MOLECULAR MODELING OF SIGMA RECEPTOR LIGANDS: A MODEL OF BINDING BASED ON CONFORMATIONAL AND ELECTROSTATIC CONSIDERATIONS

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Received 28 May 1990

Abstract

We have performed molecular modeling studies on four representative sigma receptor specific ligands, (+)haloperidol, (+)3-PPP, (+)pentazocine and progesterone, to develop a model for sigma receptor-ligand binding. The modeling studies have investigated the conformational and electrostatic properties of the ligands. Based on the complementarity of the conformational and electrostatic properties of the ligands, a model of binding has been proposed which shows that the four ligands can fit a common receptor site. Unlike the binding model for haloperidol that was previously proposed by Manallack and Andrews, our model binds haloperidol in the gauche conformation. The first site binds the fluorophenyl group and the second site the lone pair of the piperidine nitrogen. This pharmacophore can be presented by (+)3-PPP and (+)pentazocine, but for progesterone the binding model requires the ring junction of the cyclohexenyl ring A and ring B to fit the fluorophenyl region, while the lone pair of the acetylcarbonyl oxygen at ring D emulates the nitrogen lone pair of the piperidine ring. Calculations were performed using RCG5 for generating conformations, molecular mechanics for calculating steric energies, quantum mechanical methods for generating charges, and ARCHEM for calculating electrostatic potentials on the Van der Waals surface.

1. Introduction

Three types of opiate receptors have been proposed to account for the different pharmacological effects of opiates in chronic spinal dogs [1]. These receptors were named after the drugs that they bound to, namely, mu (morphine), kappa (ketocyclazocine), and sigma (SKF 10,047). Each of the receptors has been postulated to mediate a certain type of physiological response. For example, mu receptors are hypothesized to mediate analgesia, kappa receptors to mediate sedation, and sigma receptors to mediate mania and other psychotomimetic effects. The sigma ligands SKF 10,047,

cyclazocine, and pentazocine produced delirium in the dog [1] and psychotomimetic effects in man that include dysphoria and hallucinations [2–4]. The existence of delta (enkephalin) receptors has been demonstrated in vitro.

Sigma receptors were hypothesized to mediate psychotomimetic effects of certain benzomorphan opiates [1]. The prototytic drug for inducing such an effect was SKF 10,047 (N-allylnormetazocine), from which the name sigma receptor was derived. Attempts to identify sigma receptors using receptor binding assays have yielded two biochemically distinct types of binding sites. Each of the two binding sites, however, was claimed to represent sigma receptors. The first site was labeled with (^{3}H)-phencyclidine and was termed the PCP receptor [5]. The second site was labeled with (^{3}H)-SKF10047 and was termed the sigma receptor [5]. It is interesting to note, however, that although a selective ligand for each receptor exists, most ligands examined will interact with both types of receptors. In fact, the original ligands used to label these two receptors, i.e. (^{3}H)-phencyclidine and (^{3}H)SKF-10047, respectively, will interact with both types of receptors, giving ambiguity to the original biochemical identities [5]. However, which of these two receptors is responsible for the psycho-tomimetic effect induced by drugs such as phencyclidine and SKF-10047 remains an open question [5].

It is now recognized that selective ligands for the PCP receptor are MK801 [6] and TCP [7–11]. Haloperidol, d-pentazocine, (+)3-PPP, DTG [7–11], remoxipride [11], BMY14802 [12] and, interestingly, progesterone [13] are more selective ligands for the sigma receptor.

Two questions arise: Why do selective ligands for the sigma receptor encompass so many structurally dissimilar classes of substances? What is the basic difference in the structural requirement for the PCP versus the sigma receptor?

We attempt to answer the first question relating to sigma ligands by examining the molecular configurations of selective ligands for the sigma receptor using computer-assisted molecular modeling techniques.

Recently, Manallack et al. [14] have used molecular modeling and radioreceptor techniques on a wide range of PCP and sigma ligands to derive topographies of the PCP and sigma receptors. The PCP receptor model was defined using key molecules from the arylcyclohexylamine, benzomorphan, bridged benz(f) isoquinoline, and dibenzo-cycloalkeneimine drug classes. Hypothetical receptor points (R1, R2) were constructed onto the aromatic ring of each compound to represent hydrophobic interactions with the receptor, together with an additional receptor point (R3) representing a hydrogen bond between the nitrogen atom and the receptor. The superimposition of these three 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. The sigma receptor model was constructed from ligands of the benzomorphan, octahydrobenzo(f) quinolone, phenylpiperidine, and diphenylguanidine drug classes. Coordinates for the primary sigma pharmacophore are as follows:

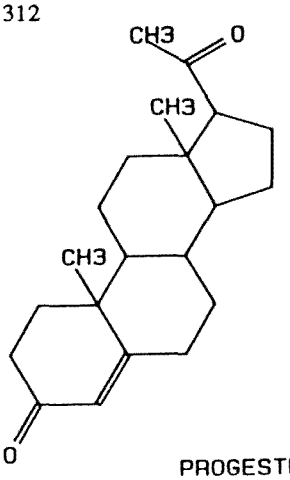
R1 (0.00, 3.50, 0.00), R2 (0.00, −3.50, 0.00) R3 (6.09, 2.09, 0.00), and N (4.9, −0.12, −1.25). Secondary binding sites for sigma ligands were proposed for the interaction of aromatic ring substituents and large N-substituted lipophilic groups with the receptor. Although the sigma model explains the compounds that were used in the study, it does not adequately explain other potent ligands such as progesterone, and some other sigma-specific drugs such as remoxipride and BMY14802. We are developing a model which is partly related to the Manallack–Andrews model, but differs in the site of activity for haloperidol and explains the competitive binding of several sigma ligands to haloperidol. The questions to be asked are: How do molecules which do not possess a nitrogen as a pharmacophore interact with the sigma or PCP receptor, and is there one pharmacophore which satisfies the requirements of binding for all sigma ligands?

