahydropyridine (**13**), and pipamperone (**14**). Torsion angles used throughout this study have been indicated (τ).

turally rigid PCP receptor ligand MK-801 was assumed to be the biologically active conformation and was employed as the template. Two possible combinations of phenyl ring and N atom were available to MK-801. The final combination of phenyl ring and N of MK-801 finally used was determined by superimposition onto the receptor points of *m*-OH-PCP [conformation based on crystal structure of PCP (62)]. The configuration that gave both the highest degree of fit to the four guide points and the highest degree of overlap of the second phenyl ring and the cyclohexane ring of *m*-OH-PCP was used. After the choice of primary aromatic ring for MK-801 (compound **4**, ring A; Fig. 1), the molecules LY 154045 and (+)-SKF 10,047 (Fig. 1) were superimposed onto the structure of MK-801, using the guide points R1, R2, R3, and N. The conformation used for *m*-OH-PCP was refined by simultaneously altering the torsion angles τ(C8, C7, C1, C2) and τ(C8, C7, N13, C14) while minimizing the geometric fit to the four guide points of MK-801. All four molecules were then superimposed and the receptor points R1, R2, R3, and N were averaged to give the coordinates of the primary PCP receptor model. An additional receptor point was defined by averaging the coordinates of the oxygen atom of the three molecules (*m*-OH-PCP, LY 154045, and (+)-SKF 10,047) in the primary model with hydroxyl groups.

7. The superimposition of the five molecules used to define the primary pharmacophore of the σ site followed a similar method to that used for the PCP site. Firstly, all possible low energy conformers for (*R*)-(+)-3-PPP, DTG, and haloperidol were determined by varying the torsion angles τ(C2, C1, C8, C9), τ(C6, C2, N1, C7), and τ(C6, C1, C9,

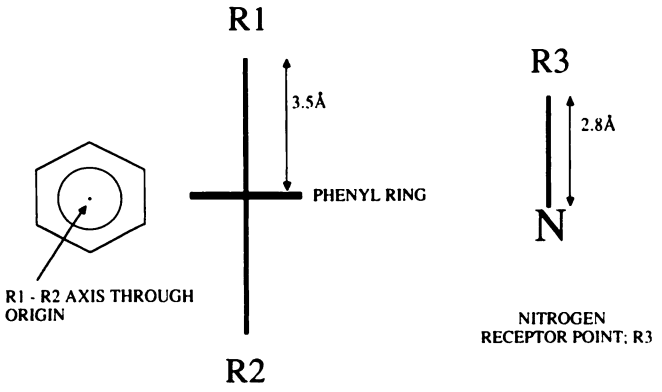

Fig. 3. Diagram detailing the position of hypothetical receptor points (R1, R2, R3) used as guide points for molecular superimpositions. Receptor points R1 and R2 were placed 3.5 Å above and below the center of phenyl rings (centroid) to represent hydrophobic interactions with the receptor and a nitrogen receptor point, R3, was located tetrahedrally, 2.8 Å from nitrogen atoms to represent a hydrogen bond with the receptor.

C8), respectively (Fig. 2). The potent and relatively structurally rigid σ receptor ligand (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ was then used as the template onto which (+)-SKF 10,047 and low energy conformers of (*R*)-(+)-3-PPP, DTG, and haloperidol were superimposed. For the molecules (*R*)-(+)-3-PPP, DTG, and haloperidol, the aforementioned torsion angles were altered to simultaneously minimize the potential energy and geometric fit to (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ. Two conformations of (+)-SKF 10,047 [one based on crystal structure of cyclazocine (63) versus another structure with nitrogen inversion] were considered for the σ site and the conformer that fitted better to (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ was finally used. All five molecules were then superimposed and the receptor points R1, R2, R3, and N were averaged to give the coordinates of the primary σ receptor model.

8. Three calculations were then performed on each compound. The first calculation gave the energy, in kilocalories/mole, above the global minimum conformation. The second calculated the RMS distance between the points R1, R2 and R3 and the N atom to the corresponding points on the test compound and, where relevant (PCP site only), the —*eta*-oxygen was included to give a five-point comparison. A good 'fit' is indicated by low values for both the RMS distance (<0.8 Å) and potential energy (<5 kcal/mol above the global minimum). The third calculation used the program OVALAP to determine the per cent molecular overlap volume of representative compounds with *m*-OH-PCP and (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ for the PCP and σ sites, respectively (64).

Materials. Chemicals were obtained from the following sources: (*R*)-(+)-[^{3}H]3-PPP (99.3 Ci/mmol) and [^{3}H]TCP (47.6 Ci/mmol), New England Nuclear, Boston, MA); (+)- and (−)-SKF 10,047, National Institute on Drug Abuse (Rockville, MD); TCP and *m*-OH-PCP, J.-M. Kamenka (Montpellier, France); LY 154045 and LY 156007, Eli Lilly (Indianapolis, IN); MK-801 and (−)-MK-801, Merck Sharp & Dohme, (Harlow, UK, and Rahway, NJ); haloperidol, Searle (Sydney, Australia); (*R*)-(+)- and (*S*)-(−)-3-PPP, Astra (Sodertalje, Sweden); (*trans*)-(±)-9-OH-*n*-Bu-OHBQ, H. Wikstrom (Goteborg, Sweden); DTG, E. Weber (Portland, OR); DPG, BDH Chemicals (Poole, England); (+)- and (−)-butaclamol, Ayerst Laboratories (Montreal, Canada); naloxone, Endo Laboratories (New York, NY); dexoxadrol and (−)-2-MDP, Upjohn (Kalamazoo, MI); PCP, D. Lodge (London, England); rimcazole, Burroughs Wellcome (Research Triangle Park, NC); ketamine, Warner Lambert (Sydney, Australia); D-AP5, DL-AP7, and L-AP4, Tocris Chemicals (Buckhurst Hill, England); L-glutamate, Calbiochem (Los Angeles, CA); L-aspartate, BDH Chemicals (Poole, England); glycine, Ajax Chemicals (Sydney, Australia); D-glutamate, kainic acid, and NMDA, Sigma Chemical Co. (St. Louis, MO); and quisqualic acid, Cambridge Research Biochemicals Ltd. (Cambridge, England). All other reagents were obtained from commercial sources.

Results

PCP Receptor Model

[^{3}H]TCP binding. As described previously (38, 65), [^{3}H]TCP bound to a single population of binding sites in rat brain membranes with a dissociation constant of 10.2 ± 5.0 nM (four experiments) and density of binding sites of 0.32 ± 0.15 pmol/mg of protein (four experiments). The drug specificity of ligands displacing [^{3}H]TCP was consistent with previous studies demonstrating that the binding site correlates with that of a PCP receptor (10, 15, 23, 38, 65), with the rank order of potency for the compounds chosen to define the primary pharmacophore being MK-801 > *m*-OH-PCP > LY 154045 > (+)-SKF 10,047 (Fig. 4; Table 1; cf. 10, 38).

Primary pharmacophore. Once the appropriate configuration of phenyl ring and N atom had been established for MK-801 (see Materials and Methods), superimpositions of *m*-OH-PCP, LY 154045, and (+)-SKF 10,047 were employed to define the coordinates of the primary receptor model for the PCP binding site. LY 154045 and (+)-SKF 10,047 are relatively rigid molecules and conformational analyses were not conducted on these molecules. The configuration of (+)-SKF 10,047 [based on crystal structure of cyclazocine (63)] and MK-801,[3] and in the case of LY 154045 the optimal structure minimized by MM2 calculations, were considered to be their biologically active conformations. The semirigid molecule *m*-OH-PCP has two variable torsion angles (Fig. 1) and the potential energy surface obtained by their rotation is shown in Fig. 5. There are two series of energetically feasible conformations available to *m*-OH-PCP, which correspond to the plane of the phenyl ring being perpendicular to the plane of the cyclohexane ring at

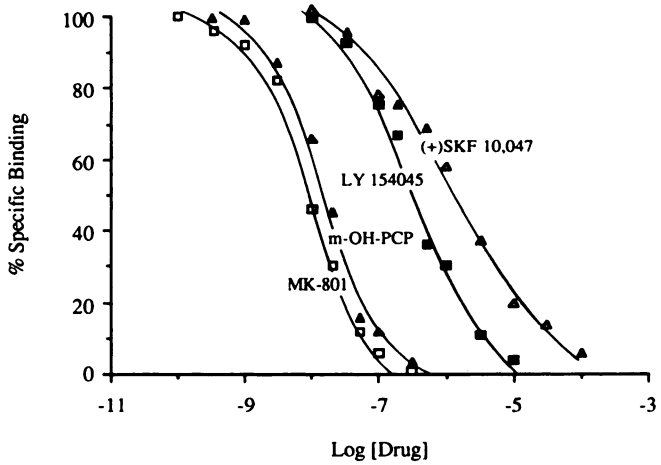

Fig. 4. Inhibition of specific [^{3}H]TCP binding to rat brain membranes by the four PCP ligands used to define the primary pharmacophore of the PCP receptor. Membranes were incubated with 2–5 nM [^{3}H]TCP and at least 10 concentrations of each drug for 30 min at 25°. Data shown are from typical experiments representing the mean of replicate determinations. Rank order of potency: MK-801 (□) > *m*-OH-PCP (▲) > LY 154045 (■) > (+)-SKF 10,047 (△).

[3] The crystal coordinates of MK-801 were kindly provided by Dr. P. S. Anderson, Merck Sharpe and Dohme, West Point, PA.

TABLE 1

Energy, distance, potency, and overlap of several PCP-like compounds

Energy above the global minimum, RMS distances, percentage overlaps, and potencies for compounds at the PCP receptor model. The following compounds (25 μM) failed to displace 50% of specific [^{3}H]TCP binding: DL-AP7, D-AP5, L-AP4, L-aspartate, L-glutamate, D-glutamate, (*R*)-(+)3-PPP, (*S*)-(−)3-PPP, kainic acid, quisqualic acid, NMDA, and glycine. NT, not tested. (±) indicates that the radioligand binding data was only available for the racemic mixture; however, the energy and fit calculations are for the isomer specified.

