0026-895X/86/060589-10$02.00/0

MOLECULAR PHARMACOLOGY, 29:589-598

Haloperidol Binding to Monoclonal Antibodies: Conformational Analysis and Relationships to D-2 Receptor Binding

MARK A. SHERMAN, D. SCOTT LINTHICUM,[1] and MICHAEL B. BOLGER

Division of Biomedicinal Chemistry, School of Pharmacy, University of Southern California, Los Angeles, California 90033

Received December 23, 1985; Accepted March 24, 1986

SUMMARY

A library of 22 monoclonal antibodies to the D-2 dopaminergic receptor antagonist haloperidol has been developed by immunizing BALB/c mice with two conformationally distinct immunogens. The two immunogens were prepared by coupling haloperidol to bovine serum albumin through the tertiary alcohol at the 4-position of the piperidine ring with a succinic acid linkage and by coupling to bovine serum albumin through the ketone group of the butyrophenone with a carboxymethyl oxime linkage. Seventeen monoclonal antibodies displayed specific, saturable, high affinity binding of [^{3}H]haloperidol which could be inhibited by a variety of neuroleptic drugs. Three monoclonal antibodies raised against the succinic acid conjugate and two monoclonal antibodies raised against the oxime conjugate were selected for detailed analysis of the molecular characteristics of binding specificity and for relationships to bovine striatal D-2 dopaminergic receptor binding. The monoclonal antibody with highest affinity for haloperidol, 185(2)-1 (raised against the succinic acid conjugate and herein referred to as mAb A), had a K_d of 3.3(±0.06) nM and primarily recognized chemical determinants in the butyrophenone ring (ring 1) and side chain. Inhibition of [^{3}H]haloperidol binding to mAb A by 16 unlabeled haloperidol analogs displayed a good correlation [$r = 0.82$, $n = 16$, $m = 1.06(\pm 0.38)$] with D-2 receptor binding affinity, suggesting that the parts of the D-2 receptor combining site which recognize butyrophenone antagonists may have molecular characteristics which are similar to those of the monoclonal antibody. Other dopaminergic ligands such as dopamine and the D-1 antagonist SCH-23390 were not recognized by monoclonal antibodies raised against the succinic acid conjugate. Monoclonal antibodies raised against the oxime conjugate such as 258(2)-1 (herein referred to as mAb D) primarily recognized chemical determinants in ring 2 and the tertiary amino group of the piperidine ring. Although the D-2 receptor and mAb D both prefer electron-withdrawing substituents in the *para* position of ring 2, the antibody was more sensitive than the receptor to changes and displayed affinities that were much lower for substituents attached to the *p*-chlorophenyl ring (ring 2), which were electron donating. In addition, dopamine was able to completely displace 4 nM [^{3}H]haloperidol from mAb D at a concentration of 6 mM.

Antibodies which bind specifically to drug molecules have proven to be invaluable tools in therapeutic drug monitoring (1). In addition, it has recently been demonstrated that these anti-drug antibodies (Ab_1) possess a variety of idiotopes which can be recognized by the paratopes carried on the immunoglobulin products of other B- and T-cell clones (2). The idiotypic antibodies (Ab_1) can then serve as templates for the formation of second antibodies (anti-idiotypes Ab_2). Anti-idiotypic antibodies ($Ab_2\beta$), which presumably recognize the internal surfaces of the combining site of Ab_1, have been shown to mimic the original drug in their ability to bind to a pharmacological receptor (2, 3). For example, anti-idiotypic monoclonal antibodies developed by immunization with anti-alprenolol antibodies (Ab_1) or anti-reovirus antibodies (Ab_1) have been shown to cross-react with the β-adrenergic receptor and have enabled investigators to perform large scale purifications of this important protein (4, 5). At the present time, many of the factors responsible for successful development of anti-idiotypic antibodies which cross-react with neurotransmitter receptors are not well defined. The molecular characteristics of the initial drug-conjugate and the specificity of the idiotypic antibody are extremely important. It is assumed that drug-protein conjugates which preserve the native structure and conformation of the free drug are most desirable (6). It is also assumed that idiotypic antibodies whose binding sites closely resemble the actual receptor-binding site will serve as the best templates for generating the anti-receptor antibodies (2, 7). We have previously reported the preparation and a limited characterization of monoclonal antibodies to haloperidol (8).