To answer the above questions, we use computer-assisted modeling which involves the investigation of preferred conformations and their energetics as the first criteria for deriving bioactive conformations, and the investigation of electrostatic properties of the ligands to determine complementarity in charge distribution of the ligands and their receptors.

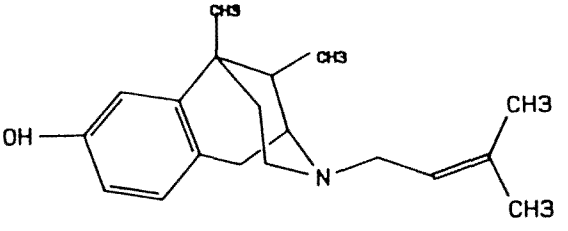
This paper will concentrate on deriving a model of binding for the sigma receptor using four ligands as model compounds, namely, haloperidol as the template, (+)3-PPP, (+)pentazocine, and progesterone.

2. Molecular modeling of sigma ligands

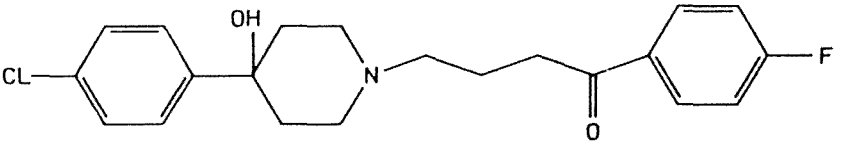
We investigated the conformational and electrostatic properties of four representative potent sigma ligands, whose structures are shown in fig. 1 and whose relative sigma potencies are listed in table 1. These compounds present several questions. Three of the four possess a phenyl group which could act as the hydrophobic site, and a nitrogen atom and a lone pair of electrons which could act as the hydrogen bonding or hydrophilic site. The published model has these two sites as being the primary points of interaction with the receptor. However, progesterone, with neither a nitrogen nor a phenyl group, is still an active sigma agonist [13,15], although it is 100-fold less active than haloperidol. The second problem is one of size or distance between the active sites within a molecule. Haloperidol is conformationally flexible and can achieve many different conformations, all of low energy. It can be fully extended as in the X-ray structure and thus extend over a large space, or it can twist to form a gauche conformation and thus assume a shorter, more globular shape. (+)3-PPP is sterically constrained but has a rotatable bond. On the other hand, the possibilities for extension over a larger spatial distance are more limited than for haloperidol. (+)Pentazocine also possesses a more fixed distance between the binding sites. Progesterone, on the other hand, is the most rigid molecule, and if one measures the backbone distance of the molecule, it is longer in length and would fit better with haloperidol in the extended conformation.



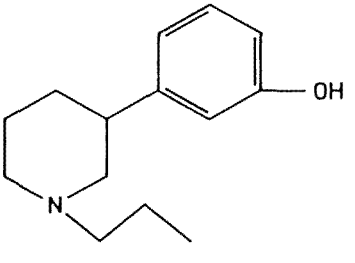
PROGESTERONE



PENTAZOCINE



HALOPERIDOL



PPP3

Fig. 1. The modeled ligands.

Table 1

Relative sigma activities of ligands

Ligand	Relative sigma activity[a)]
(+)Haloperidol	1
(d) (+)Pentazocine	15
(+)3-PPP	30
Progesterone	100

[a)]For methods of running sigma receptor binding assays on these compounds, see refs. [13] and [15].

2.1. CONFORMATIONAL STUDIES

Low-energy conformations of haloperidol, (+)3-PPP, (+)pentazocine, and progesterone were generated using the conformation generator program (RCG5) which we have implemented for the Cyber 205 supercomputer [16]. Conformations were produced by rotating the bonds or rotating the bonds of a ring with some constraints on the ring closure bond. The conformer geometries were optimized using MM2 [17,18]. We believe the active form is uncharged, since according to pka studies the nitrogen containing ligands are about 90% uncharged and progesterone is not charged. Molecular modeling studies were performed using the PS330 color vector terminal interfaced with a VAX 11-785 minicomputer. The molecules were manipulated, displayed and superimposed using the SYBYL [19] and Chemx [20] software.

For haloperidol, over one hundred conformations were generated, and those which had energies within 15 kcal of the minimum energy form were selected for further study. In general, the gauche conformers were somewhat higher in energy than the extended (2–10 kcal).

(+)3-PPP has a piperidine ring which can exist in a boat or a chair conformation. Both conformations were generated and the energies calculated. The boat conformation fits better into the model, but was 6 kcal higher in energy than the chair conformation. (+)Pentazocine fits well in the chair conformation of the piperidine ring. The boat could not be generated since it always reverted back to the chair. More studies will be performed to generate the boat. Other conformations of progesterone were generated as well. However, the X-ray structures fit as well as any of the others.