Compound	Energy[a]	Distance[b]	Distance[c]	Potency[d]	Overlap[e]
	kcal/mol	Å		nM	%
TCP	0.0	0.30		10 ± 5.0	85
m-OH-PCP	0.1	0.26	0.33	13 ± 4.4	100
PCP	0.1	0.26		71 ± 2.3	
Ketamine (*R*)-isomer	0.1			(±)1200 ± 330	
Ketamine (*S*)-isomer	0.1			(±)1200 ± 330	
(+)-SKF 10,047	0.0	0.37	0.41	1200 ± 160	70
(−)-SKF 10,047	0.0	0.61		1200 ± 56	
LY 154045	0.0	0.47	0.43	310 ± 65	72
LY 156007	NT			740 ± 170	
MK-801	0.0	0.49		6.3 ± 1.3	72
(−)-MK-801	0.0	0.38		19 ± 2.8	
Dexoxadrol	3.8	0.79		51 ± 13	
(−)-2-MDP	NT			125 ± 9.0	

[a] Energy above the global minimum conformation in kcal/mol of each compound as fitted to the PCP receptor model.
[b] Best fit distance (RMS) in Å using R1, R2, R3, and N atom as guide points.
[c] Best fit distance (RMS) in Å using R1, R2, R3, N atom, and oxygen atoms as guide points to the PCP receptor model.
[d] Potencies (nM) are represented as the affinity constant (K_d) ± standard error from three or four determinations employing [^{3}H]TCP in a radioreceptor assay with brain membranes. Further details given in Materials and Methods.
[e] Percentage molecular overlap relative to *m*-OH-PCP for selected PCP ligands.

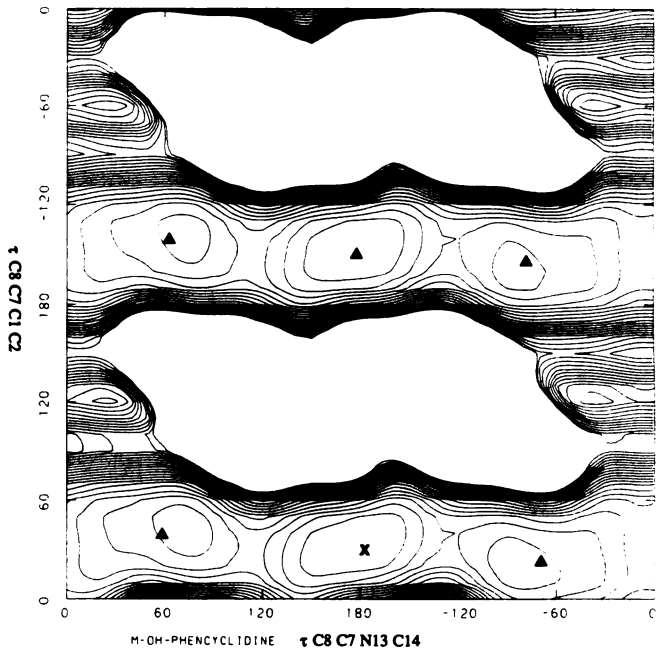

Fig. 5. Potential energy contour map obtained for the rotation of τ(C8, C7, C1, C2) and τ(C8, C7, N13, C14) of *m*-OH-PCP. Energies can be interpreted from the shape and spacing of energy contours in the map. The two large areas devoid of contour lines represent high energy conformations, whereas the symbol × represents the crystal conformation of PCP (62) and five other energetically accepted conformations of *m*-OH-PCP are indicated (▲). Two series of low energy conformers are accessible to *m*-OH-PCP, corresponding to τ(C8, C7, C1, C2) = 30° and −150°. When τ(C8, C7, C1, C2) is set to these angles, rotation of the bond τ(C8, C7, N13, C14) produces three energy minima separated by barriers of less than 11 kcal/mol. The contour interval is 2.5 kcal/mol and the first 20 contour lines are shown.

which τ(C8, C7, C1, C2) is equal to approximately 30° and −150°. These conformations are related by the 180° rotation of the phenyl ring. When τ(C8, C7, C1, C2) is set to 30° or −150°, rotation of the bond τ(C8, C7, N13, C14) produces three energy minima separated by barriers of less than 11 kcal/mol.

The four molecules were then superimposed on to each other by simultaneously minimizing the distances between the guide points R1, R2, R3, and N while altering the two torsion angles of *m*-OH-PCP. The conformation adopted by *m*-OH-PCP was close to that of its global minimum, with the two torsion angles differing by less than 4° each from the crystal conformation. Final conformations used are shown in Fig. 6. The receptor points of the R1, R2, R3, and N atoms for each molecule were averaged to give the following coordinates (in Å) of the primary model: R1 (0.00, 3.50, 0.00), R2 (0.00, −3.50, 0.00), R3 (6.66, −1.13, 0.00), and N (3.90, −1.46, −0.32). The distance from the center of the phenyl ring (origin, O) to the N atom was 4.18 Å. The angles R1-O-N and O-N-R3 were 110.5° and 150.5°, respectively, and the dihedral angle R1-O-N-R3 was 4.2°. Spatial overlap relative to *m*-OH-PCP was calculated for selected molecules revealing a high percentage of molecular volume coincidence (Table 1).

Secondary binding requirements. From our QSAR analyses (Table 2) and previous SAR studies, predictions can be made regarding the nature and location of the secondary binding sites that influence the interaction of PCP-like molecules with the PCP binding site. A site accepting a H-bond has been introduced to explain the affinities of a series of PCP derivatives possessing various aromatic substitutents (Fig. 7, a and b). Comparison of aromatic substituted PCP derivatives demonstrated that either a hydroxy or methoxy moiety was most favorable in the *meta*-position. PCP derivatives with aromatic hydroxy groups were found to have the following order of potency: *m*-OH-PCP > *N*-(1-[2-hydroxyphenyl]cyclohexyl) piperidine > *N*-(1-[4-hydroxyphenyl]cyclohexyl)-piperidine (24) (Table 2). Similarly, the potency of PCP analogues with aromatic methoxy substituents followed the same trend as their hydroxy cogeners, i.e., *N*-(1-[3-methoxyphenyl]cyclohexyl)-piperidine > *N*-(1-[2-methoxyphenyl]cyclohexyl)piperidine > *N*-(1-[4-methoxyphenyl]cyclohexyl)piperidine (25) (Table 2). Unfortunately, the lack of available ligands with various substituents in the *ortho*- and *para*-positions limited our description of the SAR requirements in these positions. Nevertheless, the *ortho*- and *para*-substituted congeners of PCP investigated were less potent than PCP itself, indicating that substitution in these positions is unfavorable. Consequently, many workers have chosen to examine the effect of various substituents in the *meta*-position of PCP on PCP-like activity (22–26, 28–30). In addition to *m*-OH-PCP being a potent PCP-like drug, the molecules (+)-SKF 10,047 and LY 154045 also have hydroxy groups corresponding to the *meta*-position of PCP. The coordinates of these oxygen atoms were averaged to give the coordinates for the optimal location of a hydroxyl oxygen atom (−1.66, 0.06, 2.20). Other arylcyclohexylamines with substituents in the *meta*-position had the following rank order of potency: hydroxy > amino > hydrogen > methoxy > flouro ≫ nitro (22–26, 28–30) (Table 2). To further investigate the effect of substituents in this position on SAR, electrostatic potential maps of *m*-OH-PCP, *N*-(1-[3-nitrophenyl]cyclohexyl)-piperidine, *m*-NH_2-PCP, and *N*-(1-[3-fluorophenyl]cyclo-