This report is a detailed, quantitative characterization of the binding sites of five monoclonal anti-haloperidol antibodies, produced by immunization with two chemically distinct immunogens. These five mAbs have been considered as candidate

This work was supported in part by Grants AG-03272 (M. B. B.) from the National Institute on Aging, BRSG SO7 RR05192 (M. B. B.) from the Biomedical Research Support Grant Program, National Institutes of Health, and NS 22448 (D. S. L.) from the National Institute of Neurological and Communicative Disorders and Stroke.

[1] Present address: Laboratory of Neuroimmunology, Department of Pathology, University of Texas Health Science Center at Houston, P. O. Box 20708, Houston, TX 77025.

ABBREVIATIONS: mAb, monoclonal antibody; BSA, bovine serum albumin; QSAR, quantitative structure-activity relationship.

idiotypes for the production of anti-idiotype antibodies which recognize the D-2 dopaminergic receptor. This study has enabled us to evaluate the chemical determinants in haloperidol which are recognized by each mAb and to predict the conformational characteristics of the initial drug-conjugates which were recognized by the immune system. Subsequent development of anti-idiotypic antibodies (currently under way), in light of the current data, will help to define the characteristics of ligand conjugates and idiotypic mAb binding specificities which will guide the future development of anti-receptor antibodies.

Materials and Methods

Chemicals

[^{3}H]Haloperidol and [^{3}H]spiperone were obtained from New England Nuclear (Boston, MA) at a specific activity of 9.4 and 23.4 Ci/mmol, respectively, and were used without purification. Haloperidol and its analogs were a gift from Dr. Pierre Laduron of Janssen Pharmaceutica (Beerse, Belgium). (+)-Butaclamol was obtained from Research Biochemicals (Wayland, MA). Dopamine (3-hydroxytyramine) and BSA were from Sigma (St. Louis, MO).

Drug-Protein Conjugates

The method for coupling of haloperidol to BSA through the tertiary alcohol at the 4-position on the piperidine ring was developed at Janssen Pharmaceutica and is described elsewhere (8). We have developed a new method of coupling haloperidol through the keto group of the butyrophenone in order to clarify the chemical determinants of haloperidol which produce monoclonal antibodies having binding affinities similar to those of the D-2 receptor. Briefly, haloperidol-carboxymethyl-oxime was prepared by reaction of haloperidol with aminooxyacetic acid in ethanol at pH 5.5. The free carboxylic acid was then activated by reaction with isobutyl chloroformate in dimethylformamide and then conjugated to BSA at pH 9.0 in a mixture of H_2O/dimethylformamide (50:50).

Anti-Haloperidol mAbs

Preparation of mAbs which specifically bind haloperidol was accomplished using the hybridoma technique of Kohler and Milstein (9) and is described elsewhere (8). For the sake of simplicity, the hybridomas selected for this study were renamed as follows: 185(2)-1 = mAb A; 189(2)-6 = mAb B; 190(2)-6 = mAb C; 258(2)-1 = mAb D; and 258(2)-11 = mAb E.

Radioligand Binding Assays

Monoclonal antibodies. Prior to determination of competitive binding affinities, the maximal binding capacity and affinity for [^{3}H]haloperidol was determined for each mAb. Titer assays, which allow determination of the dilution range over which binding is linear, were performed by incubating increasing dilutions of ascites fluid with [^{3}H]haloperidol (see protocol below). Once the titer was established, the affinity of each mAb for haloperidol was evaluated in a saturation assay by varying [^{3}H]haloperidol over a concentration range spanning 5 log units (using the protocol below). Nonspecific binding was evaluated in the presence of 30 μM unlabeled haloperidol, and the equilibrium dissociation constant (K_d) and maximal binding capacity were determined by nonlinear regression analysis.