2.2. ELECTROSTATIC STUDIES

Net atomic charges were calculated for the ligands using various quantum methods. Initially, we used MNDO from MOPAC. The net charges were then used to calculate the electrostatic potentials on the Van der Waals surface using the program ARCHEM [21,22]. The potentials are graphically coded according to the magnitude of the potential where the highest positive value indicates the most repulsive interaction with a positively charged probe. Figure 2 illustrates the results of this calculation for haloperidol, (+)3-PPP, (+)pentazocine, and progesterone. Interestingly, all the ligands showed the high potential to be located in the center of the molecule and the low potential around the periphery where the aromatic rings are located. The lowest potential was around the lone pair of the piperidine nitrogen and the lone pair of the carbonyl oxygens at ring D of progesterone. MNDO [23] is the fastest method of calculating charges and can be used for larger molecules, but it is not necessarily the most accurate, especially for phenyl rings where an incorrect sign is frequently obtained. However, for these molecules the method can be used as a first approximation to indicate consistency, in particular for the lone pair binding site. Potential derived ab initio STO3G [24–27] calculations have been

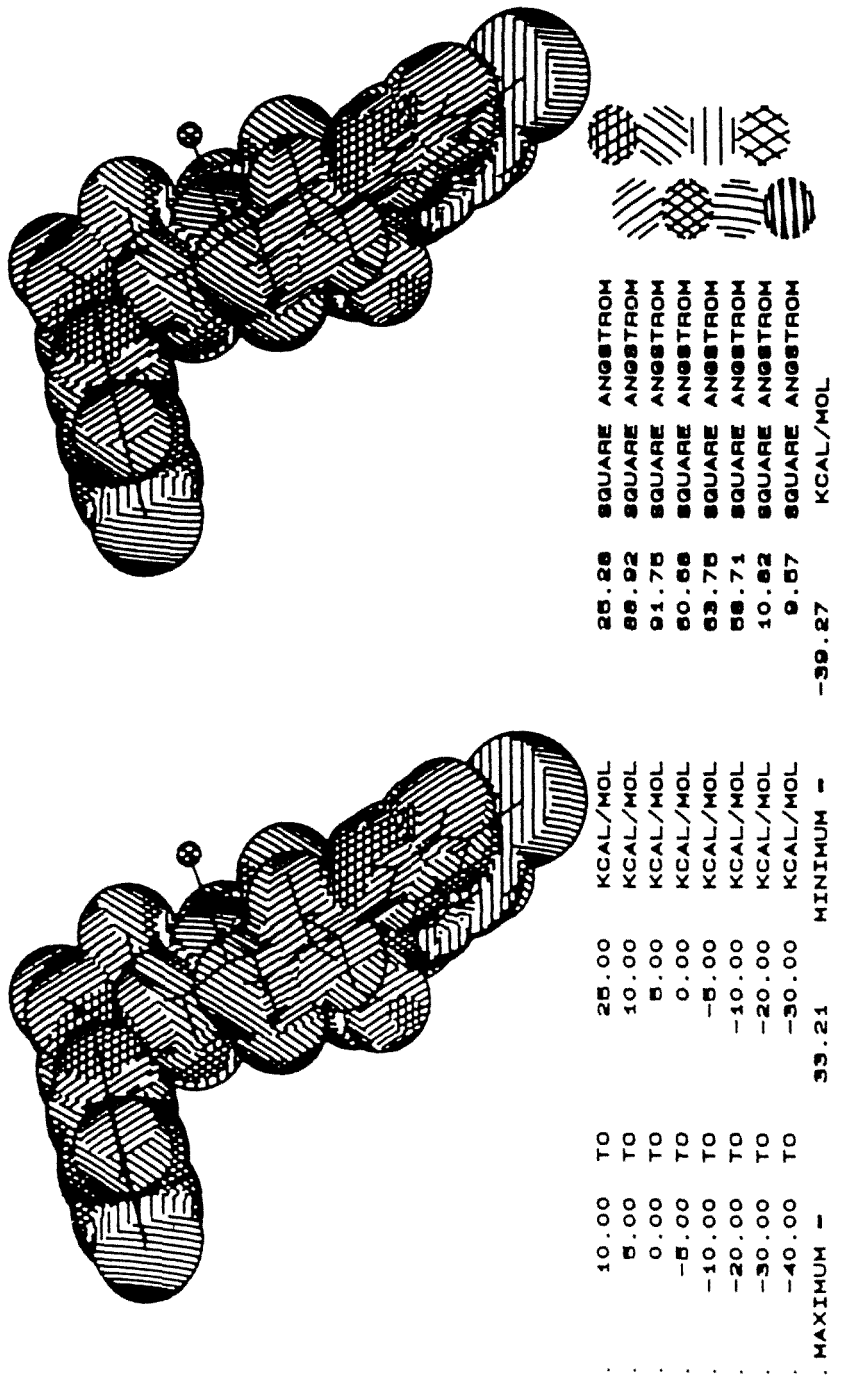
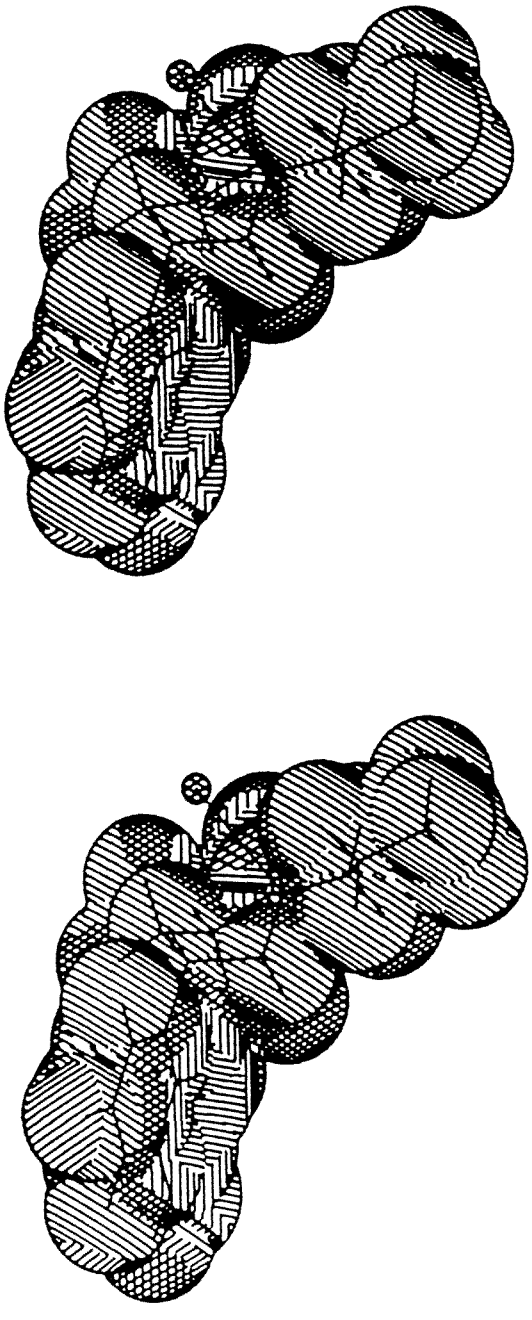
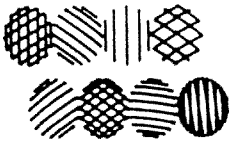