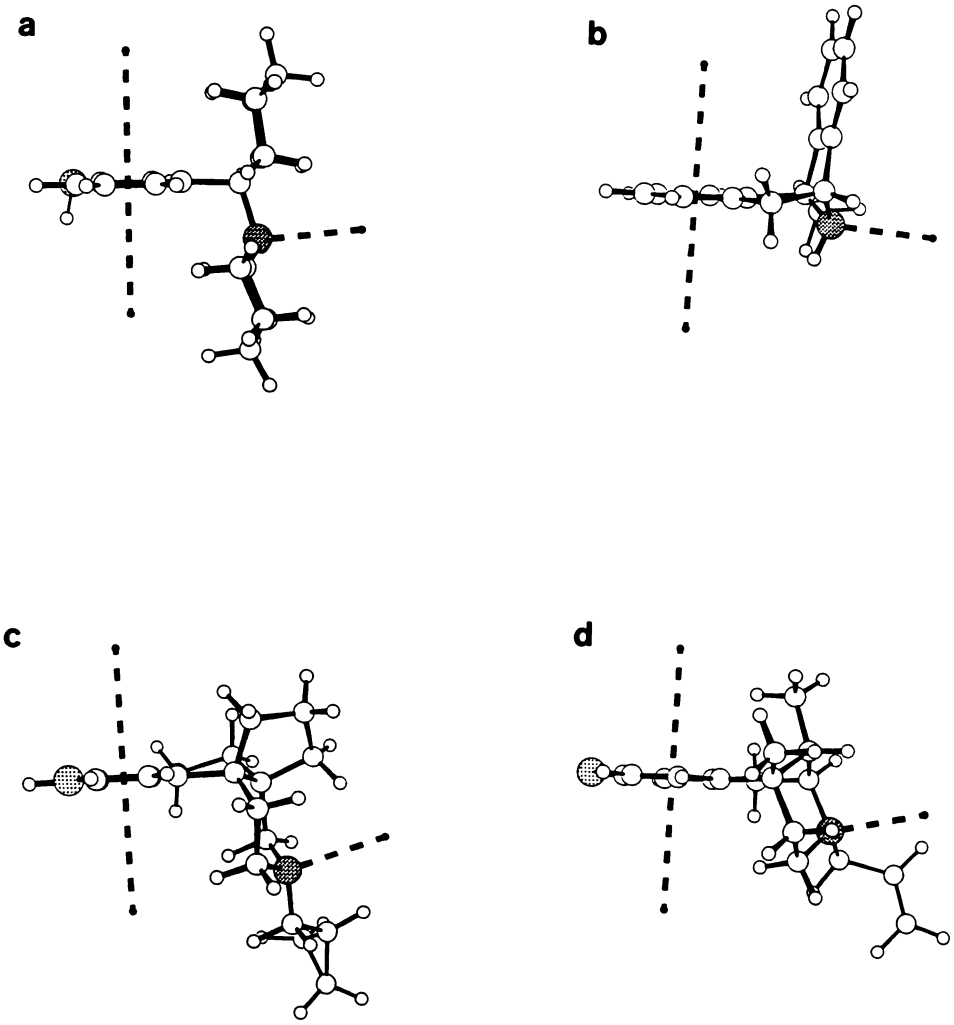

Fig. 6. Perspective drawings of the four molecules chosen to define the primary pharmacophore of the PCP receptor. a, *m*-OH-PCP; b, MK-801; c, LY 154045; d, (+)-SKF 10,047. The hydrophobic receptor points (R1, R2) are shown constructed onto the phenyl ring and the hydrogen receptor point (R3) onto the nitrogen atom. R1-R2 and N-R3 vectors are represented by *dashed lines*. Nitrogen and oxygen atoms are indicated by *dark* and *light shadings*, respectively.

hexyl) piperidine were examined (Fig. 8). *m*-OH-PCP, which is the most potent in this series, had a shallow electronegative well in the region of the *meta*-hydroxy group (Fig. 8), whereas the inactive nitro- and fluoro compounds had deeper potential wells associated with these substituents (Fig. 8). Thus, electron-withdrawing substituents in this *meta*-position are unfavorable for the PCP binding site. Presumably, the receptor may be donating or accepting a hydrogen bond with the hydroxy or amino group, thus increasing the potency of these compounds relative to PCP. Additionally, inasmuch as *N*-(1-[3-methoxyphenyl]cyclohexyl)piperidine has a similar potency to the parent molecule, this domain may accept rather than donate a H-bond.

An 'upwards' directed cleft in the receptor that accepts lipophilic substituents (Fig. 7b) is a secondary binding site to accommodate lipophilic groups in the region of the cyclohexane ring of PCP. Substitution onto the cyclohexyl ring of PCP and structural variations to this ring rarely increases the potency above that of PCP (25). Two PCP analogues with methyl substituents, (*trans*)-*N*-(1-phenyl-2-methylcyclohexyl)-piperidine and (*cis*)-*N*-(1-phenyl-4-methylcyclohexyl)piperidine do, however, possess a higher affinity than PCP (Table 2, cf. PCP 250 nM) (25). Replacement of the cyclohexyl ring with a cyclopentyl ring (i.e., *N*-(1-phenylcyclopentyl)piperidine) reduces the potency relative to PCP, and smaller ring systems again are inactive (27). Removal of the cyclohexyl ring altogether (i.e., benzylpiperidine) results in a total loss of activity (27). The molecules MK-801, (+)-SKF 10,047, and LY 154045 possess lipophilic substituents in this cleft and it appears that a lipophilic moiety in this domain is an essential requirement for activity (Fig. 7b).

Another secondary binding site for interaction of PCP ligands is likely to involve a lipophilic cleft in the 'downwards' direction, capable of accommodating *N*-alkyl substituents of PCP-like drugs (Fig. 7b). Many derivatives of PCP have been studied with substitutions on the piperidine ring or replacement of this ring with either other ring systems or alkyl substituents. *N*-(1-phenylcyclohexyl)4-methylpiperidine (Table 2) is less potent than PCP, presumably due to some steric interaction with the receptor surface. Replacement of the piperidine ring with either a pyrrolidine (28) or morpholine (28) ring reduces their potency relative to PCP (Table 2). Alkyl substitution (up to a five-carbon moiety) onto the N atom does little to vary the potency: *N*-ethyl-1-phenylcyclohexylamine is over twice as potent as PCP in radioligand binding studies (22, 28). Molecules devoid of groups in this region retain activity, e.g., 1-phenylcyclohexylamine. Indeed, MK-801 does not have any *N*-substituent and is the most potent PCP ligand to date.

Finally, increasing the distance between the phenyl ring and the cyclohexyl/piperidine rings by the addition of methylene

TABLE 2

Energy, distance, and potency of several PCP-like compounds

Energy above the global minimum, RMS distances, and potencies for several PCP-like compounds at the PCP receptor model. NT; not tested.

Compound	Energy[a]	Distance[b]	Potency[c]
	kcal/mol	*Å*	*nM*
1-Piperidinocyclohexanecarbonitrile	NT		5,500[d]
N-(1-[4-Hydroxyphenyl]cyclohexyl)piperidine	0.1	0.26	20,000[e]
N-(1-[4-Methoxyphenyl]cyclohexyl)piperidine	0.1	0.26	1,100[f]
N-(1-[2-Hydroxyphenyl]cyclohexyl)piperidine	0.1	0.26	750[e]
N-(1-[2-Methoxyphenyl]cyclohexyl)piperidine	0.1	0.26	500[f]
N-(1-[3-Nitrophenyl]cyclohexyl)piperidine	0.1	0.26	Inactive[g]
N-(1-[3-Aminophenyl]cyclohexyl)piperidine	0.1	0.26	31[h]
N-(1-[3-Methoxyphenyl]cyclohexyl)piperidine	0.1	0.26	90[f]
N-(1-[3-Fluorophenyl]cyclohexyl)piperidine	0.1	0.26	RP 0.2[g]
(*trans*)-*N*-(1-Phenyl-2-methylcyclohexyl)piperidine	0.1	0.26	110[f]
(*cis*)-*N*-(1-Phenyl-4-methylcyclohexyl)piperidine	0.1	0.26	130[f]
N-(1-Phenylcyclohexyl)-4-methylpiperidine	0.1	0.26	400[f]
N-(1-Phenylcyclopentyl)piperidine	NT		RP 0.37[i]
Benzylpiperidine	NT		Inactive[i]
1-Phenylcyclohexylamine	0.0	0.27	RP 0.29[k]
N-Ethyl-1-phenylcyclohexylamine	0.1	0.26	15[f]
1-(1-Phenylcyclohexyl)pyrrolidine	NT		65[f]
1-(1-Phenylcyclohexyl)morpholine	NT		RP 0.18[k]
1-[1-(1-Naphthyl)cyclohexyl]piperidineamine	NT		Inactive[g]
1-[1-(2-Naphthyl)cyclohexyl]piperidineamine	NT		Inactive[g]
1-[1-(1-Phenylmethylcyclohexyl]piperidine	NT		Inactive[g]
1-[1-(2-Phenylethylcyclohexyl]piperidine	NT		Inactive[g]
1-[1-(3-Phenylpropylcyclohexyl]piperidine	NT		Inactive[g]

[a] Energy above the global minimum conformation in kcal/mol.
[b] Best fit distance measured as the RMS (in Å) using R1, R2, R3, and N atom as guide points to the PCP receptor model.
[c] Potency (nM) from radioligand binding studies or potency relative to PCP from behavioral experiments.
[d] IC_{50} from binding data, employing [^{3}H]PCP, to rat brain membranes (26).
[e] Dissociation constant from binding data, employing [^{3}H]PCP, to rat brain membranes (24).
[f] IC_{50} from binding data, employing [^{3}H]PCP, to rat brain membranes (102).
[g] Potency of compound relative to PCP in producing PCP-like discriminative stimuli in the rat (22).
[h] IC_{50} from binding data, employing [^{3}H]TCP, to rat brain membranes (38).
[i] Approximate IC_{50} from binding data, employing [^{3}H]PCP, to rat brain membranes (25).
[j] Relative potency of compound to PCP in producing PCP-like discriminative stimuli in the rat (27).
[k] Potency relative to PCP to produce ataxia as measured by motor incoordination in mice (28).
[l] IC_{50} from binding data, employing [^{3}H]PCP, to rat olfactory bulb slices (23).

units [e.g., 1-[1-(1-phenylmethyl)cyclohexyl]piperidine, 1-[1-(2-phenylethyl)cyclohexyl]piperidine, and 1-[1-(3-phenylpropyl)cyclohexyl]piperidine] totally abolishes activity (Table 2) (22). The receptor model arising from our studies is depicted in Fig. 7, a and b, which detail the positions of points R1, R2, R3, and N, and secondary binding site locations.