The concentration of each butyrophenone required to displace 50% of [^{3}H]haloperidol or [^{3}H]spiperone from the mAb-binding site (IC_{50}) was determined using the following assay. Disposable glass tubes were prepared in triplicate with tritiated ligand at a concentration slightly less than the predetermined K_d of the antibody to be tested. For mAbs A and B, this was 1 nM [^{3}H]haloperidol, whereas for mAbs D and E, 3 nM was used. Since the affinity of mAb C for [^{3}H]haloperidol is rather low, (K_d = 810 nM), competition assays for this mAb were performed using 30 nM [^{3}H]spiperone, a butyrophenone whose affinity is somewhat greater (K_d = 210 nM). An initial screening assay was used to determine an approximate IC_{50} using the unlabeled competing drug at concentrations of 0.01 nM–0.1 mM. For the final IC_{50} determination, this concentration range was narrowed to 1.5 log units above and below the approximate value determined in the screening assay. Finally, ascites fluid or tissue culture supernatant was added so that the final concentration of antibody-binding sites was equal to the K_d for the tritiated ligand being used. This dilution was 1/10,000 for mAb A, 1/20,000 for mAb B, 1/20 for mAb C (tissue culture supernatant), 1/15,000 for mAb D, and 1/2,500 for mAb E. All dilutions were made with PBS buffer (50 mM sodium phosphate, 140 mM NaCl, pH 7.4) so that the final incubation volume was 0.5 ml. Following a 2-hr incubation at 25°, tubes were quickly chilled on ice for several minutes prior to separation of bound drug from free drug. BSA-charcoal [62 μl of a well stirred, homogeneous suspension containing 10% (w/v) activated charcoal and 2% (w/v) BSA] was then added to each tube, vortexed, and permitted to settle for exactly 10 min. Tubes were then centrifuged at 4°, 2000 × g for 10 min to pellet the charcoal which adsorbs any unbound radioligand. Determination of bound radioligand was assessed by counting a 0.3-ml aliquot of the supernatant from each tube pipetted into 5 ml of liquid scintillation fluid (Liquiscint, National Diagnostics, Somerset, NJ) using a Beckmann LS 7500 counter. The speed and simplicity of the charcoal technique as well as the high affinity of our antibodies for the tritiated ligands persuaded us to adopt this procedure. Similar results were obtained by separating bound and free counts by gel filtration (Sephadex G-25 minicolumns at 4°). After plotting cpm bound versus log [drug concentration], IC_{50} values were obtained by performing weighted nonlinear regression analysis with an IBM personal computer using the program PLOT4U developed by Bolger and Muir.[2] Typical displacement curves generated from the above protocol can be seen in Fig. 1.

Membrane-bound D-2 RECEPTOR. Homogenates of bovine striatal membranes were prepared by the method of Kuno *et al.* (10) as follows. Freshly dissected tissue was homogenized in 20 volumes of ice-cold 50 mM Tris buffer (pH 7.7) with a Potter-Elvehjem-type tissue grinder (five passes). The homogenate was then centrifuged three times at 50,000 × g for 10 min with resuspension of the intermediate pellet in fresh buffer. The final pellet was resuspended in 2 volumes of the same buffer and frozen at −70° for up to 1 month. For binding assays, [^{3}H]spiperone (0.2 nM), an unlabeled haloperidol analog (0.1 mM–0.01 nM), and the thawed membrane preparation were added to each tube (0.05 mg/ml of protein final) and incubated in 50 mM Tris, pH 7.4, containing 0.1% (w/v) ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. After 15 min at 37°, bound ligand was separated from free ligand by rapid filtration through Whatman GF/C filters using a Brandel Cell Harvester (Gaithersburg, MD). Filters were then washed three times with ice-cold 50 mM Tris, pH 7.4, and then counted as above in 5 ml of liquid scintillation fluid after soaking for 3 hr at room temperature. Nonspecific binding was evaluated in the presence of 10 μM (+)-butaclamol.