Fig. 2. (a) Haloperidol.





6.99 SQUARE ANGSTROM
10.49 SQUARE ANGSTROM
136.85 SQUARE ANGSTROM
76.64 SQUARE ANGSTROM
31.45 SQUARE ANGSTROM
30.42 SQUARE ANGSTROM
2.63 SQUARE ANGSTROM
0.00 SQUARE ANGSTROM
KCAL/MOL
−22.82

10.00 TO 25.00 KCAL/MOL
5.00 TO 10.00 KCAL/MOL
0.00 TO 5.00 KCAL/MOL
−5.00 TO 0.00 KCAL/MOL
−10.00 TO −5.00 KCAL/MOL
−20.00 TO −10.00 KCAL/MOL
−30.00 TO −20.00 KCAL/MOL
−40.00 TO −30.00 KCAL/MOL
MAXIMUM = 17.12 MINIMUM =

Fig. 2 (b) (+)3-PPP.

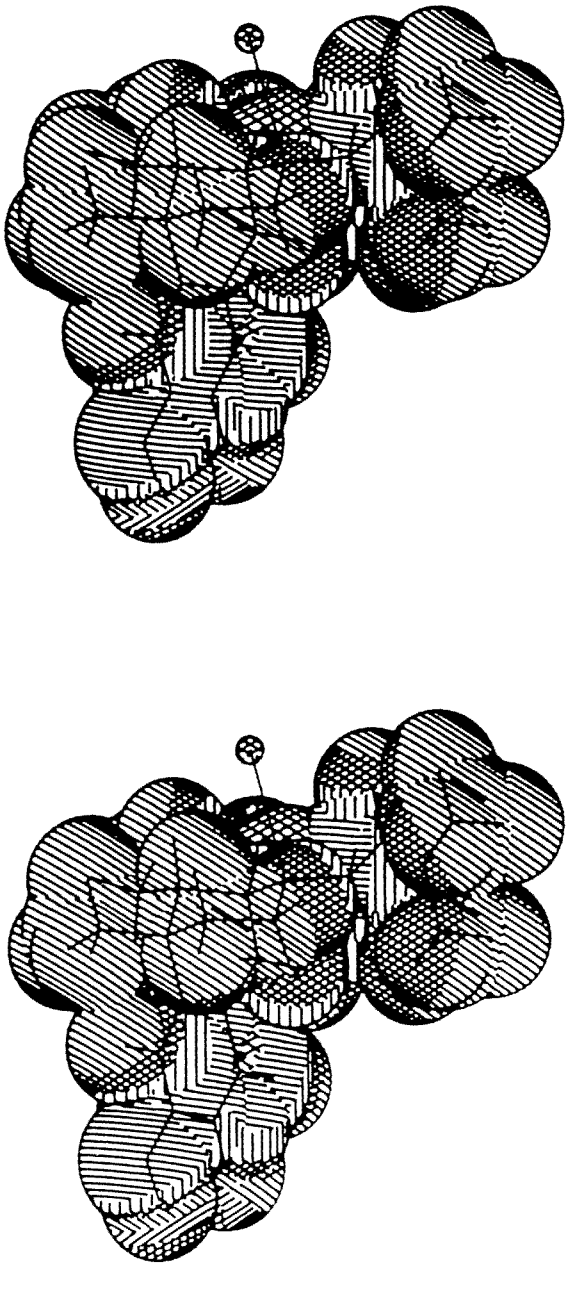


Fig. 2 (c) (+)Pentazocine.