σ Receptor Model

(*R*)-(+)-[^{3}H]3-PPP binding. (*R*)-(+)-[^{3}H]3-PPP bound to washed rat brain membranes with a dissociation constant of 30.8 ± 1.5 nM (four experiments) and a density of binding sites of 0.55 ± 0.03 pmol/mg of protein (four experiments). The Hill coefficient for (*R*)-(+)-[^{3}H]3-PPP binding was close to unity, indicating that binding was to a single population of sites. The displacement of (*R*)-(+)-[^{3}H]3-PPP binding by a range of σ receptor ligands (Fig. 9) also indicated that the nature of the binding site was essentially identical to the previously described σ binding site (38–40). The majority of compounds displaced (*R*)-(+)-[^{3}H]3-PPP with a slope factor of unity, although some drugs exhibited slope factors significantly less than one. More detailed curve fitting analyses were performed on these drugs by comparing one- and two-site models for ligand interaction,

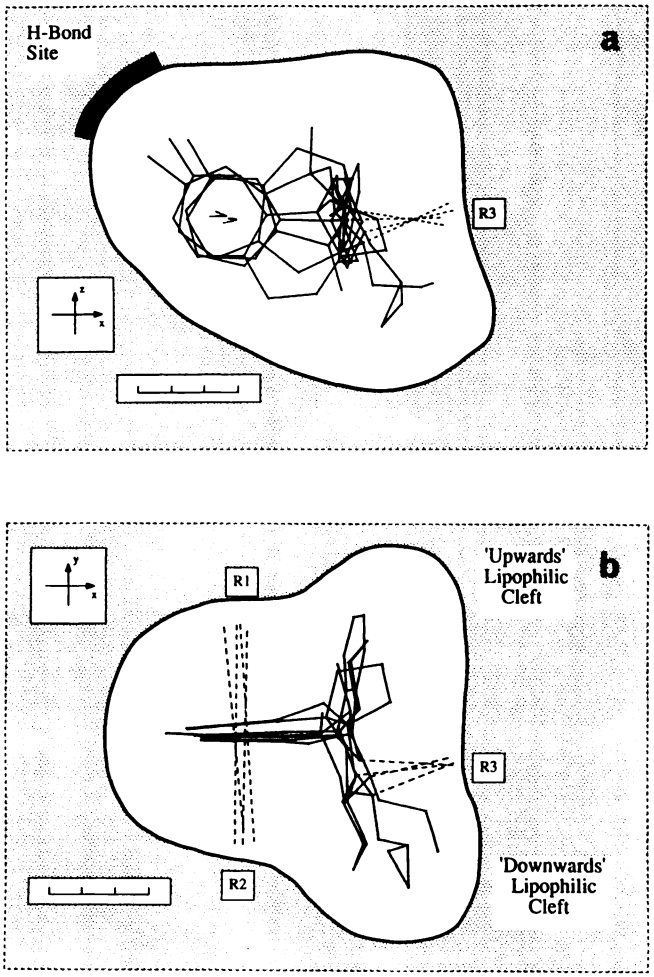

Fig. 7. Diagrammatic representation of the PCP receptor model, detailing the position of receptor points R1, R2, and R3, lipophilic clefts, and a hydrogen bond site. R1-R2 and N-R3 vectors are represented by *dashed lines* and hydrogen atoms have been deleted for clarity. Each unit on the scale bar represents 1Å. a, PCP receptor model viewed down the *y* axis, showing the molecules MK-801, *m*-OH-PCP, LY 154045, and (+)-SKF 10,047 in their best fit low energy conformations to the primary pharmacophore. b, PCP receptor model viewed down the *z* axis, detailing the position of the 'upwards' and 'downwards' lipophilic clefts.

with the affinity of (*R*)-(+)-[^{3}H]3-PPP for each site constrained to the K_G of 30.8 nM. Displacement data for these drugs fitted significantly better to a model in which the radioligand was displaced by the drug competing for two sites (Table 3). Interestingly, these compounds also display moderate affinity for the PCP binding site.

Primary pharmacophore. (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ is a relatively rigid molecule and the crystal structure of its tricyclic backbone was considered to be its biologically active conformation (66). The crystal structures of (*R*)-(+)-[^{3}H]3-PPP (67), (+)-SKF 10,047 [from structure of cyclazocine (63)], and haloperidol (68) were used, whereas DTG was constructed using standard bond lengths and angles. The potent σ receptor ligand (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ was used as the template onto which the four other σ ligands were superimposed. For the molecules DTG and (*R*)-(+)-[^{3}H]3-PPP, the torsion angles indicated in Fig. 2 were altered to locate all low energy conformers and to minimize the fit between the receptor points R1, R2, R3, and N and the corresponding points on (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ. Rotation of the

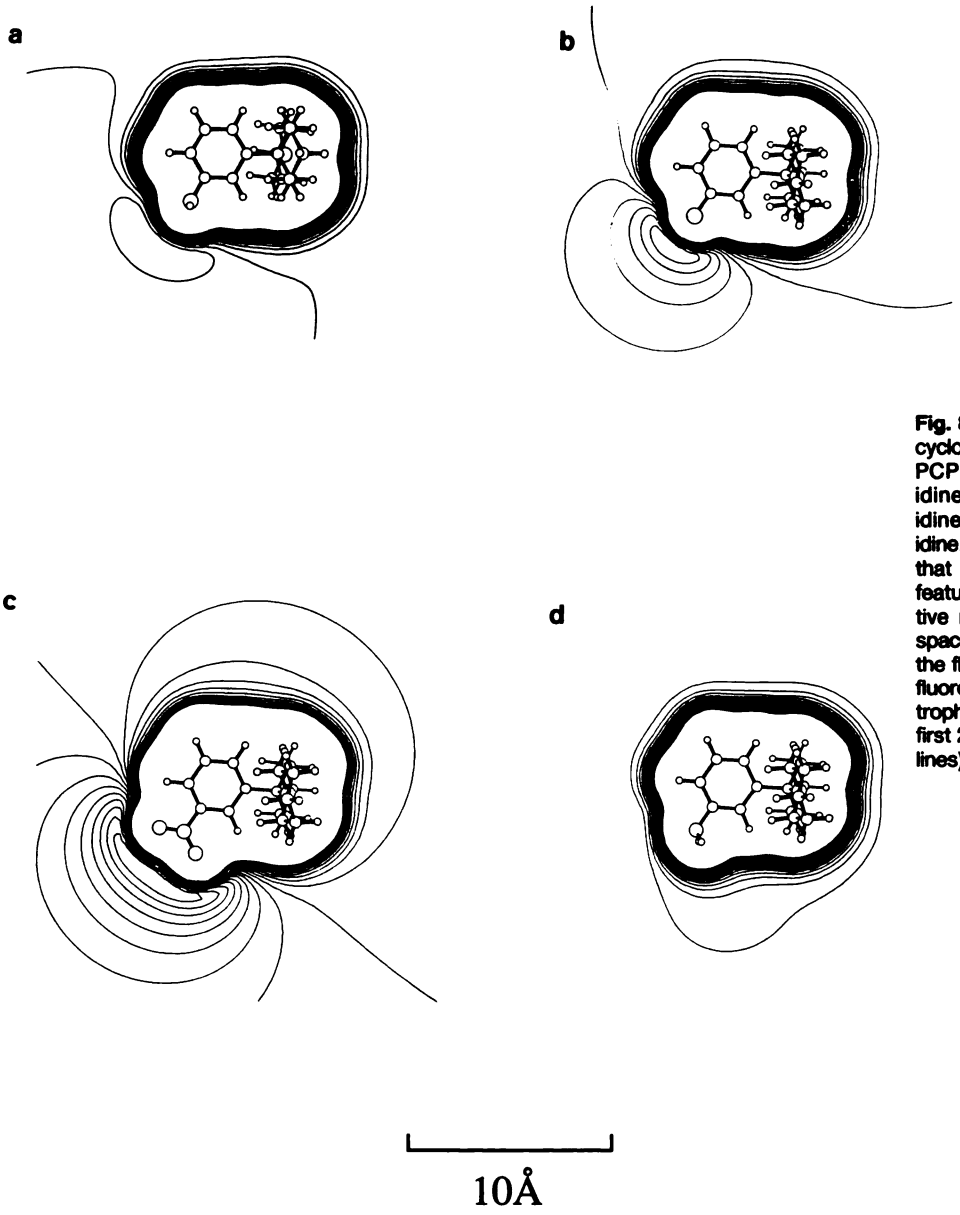

Fig. 8. Electrostatic potential maps for some arylcyclohexylamine derivatives of PCP. a, *m*-OH-PCP; b, *N*-(1-[3-fluorophenyl]cyclohexyl)piperidine; c, *N*-(1-[3-nitrophenyl]cyclohexyl)piperidine; d, *N*-(1-[3-aminophenyl]cyclohexyl)piperidine. In each case the potential surface shown is that in the plane of the phenyl ring. The main features of the potential energy maps are a positive region extending over the whole molecular space and regions of negative energy adjacent to the fluoro (b) and nitro (c) substituents of *N*-(1-[3-fluorophenyl]cyclohexyl)piperidine and *N*-(1-[3-nitrophenyl]cyclohexyl)piperidine, respectively. The first 20 contour lines (2 kcal/mol between contour lines) are shown. The *scale bar* represents 10 Å.

bond τ(C2, C1, C8, C9) for (*R*)-(+)-3-PPP gave two low energy conformers at τ(C2, C1, C8, C9) = 60° and 240°. The minimal RMS distance for (*R*)-(+)-3-PPP fitted to (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ occured at τ(C2, C1, C8, C9) = 163° and the potential energy of this conformation was less than 5 kcal/mol above the global minimum conformation. Haloperidol possesses two aromatic rings and the 4-phenylpiperidine moiety was used as the primary aromatic and N atom (40). The torsion angle indicated on haloperidol was also altered to locate all low energy conformers and to minimize the fit between the receptor points R1, R2, R3, and N and the corresponding points on (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ. The structure of (+)-SKF 10,047 [based on crystal structure of cyclazocine (63)] did not, however, fit well to the receptor points of (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ (RMS 0.85 Å). An alternative conformation of (+)-SKF 10,047 is that with an inversion of the N atom (cf. Fig. 1 and Fig. 2) (69). Potential energy differences between the two conformers of (+)-SKF 10,047 (*N*-invert was 2.7 kcal/mol above the crystal conformation) were such that the molecule was able to 'flip' easily between each conformation. N inversion alters the N lone pair vector orientation considerably and the conformer that gave the better fit to (*trans*)-(4a*R*,10b*R*)-9OH-*n*-Pr-OHBQ was that where the N atom had undergone inversion (Fig. 10, Table 4). The *N*-inverted conformation of (+)-SKF 10,047 was therefore considered to be the σ-active conformation. The five molecules were then superimposed onto each other by simultaneously minimizing the distances between the guide points R1, R2, R3, and N and by altering the torsion angles labeled in Fig. 2. The final conformations of haloperidol [τ(C6, C1, C9, C8) = 77.6°], DTG [τ(C6, C2, N1, C7) = 115°], (*R*)-(+)-3-PPP [τ(C2, C1, C8, C9) = 163°], (+)-SKF 10,047 (*N*-invert), and (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ are shown in Fig. 10. The receptor points R1, R2, R3, and N from each molecule were averaged to give the following coordinates (in Å) of the primary model for the σ binding site: R1 (0.00, 3.50, 0.00), R2 (0.00, −3.50, 0.00), R3 (6.09, 2.09, 0.00), and N (4.9, −0.12, −1.25). The distance from the center of the phenyl ring (origin, O) to N atom was 5.06 Å.