Physico-chemical Parameters

The binding affinities of 16 haloperidol analogs were correlated with physico-chemical parameters using multiple linear regression analysis. Log P values, where P is the 1-octanol/water partition coefficient, were kindly supplied by Dr. Pierre Laduron, Janssen Pharmaceutica. The true value of a substituent's lipophilicity (π) was obtained by subtracting the log P of the chosen haloperidol analog from the log P of haloperidol itself, this value being labeled $\Delta\pi$. Intramolecular electronic effects were evaluated using σ_p values (11), while intermolecular electronic effects were measured using substituent dipole moments, μ (12). Steric effects were evaluated using molar volumes, calculated according to the method of Moriguchi *et al.* (13), or Verloop's Sterimol param-

[2] M. B. Bolger and K. T. Muir. PLOT4U: an interactive graphics and nonlinear regression program for the analysis of experimental and simulated data. Submitted to the Society for Computer Simulation for presentation at the Summer Computer Simulation Conference, July 1986, Reno, Nevada.

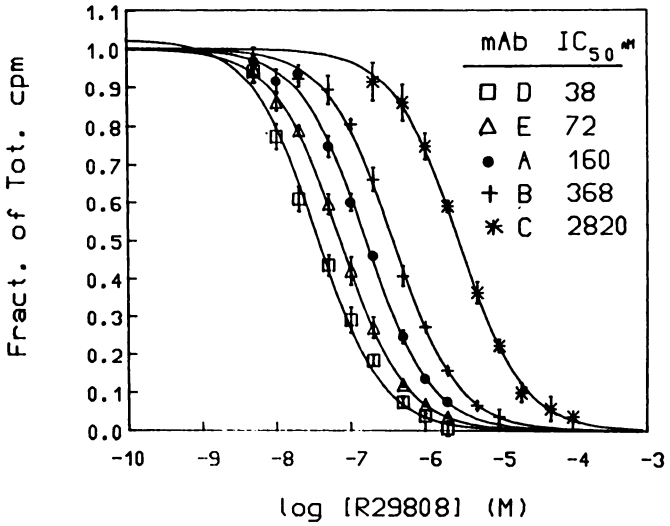


Fig. 1. Tritiated ligand displacement by R29808 for mAbs A–E. A competitive ligand-binding assay was performed in triplicate as described in Materials and Methods. For each mAb, IC_{50} values were determined by weighted nonlinear regression analysis using PLOT4U.EXE running on the IBM personal computer (10). Total counts bound were fitted to an equation describing a single independent binding, site with parameters for nonspecific binding, maximal binding, and IC_{50} as follows:

$$\text{Tot. cpm Bound} = \text{Nonspec.} + \frac{B_{max}}{1 + [\text{R29809}]/IC_{50}}$$

The following parameters and coefficients of variation were determined separately and then replotted above as a fraction of the total cpm specifically bound. CD% is defined as the ratio of standard error for a parameter estimate divided by the value of the estimated parameter.

mAb	Nonspec. (cpm)	CD%	B_{max} (cpm)	CD%	IC_{50} (nM)	CD%
	Displacement of [^{3}H]haloperidol by R29808					
D	503	7.3	2,895	4.4	38.4	14.4
E	234	5.9	3,837	0.9	72.4	3.6
A	102	22.2	1,872	1.8	160.9	8.2
B	75	10.5	1,386	1.5	368.7	5.6
	Displacement of [^{3}H]spiperone by R29808					
C	7387	3.3	26,232	2.0	2820.2	7.7

eters, which measure the actual dimensions of a substituent in angstroms (14). Values for molar refractivity, a parameter which evaluates both bulk and polarizability, were taken from Hansch and Leo (11). pK_a values for the analogs in question do not vary more than 0.2 unit and were therefore ignored in the correlations.

Quantitative Structure-Activity Relationships (QSARs)

Binding affinities of the various haloperidol analogs were correlated with the free energy-related physico-chemical substituent parameters of the analogs using the Hansch approach (15, 16), whose model takes the form:

$$\text{Log } 1/IC_{50} = a(\text{hydrophobic}) + b(\text{electronic}) + c(\text{steric}) + d$$

Stepwise multiple linear regression was performed on an IBM personal computer using the Stat-Systems software package (Psychological Assessment Research Inc, Odessa, FL).