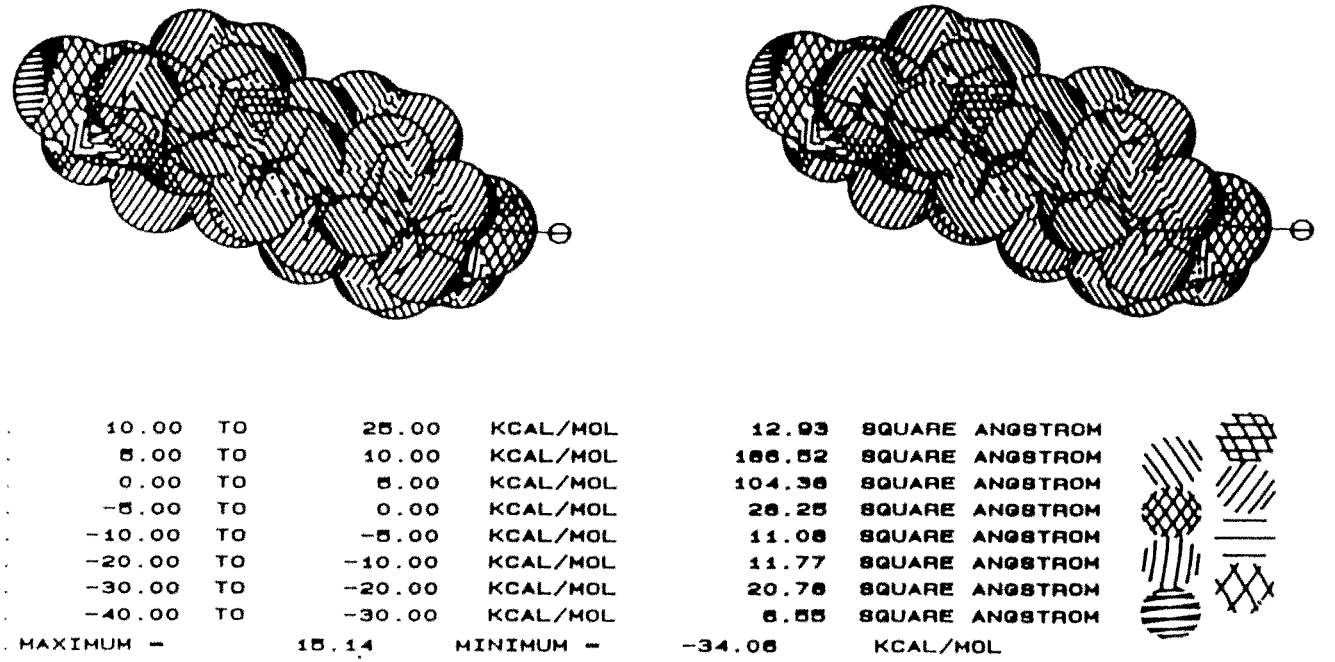


Fig. 2(d) Progesterone

Fig. 2. Electrostatic potentials on the Van der Waals surface calculated using ARCHEM. Small circle extensions indicate lone pairs. Pattern coded according to size of potential. (a) Haloperidol, potential calculated using MNDO partial charges. (b) (+)3-PPP, potential calculated using MNDO partial charges. (c) (+)Pentazocine, potential calculated using MNDO partial charges. (d) Progesterone, potential derived using MNDO partial charges.

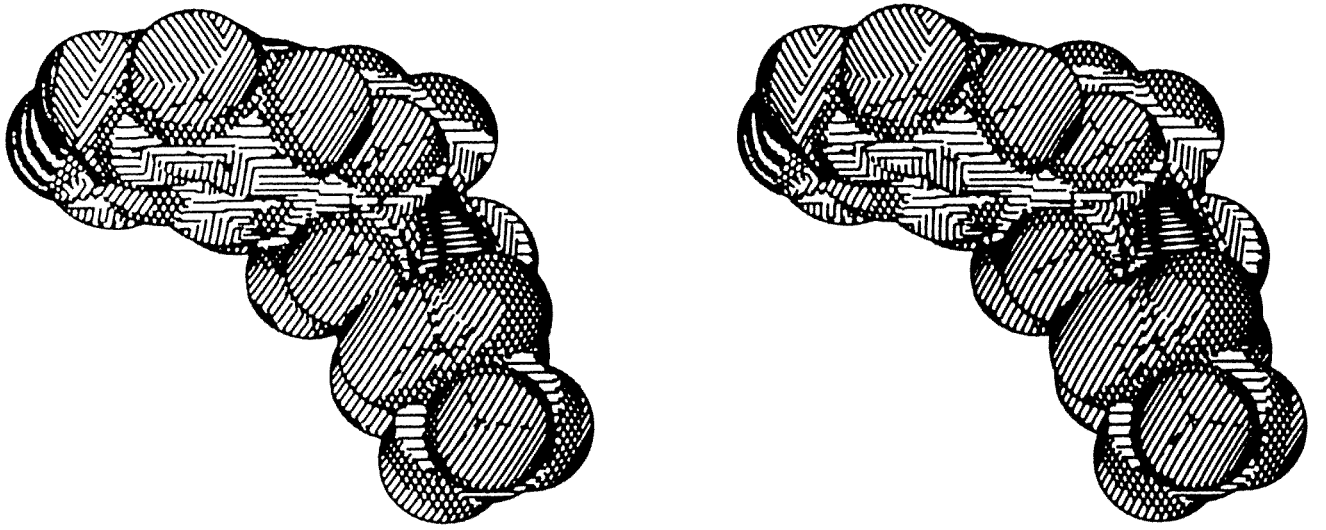


Fig. 3. Electrostatic potential on the Van der Waals surface of (+)3-PPP derived from the wave function using Gaussian ab initio charges at the STO3G level. Pattern coded according to size of potential.

performed for (+)3-PPP and are shown in fig. 3. The predictions are similar to those from MNDO-derived charges. Consistency in position of charge for the ligands is observed, e.g. the lowest potentials around the nitrogen or oxygen atoms and their lone pairs, more positive areas in the middle of the molecules, and lower potentials around the hydrophobic regions, phenyl or cyclohexynyl regions. These calculations will be compared with higher basis set calculations. Larger ligands present a problem, since the molecules need to be dissected into smaller fragments for calculation and then rebuilt. In rebuilding, approximations are introduced which provide for some inaccuracies. Nevertheless, ab initio potential derived charges will be calculated for the other ligands as well.