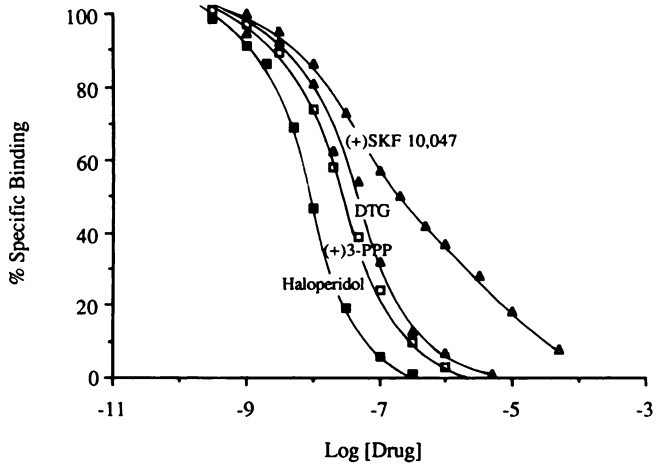

Fig. 9. Inhibition of specific (*R*)-(+)-[^{3}H]3-PPP binding to rat brain membranes by the four ligands used to define the primary pharmacophore of the σ receptor. Membranes were incubated with 3 nM (*R*)-(+)-[^{3}H]3-PPP and at least 10 concentrations of each drug for 90 min at 25°. Data shown are from typical experiments representing the mean of replicate determinations. Rank order of potency, haloperidol (■) > (*R*)-(+)-3-PPP (□) > DTG (▲) > (+)-SKF 10,047 (△).

The angles R1-O-N and O-N-R3 were 91.3° and 106.4°, respectively, and the dihedral angle R1-O-N-R3 was 34.1°. Spatial overlap relative to (*trans*)-(4a*R*,10b*R*)-9OH-*n*-Pr-OHBQ was calculated for selected molecules, revealing a high percentage of molecular volume coincidence (Table 4).

Secondary binding requirements. Although the σ binding site accepts many structurally unrelated compounds, it displays stereoselectivity for a number of these drug classes. (*S*)-(−)-3-PPP is approximately 5 times less potent than the (*R*)-isomer (Table 3) consistent with the slightly reduced fit of the (*S*)-isomer to the primary pharmacophore [RMS 0.45 and 0.12 Å for (*S*)- and (*R*)-isomers, respectively]. This result may also be due to steric interactions with the receptor surface by the altered position of the *N*-*n*-propyl group. The benzomorphans also display a mild stereoselectivity, with the prototypical σ ligand (+)-SKF 10,047 being approximately 6 times more potent than the (−)-isomer (RMS 1.04 and 0.55 for (−)- and (+)-isomers, respectively) when the K_H values determined from two-site analyses are compared. The OHBQ series share a stereoselectivity similar to that of 3-PPP, in that (*trans*)-(4a*R*,10b*R*)-9OH-*n*-Pr-OHBQ is about 25 times more potent than (*trans*)-(4a*S*,10b*S*)-9-OH-*n*-Pr-OHBQ (RMS 0.28 and 0.69 for (4a*R*,10b*R*)- and (4a*S*,10b*S*)-isomers respectively) (40). The potency difference may again be explained by the reduced fit of the less active (4a*S*,10b*S*)-isomer (Table 4) or due to an unfavorable location of its *N*-alkyl substituent. Indeed, the *n*-propyl groups of the less active (*S*)-(−)-[^{3}H]3-PPP and (*trans*)-(4a*S*,10b*S*)-9OH-*n*-Pr-OHBQ coincide and are placed in a region that is not shared by the five molecules chosen for the primary receptor model. In addition to the stereoselectivity displayed for various σ ligands, the OHBQ series of molecules can adopt either a (*cis*)- or (*trans*) configuration, of which the (*trans*)-isomers have the higher affinity (40, 42, 43). Again, the reduced potency may be due to the suboptimal fit of the (*cis*)-isomers (RMS 0.69 Å) to the σ receptor model (Table 4).

The nature and placement of substituents on the aromatic ring influences σ activity. Substitution of a hydroxyl group on to the 8- or 9-position in the (*trans*)-(4a*R*,10b*R*)-n-Pr-OHBQ series of compounds increases affinity, with the rank order of potency likely to be 8-OH- > 9-OH- > 7-OH. (Table 4) (40). Similar alterations to the PPP backbone have demonstrated that the 4-hydroxy derivative is much less potent than (*R*)-(+)-3-PPP (40). This result is, however, inconsistent with the OHBQ series, in which the 8-OH-position corresponds to the 4-OH-position on the PPP structure (Fig. 11). (+)-SKF 10,047 also has a hydroxyl group located in the region of the 8-position on the (*trans*)-(4a*R*,10b*R*)-OHBQ backbone (Fig. 11). Compounds possessing σ activity with substituents other than hydroxyl groups include DTG, (±)-3-(3-fluorophenyl)-*N*-*n*-propylpiperidine, haloperidol, and bromoperidol. (±)-3-(3-Fluorophenyl)-*N*-*n*-propylpiperidine is of a similar potency to (*R*)-(+)-3-PPP, indicating that a halogen substituent is an acceptable alternative to a hydroxyl group (40). Bromoperidol and haloperidol are highly potent σ receptor ligands and the position of their chlorine and bromine atoms bisects the 8- and 9-positions of the (*trans*)-(4a*R*,10b*R*)-OHBQ backbone (Fig. 11). DTG possesses a higher affinity than its diphenyl derivative DPG, presumably due to the reduced conformational freedom imparted by the methyl groups, resulting in DTG being constrained to a conformation that is acceptable for σ activity. Greater conformational freedom, as exhibited by DPG, would

TABLE 3

Binding characteristics of several σ ligands

Potencies, high and low affinity binding components, receptor concentration for high and low affinity components, and slope factors of various ligands for inhibition of (*R*)-(+)[^{3}H]3-PPP binding sites to brain membranes. Experimental details are given in Materials and Methods. Displacement data for (+)- and (−)-SKF 10,047, PCP, and dexoxadrol fitted better to a two-site model. The values are presented as the mean ± standard error from three or four determinations. The following compounds (10 μM) failed to displace 50% of specific binding: MK-801, ketamine, naloxone, quisqualic acid, kainic acid, L-AP4, DL-AP7, D-AP5, L-glutamate, and glycine.

Compound	K_G	K_H	K_L	R_H	R_L	Slope
		nM		%		
(*R*)-(+)-3-PPP	30.8 ± 1.5					1.07 ± 0.07
(*S*)-(−)-3-PPP	140 ± 15					0.81 ± 0.03
Haloperidol	12 ± 0.7					0.84 ± 0.05
Rimcazole	1,800 ± 210					0.98 ± 0.04
(*trans*)-(±)-9OH-*n*-Bu-OHBQ	11 ± 2.6					0.94 ± 0.12
(+)-SKF 10,047	1,500 ± 320	48 ± 9.6	6,300 ± 880	60	40	0.43 ± 0.02
(−)-SKF 10,047	1,950 ± 270	300 ± 200	6,900 ± 3,000	62	38	0.60 ± 0.03
DTG	48 ± 7.7					0.98 ± 0.08
DPG	200 ± 34					0.93 ± 0.02
(+)-Butaclamol	20,000 ± 2,300					1.07 ± 0.23
(−)-Butaclamol	870 ± 110					0.79 ± 0.01
PCP	4,100 ± 900	580 ± 620	14,000 ± 9,500	65	35	0.64 ± 0.01
Dexoxadrol	6,500 ± 1,000	1,000 ± 1,900	11,500 ± 6,200	43	57	0.77 ± 0.04