Results

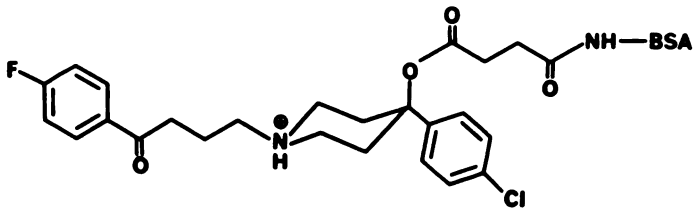
Five monoclonal anti-haloperidol antibodies possessing high affinity for free [^{3}H]haloperidol in our soluble ligand-binding assay were selected from a library of 22 mAbs for detailed molecular characterization of their binding sites. mAbs A, B, and C were raised against a haloperidol-BSA conjugate in which the drug was linked to the protein carrier via a succinic acid linker arm through the tertiary alcohol function on the piperidine ring (succinic acid conjugate). The remaining two mAbs, D and E, were also raised against haloperidol coupled to BSA, but via an aminooxyacetic acid arm through the keto group of the butyrophenone chain (oxime conjugate). The structures of both conjugates are displayed in Fig. 2.

In order to probe the various hydrophobic, electronic, and steric constraints present at the antibody-binding site, 16 analogs of haloperidol bearing simple ring or chain substitutions were selected. The structures of these analogs, their physicochemical properties, and their ability to displace [^{3}H]haloperidol from the mAb-binding site as evaluated by IC_{50} values are displayed in Table 1. Surprisingly, mAb C had a greater affinity for the related neuroleptic spiperone (K_d = 210 nM) than for the original immunogen, haloperidol (K_d = 810 nM) (8). Therefore, the IC_{50} values displayed in Table 1 for mAb C represent displacement of [^{3}H]spiperone by the various haloperidol analogs.

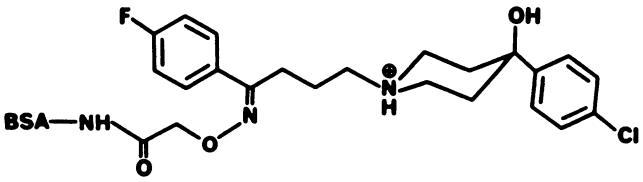
mAbs A and B, with K_d values for haloperidol of 3.3 and 4 nM, respectively, were raised against the succinic acid conjugate and appear to have very similar binding sites as shown by the series of equations in Table 2 which correlate changes in structure with changes in binding affinity. Both mAbs are highly sensitive to shortening or lengthening of the methylene chain connecting ring 1 and the piperidine ring, with the compound shortened by one methylene group having the poorest affinity. The second most heavily weighted variable is the parameter which evaluates the dimensions of the group used to replace the keto group of haloperidol. The narrow sp^2 carbonyl function of haloperidol possesses a favorable width of 3.80 Å, whereas the analogs bearing wide, sp^3-hybridized groups (sec-OH, cyclic ketal, methyl) show little affinity for the binding site. Both mAb A and B binding sites are also sensitive to the size of the substituent replacing the fluorine of haloperidol, as evaluated by its length in angstroms for mAb A and its molar volume for mAb B.

However, the binding site of mAb A differs from that of mAb B in that mAb A requires that the substituent replacing the chlorine on ring 2 of haloperidol have a negative dipole (electron density oriented toward the substituent and away from the ring), whereas no such dependency is observed for the binding site of mAb B. Overall, the binding site of mAb A appears to be a bit more restrictive than that of mAb B, since IC_{50} values tend to change much more drastically with changes in analog structure. The correlation between binding affinity and the multiple regression parameters discussed above is high, 0.93 and 0.88 for mAb A and B, respectively, both correlations being highly significant ($p < 0.001$).