3. Model of binding

Our model of binding is based on conformational and electrostatic considerations. The four molecules have been superimposed to achieve the best fit at the hydrophobic region (fluorophenyl of haloperidol, phenyl of (+)3-PPP, phenyl of (+)pentazocine, and ring junctions of cyclohexenone of progesterone (ring A and ring B)), and the hydrophilic regions (piperidine nitrogen and lone pairs of haloperidol, (+)3-PPP, (+)pentazocine and acetyl carbonyl and its lone pair). Progesterone presents a special problem, since it possesses neither a phenyl ring for the hydrophobic pocket nor a nitrogen for the hydrophilic pocket. However, progesterone contains ring A and B junctions which could fit into the hydrophobic pocket, and an oxygen lone pair on the acetyl carbonyl group of ring D which could coincide with the lone pair of the nitrogen lone pair. As stated earlier, the best fit is a compromise of length and size since (+)3-PPP and (+)pentazocine are shorter in length than haloperidol and progesterone. Haloperidol can, however, shorten its conformation by twisting to achieve the gauche conformation. Figure 4(a) shows the fitting of haloperidol (gauche) with progesterone in stereo. Atoms are fitted to produce the best skeletal fit with the fluorophenyl group fitted at the cyclohexenyl ring A and B ring junctions of progesterone and the piperidine nitrogen lone pair fitted with the carbonyl oxygen lone pair at ring D of progesterone. Figure 4(b) shows haloperidol, (+)3-PPP and (+)pentazocine. The circles indicate the points of attachment, which are summarized in table 2. Figure 4(c) shows the fitting of haloperidol, (+)3-PPP, (+)pentazocine and progesterone in the best compromise fit. Fitting is done on an atom-by-atom basis to achieve the best fit of fluorophenyl (haloperidol), phenyl of (+)3-PPP and (+)pentazocine and ring junctions A and B of progesterone, and the best fit of the nitrogen lone pair of haloperidol, (+)3-PPP and (+)pentazocine and the carbonyl oxygen lone pair of progesterone in ring D. The fitting points are again given in table 2. As can be seen, the molecules fit together quite well. The boat conformation of (+)3-PPP fits better than the chair, although it is 6 kcal higher in energy. Haloperidol is fit in a gauche conformation which is 2 kcal higher in energy than the extended X-ray structure. From the best fitting points of superimposition, a pharmacophore model has been produced, whose coordinates are given in table 2 and whose fitting

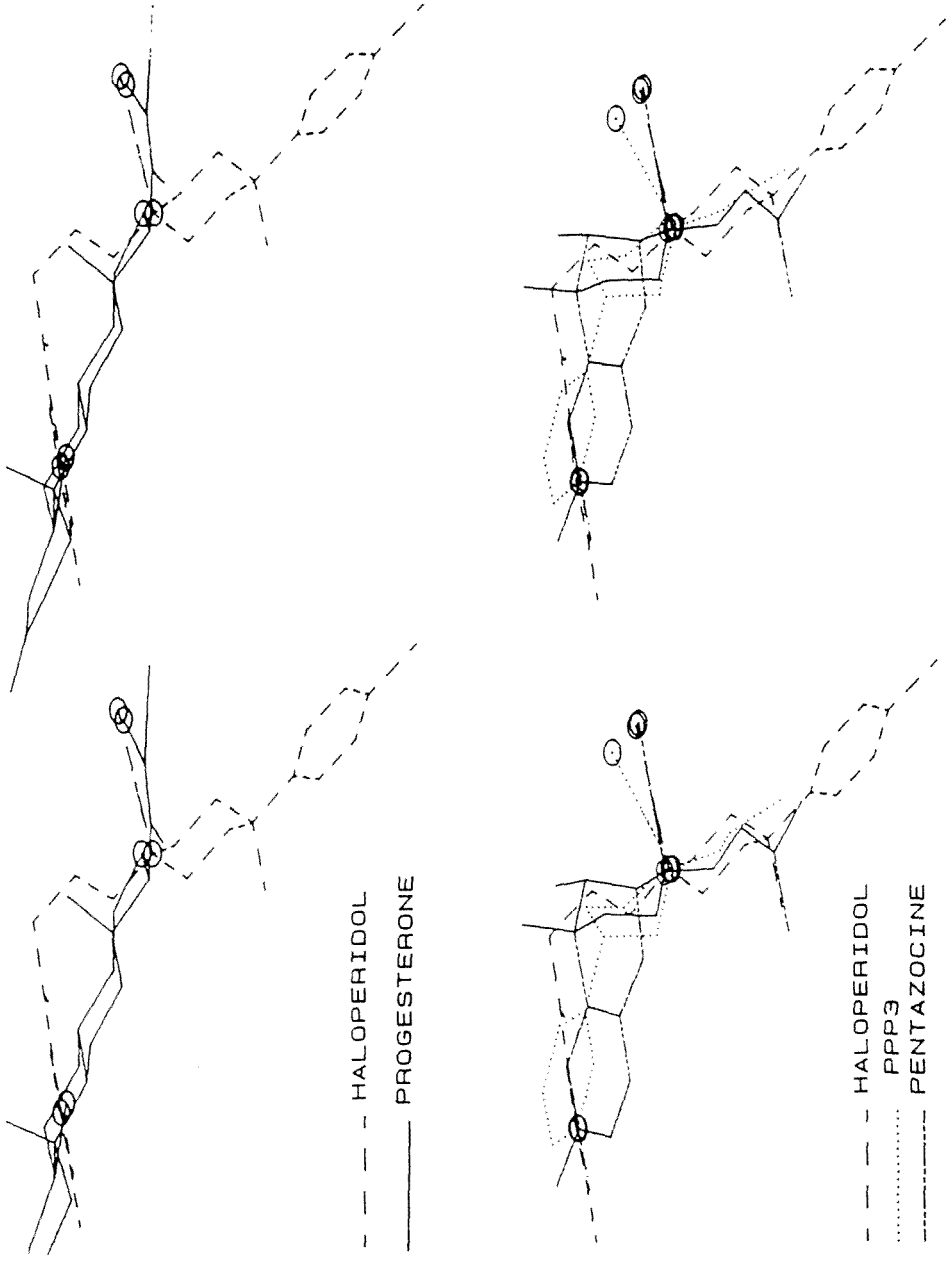


Fig. 4. Superposition of ligands at sites indicated by circles and given in table 2. (a, top) Haloperidol (gauche) and progesterone (X-ray). (b, bottom) Haloperidol (gauche), (+)3-PPP and (+)pentazocine.