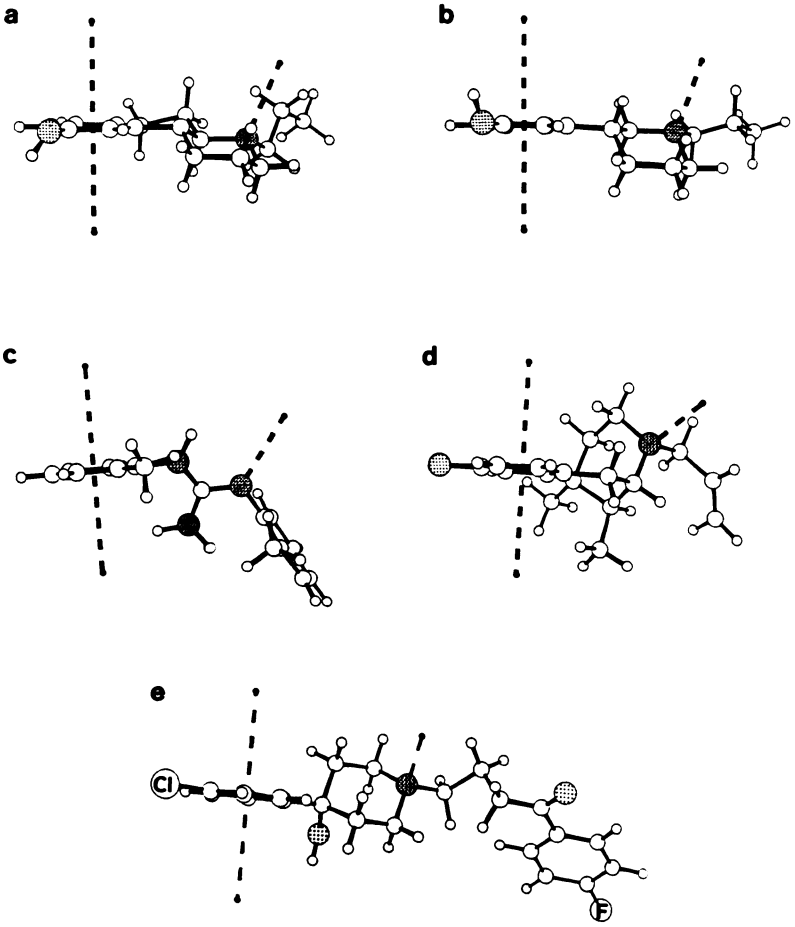

Fig. 10. Perspective drawings of the five molecules chosen to define the primary pharmacophore of the σ receptor. a, (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ; b, (*R*)-(+)-3-PPP; c, DTG; d, (+)-SKF 10,047 (*N*-invert); e, haloperidol. The hydrophobic receptor points (R1, R2) are shown constructed onto the phenyl ring and the hydrogen receptor point (R3) onto the nitrogen atom. R1-R2 and N-R3 vectors are represented by *dashed lines*. Nitrogen and oxygen atoms are indicated by *dark* and *light shadings*, respectively.

produce weaker receptor binding because of the cost in entropy (70).

A region near the primary aromatic ring that will accept electronegative substituents was hypothesized from analyses of electrostatic potentials and SAR data (Fig. 12a). The electrostatic potentials of the five compounds chosen to define the primary σ receptor site model were calculated to further investigate the nature of aromatic substitution on σ receptor ligands. Haloperidol, which is the most potent in this series, had a deep potential well in the region of its chlorine atom, whereas DTG did not produce a negative potential well (Fig. 13). (+)-SKF 10,047 (*N*-invert), (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ and (*R*)-(+)-3-PPP had either a shallow or negligible electronegative well in the region of their hydroxyl substituents (Fig. 13). The high potency of bromoperidol and haloperidol may be explained in part by the electronegative nature of their ring substituents; however, DTG does not share this property. In addition, *N*-1-*n*-propyl-4-phenyl-1,2,5,6-tetrahydropyridine also possesses high affinity (IC_{50}, 15 nM) (40) for the σ site but does not possess a substituent on the aromatic ring. Unlike the electrostatic potential analyses performed on the PCP derivatives, this analysis of σ ligands has used compounds that differ structurally, making the interpretation of this examination a subjective rather than quantitative analysis. Until additional compounds become available, it would appear that electronegative substituents, at least in the C9-position of (*trans*)-(4a*R*,10b*R*)-OHBQ backbone, possibly impart a higher activity at the σ binding site.

A large lipophilic cleft has been introduced to explain the alterations in affinity produced by variation to the *N*-alkyl groups of σ ligands. When examining *N*-alkyl substitution on the (*R*)-(+)-3-(3-hydroxyphenyl)piperidine backbone, the most potent compounds in this series are the *N*-*n*-butyl and *N*-phenethyl derivatives, with a rank order of potency of phenethyl ≥ *N*-*n*-butyl > *N*-*n*-propyl > *N*-ethyl > *N*-methyl (Table 4) (40). This has also been demonstrated in the (*trans*)-(4a*R*,10b*R*)-OHBQ series, in which the large *N*-alkyl substituents impart the highest affinity (e.g., (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Bu-OHBQ) (Table 4) (40). All secondary binding requirements have been incorporated with the primary pharmacophore in the σ receptor model (Fig. 12, a and b).

Discussion

Our QSAR investigations have used computer graphics in conjunction with energy, degree of fit, and overlap procedures to develop receptor models for the PCP and σ binding sites, consisting of a primary pharmacophore and secondary binding sites. Data from radioreceptor and other pharmacological studies aided the definition of the models. For selectivity a compound must not only 'fit' the respective primary pharmacophore, but its secondary binding groups should also be in favorable locations.

Ligands binding to the PCP site have a requirement for either a phenyl or a thienyl ring in the primary aromatic region (23, 26, 28). Molecules devoid of an aromatic group in this region (e.g., 1-piperidinocyclohexanecarbonitrile) have a very

TABLE 4

Energy, distance, potency, and overlap of several σ ligands

Energy above the global minimum, RMS distances, potencies (in nM), and percentage overlaps for compounds at the σ receptor model. The atomic coordinates for naloxone were obtained from Ref. 103. (±) indicates that the radioligand binding data was only available for the racemic mixture; however, the energy and fit calculations are for the isomer specified. NT, not tested.

Compound	Energy[a]	Distance[b]	Potency	Overlap[c]
	kcal/mol	Å	nM	
Haloperidol	2.9	0.55	11.7	69
Rimcazole	NT		1,770	
Perphenazine	NT		26[d]	
Pipamperone	NT		148[d]	
Bromoperidol	2.9	0.55	6[d]	
Lenperone	1.8	0.82	35[d]	
(+)-SKF 10,047 (*N*-invert)	2.7	0.55	47.9	65
(−)-SKF 10,047	0.0	1.04	297	
(+)-Cyclazocine (*N*-invert)	2.7	0.55	118[d]	
Naloxone	0.0	1.10	>10,000	
DTG	1.2	0.35	47.6	65
DPG	1.7	0.33	199	
(+)-Butaclamol	0.0	0.61	19,894	
(−)-Butaclamol	0.0	1.10	865	
N-1-*n*-Propyl-4-phenyl-1,2,5,6-tetrahydropyridine	2.7	0.41	15[d]	
RU 38796	3.5	0.53	41[d]	
(*R*)-(+)-3-PPP	3.6	0.12	30.8	84
(*S*)-(−)-3-PPP	2.0	0.45	140	
(*R*)-(+)-3-(4-Hydroxyphenyl)-*N*-*n*-propylpiperidine	3.6	0.12	(±)276[d]	
(*R*)-(+)-3-(3-Fluorophenyl)-*N*-*n*-propylpiperidine	3.6	0.12	(±)49[d]	
(*R*)-(+)-3-(3-Hydroxyphenyl)-*N*-methylpiperidine	3.6	0.12	373[d]	
(*R*)-(+)-3-(3-Hydroxyphenyl)-*N*-ethylpiperidine	3.6	0.12	111[d]	
(*R*)-(+)-3-(3-Hydroxyphenyl)-*N*-*n*-butylpiperidine	3.6	0.12	9[d]	
(*R*)-(+)-3-(3-Hydroxyphenyl)-*N*-phenethylpiperidine	3.6	0.12	8[d]	
(*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ	0.0	0.28	21[d]	100
(*trans*)-(4a*S*,10b*S*)-9-OH-*n*-Pr-OHBQ	0.0	0.69	520[d]	
(*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Bu-OHBQ	0.0	0.28	(±)10[d]	
(*trans*)-(4a*R*,10b*R*)-7-OH-*n*-Pr-OHBQ	0.0	0.28	48[d]	
(*trans*)-(4a*R*,10b*R*)-8-OH-*n*-Pr-OHBQ	0.0	0.28	(±)10[d]	
(*cis*)-(4a*S*,10b*R*)-7-OH-*n*-Pr-OHBQ	0.0	0.69	197[d]	
(*cis*)-(4a*S*,10b*R*)-9-OH-*n*-Bu-OHBQ	0.0	0.69	35[d]	

[a] Energy above the global minimum conformation in kcal/mol.
[b] Best fit distance measured as the RMS in Å using R1, R2, R3, and N atom as guide points.
[c] Percentage overlap relative to (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ for the σ receptor site.
[d] Potency (in nM) from radioligand binding data (40).

low potency at the PCP binding site (23, 26, 28). Molecules with groups larger than a phenyl ring, such as 1-[1-(1-napthyl)cyclohexyl]piperidineamine and 1-[1-(2-napthyl) cyclohexyl]-piperidineamine, possess no activity (22). These data indicate that the size of the aromatic moiety of PCP-like drugs is restricted to being no larger than a phenyl ring for optimal PCP-like activity. Furthermore, substitution onto the phenyl ring of PCP appears to be most favorable with a H-bond-donating group in the *meta*-position. Several workers have exploited this information, attempting to synthesize irreversible ligands for the PCP receptor. Indeed, azido-PCP [*N*-(1-[3-azidophenyl]cyclohexyl]piperidine (71)] and metaphit [1-[1-(3-isothiocyanatophenyl)cyclohexyl]piperidine, (72)] label PCP binding sites via substituents in the *meta*-position. This further demonstrates the presence of a group within the PCP receptor capable of interacting with *meta*-substituents on PCP-like drugs.

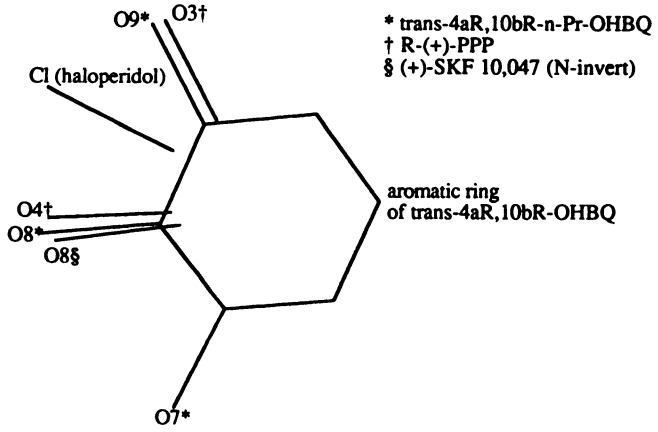

Fig. 11. Diagrammatic representation of the location of various aromatic substituents of molecules from the benzomorphan, phenylpiperidine, and OHBQ drug classes. For reference purposes, the phenyl ring of (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ and the carbon-oxygen or carbon-chloride bonds for each molecule are shown. Optimal σ receptor affinity appears to require electronegative aromatic substitutents residing in the O8-O9 region of (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ.