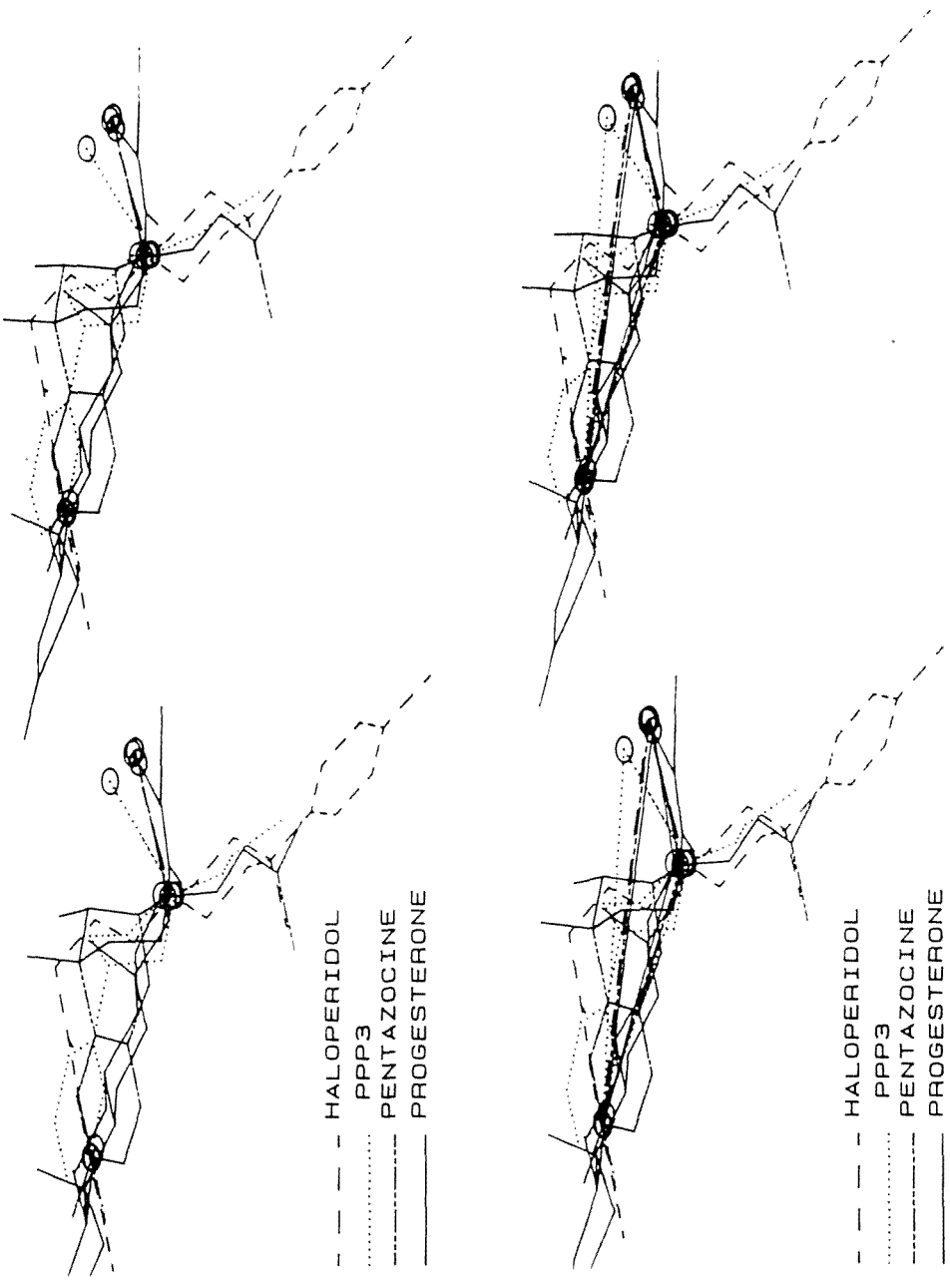
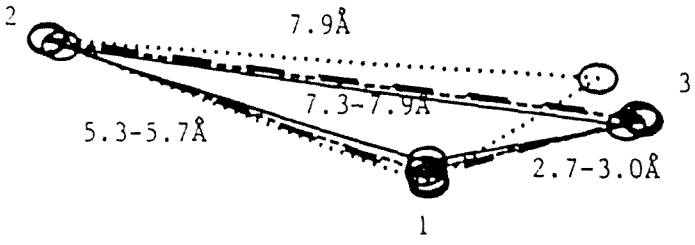


Fig. 4 (continued). Superposition of ligands at sites indicated by circles and given in table 2. (c, top) Haloperidol (gauche), (+)3-PPP, (+)pentazocine and progesterone. (d, bottom) Haloperidol (gauche), (+)3-PPP, (+)pentazocine and progesterone showing the pharmacophoric pattern superimposed with the four ligands.