The importance of alkyl groups residing in the space occupied by the cyclohexyl ring of PCP and the requirement for an 'upwards' lipophilic cleft has already been outlined. The lipophilic requirements of this site were emphasized by Kamenka and co-workers (24), who demonstrated that substitution of a hydroxy group onto the cyclohexyl ring of PCP resulted in a loss of activity (e.g., 4-phenyl-4-(1-piperidinyl)cyclohexanol, IC_{50} = 8300 nM). In addition, the cyclohexyl ring of PCP plays an important role in orienting the phenyl and piperidine rings into a T shape (Fig. 6a).

Although we have postulated a 'downwards' lipophilic cleft in our PCP receptor model, the molecules MK-801 and PCA are devoid of *N*-alkyl substituents yet retain good affinity for the PCP binding site. Substitution of the piperidine ring of PCP with a morpholine ring reduces activity, with the acidic morpholine ring probably changing the acid/base nature of the molecule. In a study examining the convulsant and anticonvulsant effects of PCP analogues, PCA behaved in a fashion that differed slightly from that of the other compounds tested (6). PCA produced a greater anticonvulsant action at the higher doses tested and eliminated all motor signs of the electrically induced seizure. Lecesse and co-workers (6) also noted that PCA failed to induce convulsions and suggested that the amine group of PCA contributed to its increased anticonvulsant activity. MK-801 may also have a potent anticonvulsant action (7), due to the lack of an *N*-substituent in the downward direction. Future drug design should exploit this feature to design potentially more potent anticonvulsants.

Cone and co-workers (22) have already proposed a receptor model for the PCP binding site using SAR analyses and Dreiding molecular models. Unfortunately, *ad hoc* molecular modelling using Dreiding models provide less precise information concerning energetically acceptable conformations and molecular geometries. In addition, Cone and his colleagues (22)

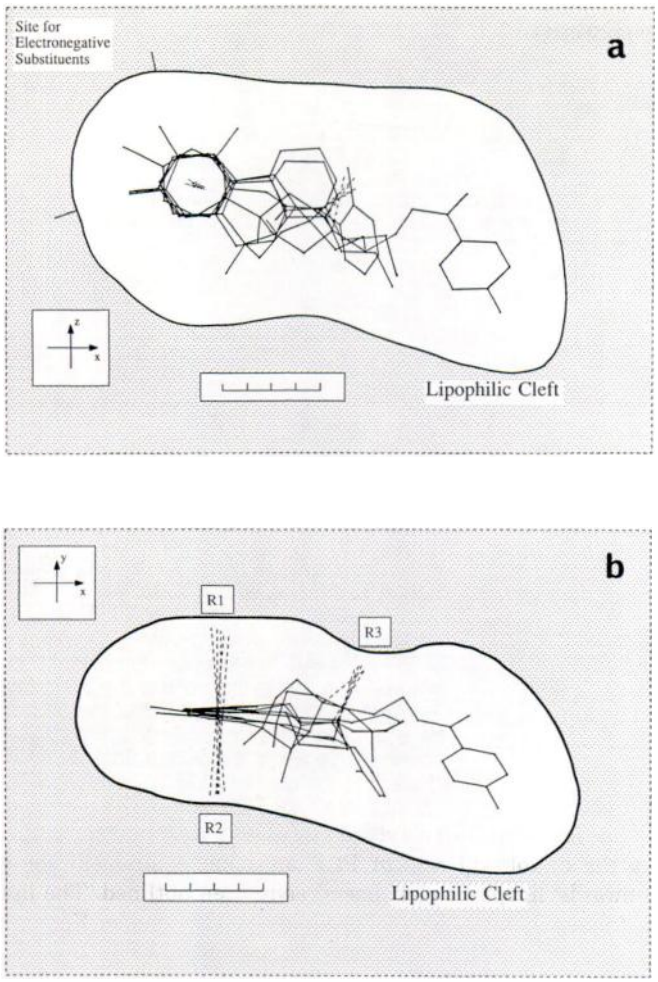

Fig. 12. Diagrammatic representation of the σ receptor model detailing the position of receptor points R1, R2, and R3, lipophilic clefts, and a site for electronegative substituents. R1-R2 and N-R3 vectors are represented by *dashed lines* and hydrogen atoms have been deleted for clarity. Each unit on the scale bar represents 1 Å. a, σ Receptor model viewed down the *y* axis, showing the molecules haloperidol, (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ, (*R*)-(+)-[^{3}H]3-PPP, DTG, and (+)-SKF 10,047 in their best fit low energy conformations to the primary pharmacophore. b, σ Receptor model viewed down the *z* axis, detailing the position of the receptor points R1, R2, and R3 and the lipophilic cleft.

modelled haloperidol to their hypothetical receptor site and demonstrated a reasonable fit. Radioligand binding studies have since demonstrated that haloperidol does not interact with the PCP site (38) and, thus, the model described by Cone *et al.* is now inappropriate.

A recent report detailing the absolute configuration of dexoxadrol[4] (73) allowed us to fit this interesting molecule to our PCP receptor model. Dexoxadrol possesses good affinity for the PCP-binding site and generalizes to PCP in drug discrimination paradigms (73, 74). Dexoxadrol fitted moderately well to the PCP receptor model [τ(O5, C4, C6, C7) = −97.5°; τ(O5, C1, C12, C13) = 78.1°; RMS 0.79 Å, 3.8 kcal/mol), whereas levoxadrol fitted very poorly (RMS 1.30 Å). The poor fit of levoxadrol correlates with its weak potency at the PCP binding site (38). Further, the second phenyl ring of dexoxadrol is not located near the lipophilic clefts described above for the PCP receptor model. When the two phenyl rings are constrained to reside within the primary aromatic region and the 'upwards' lipophilic cleft (see Fig. 7b), the fit is reduced considerably (RMS > 1.2 Å). This poor molecular concordance may be related to anomalous findings reported in radioreceptor studies with [^{3}H]dexoxadrol. One ligand binding study found an order of potency of a number of PCP analogues using [^{3}H]dexoxadrol (75) slightly different from that observed with [^{3}H]TCP (38, 65). In addition, autoradiographic studies of the distribution of [^{3}H]dexoxadrol binding sites showed regional differences to the sites labeled by [^{3}H]PCP (75). Another molecule that may share properties similar to those of dexoxadrol is (−)-2-MDP, which also has a diphenyl moiety in its structure (76). Unfortunately the absolute configuration for (−)-2-MDP is unknown, but this molecule has only moderate affinity for the PCP site and conceivably it could interact with the PCP binding site in a similar fashion to dexoxadrol.

The actions of PCP-like drugs to block the NMDA receptor are voltage dependent and have an absolute requirement for the channel to be in an activated state (77, 78). This requirement for the channel to be in an activated state has also been demonstrated in radioligand binding studies, whereby NMDA receptor agonists are needed for the binding of radiolabeled PCP ligands (79, 80). The binding site of PCP-like drugs has been postulated to be approximately midway through the ion channel of the NMDA receptor-ionophore complex (14). Although PCP-like compounds require the ion channel to be in an activated state for binding (79, 80), these drugs can become trapped inside the channel when it closes (14). Our own studies, therefore, provide insights into the topographic requirements of this domain of the PCP receptor and hence the ion channel of the NMDA receptor.

Our molecular modelling studies, which predict very different receptor models for PCP and σ ligands both in terms of primary pharmacophore and secondary binding locations, thus strongly suggest that these two classes of drugs exert their psychotomimetic effects through discrete receptors. Similarities between the actions of (+)-SKF 10,047 and PCP led to the concept of a single psychotomimetic recognition site termed the σ/PCP receptor (37), with several lines of evidence linking both the PCP-like and psychotomimetic benzomorphan classes of molecules. Recent behavioral evidence, which addresses the stereoselectivity of SKF 10,047 for the σ binding site, suggests that the effects of the psychotomimetic benzomorphans are exerted through σ recognition sites (81–83). The interpretation of behavioral results should be aided by the definition of σ receptor ligands into agonists and antagonists. Indeed, our finding of discrete low and high affinity states for some σ ligands, plus a recent report of GTP shifts in potency (84), may assist in the delineation of σ agonists and antagonists. Additional studies should also attempt to determine the receptor through which the behavioral actions of these drugs are mediated, by using selective σ ligands (42, 43) and more specific PCP ligands (e.g., TCP or *m*-NH_2-PCP).

Other evidence now exists that delineates PCP and σ sites, thus making the term 'σ/PCP' redundant. This evidence arises from radioreceptor, autoradiographic, and electrophysiological experiments. Largent and co-workers (38) first demonstrated that (+)-[^{3}H]SKF 10,047 bound to two distinct sites, of which the lower affinity site corresponds to the PCP receptor and the high affinity site to the σ receptor. Our radioreceptor data

[4] Tables of atomic coordinates of dexoxadrol were provided by Drs. J. V. Silverton, K. C. Rice, and A. E. Jacobson, National Institutes of Health, Bethesda, MD.