Table 2

Pharmacophore and fitting sites of ligands



	(+)Pentazocine	(+)3-PPP	Haloperidol	Progesterone
Fitting sites				
(1)	N-32	N-10	N-24	C-8
(2)	C-7	C-2	Center of F-phenyl ring	Center of B-ring
(3)	LP(N-32)	LP(N-10)	LP(N-24)	LP (O at ring D)
Distance in Å between groups				
(1)	N-32 to LP	N-10 to LP	N-24 to LP	C-8 to LP
	3.0	3.0	3.0	2.7
(2)	C-7 to LP	C-2 to LP	Center of F-ring to LP	Center of B-ring to LP
	7.9	7.3	7.9	7.5
(3)	C-7 to N-32	C-2 to N-10	Center of F-ring to N-24	Center of B-ring to C-8
	5.5	5.7	5.6	5.3

pattern is shown in fig. 4(d) as the triangle fitted onto the superimposed molecules. The triangle represents distances between the nitrogen or a carbon in progesterone, lone pair on nitrogen or carbonyl oxygen and the corresponding carbon in the hydrophobic pocket. Namely, fitting position **1** constitutes the nitrogens of (+)pentazocine (N-32), (+)3-PPP (N-10), haloperidol (N-24) and C-8 of progesterone. Fitting position **2** includes carbons C-7 and C-2 of (+)pentazocine and (+)3-PPP and the center of the fluorophenyl ring of haloperidol and the center of ring B in progesterone. Fitting position **3** constitutes the lone pairs of the piperidine nitrogens (N-32, N-10, N-24) for (+)pentazocine, (+)3-PPP, haloperidol and the carbonyl oxygen lone pair of ring D in progesterone. The corresponding distances between groups

are 5.3–5.7 Å for the N . . . C distance (sites 1 . . . 2), 7.3–7.9 Å for C . . . lone pair (sites 2 . . . 3), and 2.7–3.0 Å for N or C . . . lone pair (sites 1 . . . 3).

4. Discussion

The model presented represents the initial attempt to show that four molecules which are quite different in structure and size can be made to fit the same receptor site. The initial idea of Manallack and Andrews that a hydrophobic site represented by a phenyl group and a hydrophilic site represented by the nitrogen and lone pair in haloperidol, (+)3-PPP, and (+)pentazocine is needed is still retained, but with progesterone the idea that the hydrophobic group and a nitrogen is needed is negated. A better explanation may be that a group is needed to fit the phenyl pocket, but it does not necessarily need to be aromatic. In fact, when group A of progesterone is aromatized, activity at the sigma receptor is destroyed. The lone pair is emerging as the second important site for binding. The fitting of all four molecules probes the fluorophenyl of haloperidol as the important site for primary binding to the sigma receptor. It does not disprove the chlorophenyl region as being important, but with progesterone a better fit is obtained at the fluorophenyl. More work needs to be done to definitely assign the important sites of binding. This model fits in terms of structural and electrostatic parameters in that electrostatically the molecules fit a common pattern of electron density topography.

In table 1, a large variation in sigma activities is given. This model explains the variation in activities. Haloperidol is the most potent ligand. Due to its flexibility, it can achieve the desired conformation most easily. Also, studies of BMY 14802 derivatives indicate that both phenyl groups may be important to activity [28]. (+)Pentazocine superimposes with haloperidol better than (+)3-PPP or progesterone. The lone pairs and the nitrogen of (+)pentazocine and (+)haloperidol fit very well. (+)Pentazocine can also fit in its lower energy chair piperidine conformation. (+)3-PPP is thirty times less potent than (+)haloperidol. This activity can be explained in terms of lesser fit. The distance between phenyl and N-lone pair is less. When fitted, the lone pair does not fit as well as pentazocine and fit is achieved from the higher energy state boat conformation (6 kcal higher in energy than the chair). Progesterone is one hundred times less active than haloperidol. This activity difference can be explained in terms of not possessing a phenyl group to fit the hydrophobic site and not possessing a nitrogen which could superimpose with the piperidine nitrogen of haloperidol. Progesterone has only a lone pair of oxygen which can superimpose with a nitrogen lone pair. All these factors can be responsible for the different activities that have been measured.

It is not clear how solvent will affect the conformations that we have chosen for binding. Solvent effects will be computed in the near future. It also is not clear what effects the receptor has in stabilizing conformations, but we have shown that there are conformations that could fit the same receptor site. More work needs to

be done in further clarifying the groups that are needed, as well as their relative hydrophobicity and hydrophilicity.

5. Conclusion

We have derived a model of binding for four representative sigma ligands which differ in size and length between active sites of binding. An alternative mode of binding to haloperidol has been described. The model is based on a gauche conformation of haloperidol being the active conformation and fitting to (+)3-PPP in the boat conformation and (+)pentazocine in the chair conformation. Progesterone fits in its X-ray conformation. A compromise of fit is achieved where the important parameters are the fit at the fluorophenyl, phenyl and cyclohexynyl moieties and the nitrogen lone pair of the piperidine rings with the carbonyl lone pairs of the progesterone ring D.

Future work will deal with refining the pharmacophore model and investigating the role of extended length in the ligand, as well as the role of the carbonyl side chain in haloperidol.

This work has demonstrated the utility of mathematical modeling for gaining insights into biological problems dealing with mechanism and potency.

Acknowledgements

We wish to thank the NJ Governors Commission on Science and Technology (Telematics Division), the John von Neuman National Supercomputer Center, Princeton, NJ, and the National Science Foundation for supporting this work. We thank Tripos Associates for a grant of the SYBYL software.

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