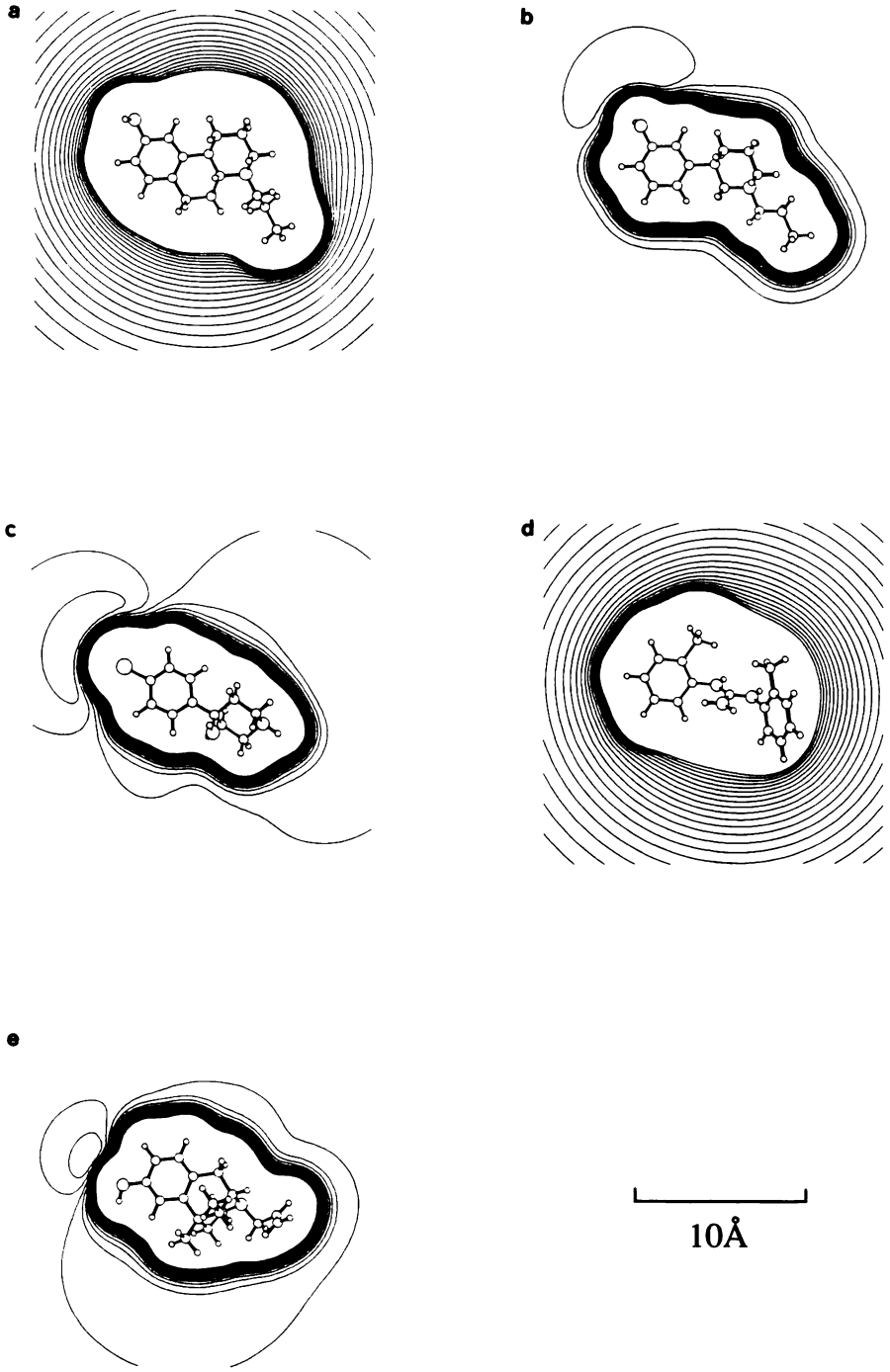

Fig. 13. Electrostatic potential maps for the five molecules chosen to define the primary pharmacophore of the σ receptors. a, (*trans*)-(4a*R*,10b*R*)-9-OH-*n*-Pr-OHBQ; b, (*R*)-(+)-[^{3}H]3-PPP; c, haloperidol (phenylpiperidine moiety); d, DTG; e, SKF 10,047 (*N*-invert). In each case the potential surface shown is that in the plane of the phenyl ring. The main features of the potential energy maps are a positive region extending over the whole molecular space and a region of negative energy adjacent to the chlorine atom of haloperidol. The first 20 contour lines (2 kcal/mol between contour lines) are shown. The *scale bar* represents 10 Å.

confirm other evidence with selective PCP and σ ligands (85) that the two sites have very different pharmacological profiles (31, 38, 39). Autoradiographic studies have also demonstrated a differential location of PCP and σ binding sites (38, 86–89). In electrophysiological experiments, NMDA antagonism has been demonstrated for several classes of PCP-like drugs (12) with the rank order of potency correlating with their affinity for the PCP binding site (3, 18). The potent σ ligands haloperidol and (*R*)-(+)-3-PPP do not, however, alter the responses to *N*-methylaspartate (90, 91). Additionally, endogenous ligands for PCP and σ receptors possessing differing physicochemical properties have been isolated (92).

From the SAR studies conducted on σ ligands to date, it is clear that a wide variety of compounds are able to interact with the σ binding site (31, 38, 42, 43). Indeed, the five compounds chosen to define the primary pharmacophore for the σ binding site have extremely diverse structures. Unlike the PCP binding site, the σ site appears to have less strict SAR requirements. For example, substitution onto the primary aromatic ring was optimal with an electronegative substituent in the region corresponding to the chlorine atom of haloperidol (Fig. 11), although this was not essential for σ potency. More recently, two DTG analogues have been synthesized as affinity labels for σ receptors, 1-(4-azido-2-methyl[6-^{3}H]phenyl)-3-(2-methyl [4,6^{3}H]phenyl)guanidine (93) and 1-(2-methyl-4-isothiocyanatophenyl)-3-(2-methylphenyl)guanidine (94). The azido and isothiocyanato groups on these molecules have been placed in the *para*-position and correspond closely to the chlorine atom

of haloperidol. This indicates the existence of a site for electronegative substituents within the σ receptor, capable of interacting with the azido and isothiocyanato moieties. The requirement for a protonated nitrogen atom also seems unnecessary, inasmuch as Su and co-workers (95) have demonstrated that various endogenous steroids had moderate potency for the σ receptor in brain and peripheral tissues. In contrast, substitution onto the N atom follows strict SAR requirements (40, 42, 43), with increased lipophilicity enhancing σ potency.

In view of the variety of compounds possessing moderate affinity for σ sites, we examined several structurally unrelated ligands in our model. The following molecules displayed a reasonable fit: lenperone (RMS 0.82 Å), *N*-*N*-1-*n*-propyl-4-phenyl-1,2,5,6-tetrahydropyridine (RMS 0.41 Å), and RU 38796 (RMS 0.53 Å). Although the majority of σ ligands fitted our model, there were a number of compounds that fitted poorly (both geometrically RMS > 1.0 Å or energetically > 5 kcal/mol) to the primary pharmacophore, including rimcazole and perphenazine. The postulated lipophilic cleft may well be important to the σ activity of these diverse ligands. Interestingly, rimcazole, a selective σ ligand of moderate affinity, displays very weak activity at dopamine receptors (96). Conceivably these compounds may interact with the receptor, via their N atom, and the lipophilic cleft, via their tricyclic ring systems, but not with the primary aromatic site. Largent and co-workers (38) also demonstrated that pipamperone has a reasonable affinity for the σ binding site, although it does not have the 4-phenylpiperidine ring system that initially appeared to be important for σ activity. In addition, (−)-butaclamol does not fit well to the primary σ pharmacophore and it too may be binding to the receptor site with both phenyl rings interacting with the lipophilic cleft rather than the primary aromatic site. Future drug design strategies for potent σ ligands should concentrate on ligands that interact with the primary pharmacophore.

Some compounds that bind to the σ binding site also interact with the D2 subtype of dopamine receptor, e.g., haloperidol, butaclamol, perphenazine, and 3-PPP (40, 42, 43, 97). The σ site, however, differs in pharmacological profile (42, 43, 98, 99) from the D2 receptor. Two recent studies have investigated σ/dopamine activity of a number of OHBQ and PPP derivatives (42, 43). They demonstrated that large *N*-substituents enhanced σ activity, whereas the compound (*trans*)-(4a*R*,10b*R*)-9-OH-4-*n*-Bu-OHBQ is extremely weak at the D2 receptor. From several studies into *N*-substitution of dopamine agonists, a lipophilic group of greater size than *n*-propyl in the 'downwards' direction (see Refs. 100 and 101) results in a loss of activity. The two receptor sites also differ in their stereochemical demands, with the general trend being (for the OHBQ series) that the (4a*R*,10b*R*)-isomers are more potent at σ sites (see Refs. 42 and 43). Other compounds that show a reversal of stereoselectivity for σ and D2 receptors include (*R*)-(+)- and (*S*)-(−)-3-PPP and (+)- and (−)-butaclamol (cf. Refs. 40 and 97). Recently, we described a receptor model for D2 agonist activity that differs from the σ site determined here both in the position of primary and secondary binding sites (101). This model for the D2 dopamine receptor explains the relative activity of both (*R*)-(+)- and (*S*)-(−)-3-PPP and both isomers of the OHBQ series. Largent and co-workers (see Ref. 40) tentatively suggested a possible evolutionary relationship between dopaminergic and σ binding sites and noted that σ compounds affect dopamine function. This result may not be surprising, inasmuch as Lloyd and Andrews (59) found that drugs from 14 different pharmacological classes had in common a phenyl ring and N atom, possibly suggesting an evolutionary link between many CNS receptors.

In conclusion, the features considered important for potent PCP or σ binding include an aromatic ring, a protonated N atom, and secondary binding groups. The receptor models of the σ and PCP binding sites differ in the position of N atoms and direction of N-R3 vectors. Furthermore, the placement and nature of secondary binding sites such as ring substituents and lipophilic clefts differ between the two models. These models, along with evidence from radioreceptor, autoradiographic, and electrophysiological studies, clearly demonstrate that the two receptor sites are distinct entities. With the use of these receptor models, in conjunction with computer-aided drug design techniques, it is possible to both predict the activity and specificity and to design novel ligands for the σ and PCP binding sites.

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