The specifications displayed by the binding sites of mAb D and E, both raised against the oxime conjugate, are entirely different from that of mAbs A and B. Antibodies raised against the oxime conjugate appear to bind the *p*-chlorophenyl ring (ring 2) of haloperidol, in contrast to the anti-succinic acid conjugate antibodies which favor the butyrophenone ring (ring 1). mAb D, with a K_d for haloperidol of 4.4 nM, possesses a binding site which is highly sensitive to the electron-withdrawing characteristics of the substituents on ring 2, as shown by



SUCCINIC ACID CONJUGATE



OXIME CONJUGATE

Fig. 2. Haloperidol coupled to BSA through the tertiary alcohol at the 4-position of the piperidine ring with a succinic acid linkage (*succinic acid conjugate*) and through the ketone group of the butyrophenone with a carboxymethyl oxime linkage (*oxime conjugate*).

the series of equations in Table 3. As with mAbs A and B, the width of the substituent replacing the keto group of haloperidol contributes to binding affinity, but in this case, to a much lesser extent. Once again, the number of methylene units between ring 1 and the piperidine ring tends to influence binding affinity. However, analogs with chains longer than that of haloperidol are no longer poor binders as with mAbs A and B. It appears that any analog with at least three methylene groups will bind well. Unlike mAbs A and B, substitutions on ring 1 have no influence on binding affinity. A highly significant ($p < 0.001$) correlation coefficient of 0.88 was obtained when the above three variables were regressed against binding affinity.

mAb E, with a K_d for haloperidol of 16 nM, possesses a binding site similar to that of mAb D in that substitutions on ring 2 have the greatest influence on binding affinity. However, the combining site of mAb E appears to be much less specific, with only one variable entering significantly into the regression analysis: the minimum radius of the substituent replacing the chlorine atom in haloperidol. A significant ($p < 0.001$) correlation coefficient of 0.85 was obtained using this variable alone.

The binding site of mAb C is perhaps the most interesting in that it fails to resemble that of mAbs A and B even though it also was raised against the succinic acid conjugate. In addition, mAb C has a rather poor affinity for haloperidol (K_d = 810 nM), yet better affinity for the neuroleptic spiperone (K_d = 210 nM) (8). The binding affinity of the 16 analogs tested is most highly correlated with the electron-donating characteristics of the substituent on ring 1 as evaluated by σ_p of the group replacing the keto group of haloperidol, and demonstrated by the equations in Table 4. Unlike mAbs A and B, the width of this substituent seems to have little influence on binding affinity. Also, the size of the substituent replacing haloperidol's fluorine has little effect. The above-mentioned variable provides a correlation coefficient of 0.80, which is significant at the $p < 0.001$ level. As seen in Table 4, the correlation can be improved to 0.92 by adding variables which evaluate chain length, the dipole moment of substituents on ring 2, and the hydrophobicity of substituents on ring 1. However, as indicated by the F test values, these additional equations do not prove to be any more significant than the first equation.

Correlations of analog binding between mAbs and the D-2 dopaminergic receptor. In order to determine the degree to which the above antibody-binding sites resemble the binding site of the D-2 dopaminergic receptor, IC_{50} values were generated for the same 16 haloperidol analogs using [^{3}H]spiperone to label the receptor in homogenates of bovine striatal tissue. These IC_{50} values and the corresponding QSAR equations are displayed in Table 5. [^{3}H]Haloperidol was not used as the radioligand for receptor binding studies because of the presence of binding sites which could be inhibited with unlabeled haloperidol but which were determined to be non-D-2-receptor related as defined by 10 μM (+)-butaclamol. This type of displaceable, non-receptor binding has been observed previously for haloperidol (17).

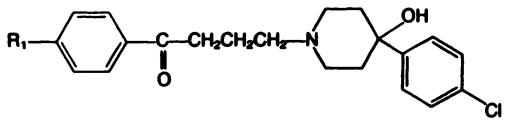
Inhibition of [^{3}H]haloperidol binding to mAb A by 16 unlabeled haloperidol analogs displayed a good correlation [$r = 0.82$, $n = 16$, $m = 1.06(\pm 0.38)$, Fig. 3] with inhibition of [^{3}H]spiperone binding to D-2 receptor binding, suggesting that the parts of the D-2 receptor combining site which recognized butyrophenone antagonists may have molecular characteristics which are similar to those of the mAb. The other monoclonal antibodies displayed poorer correlations when compared to the receptor (mAb B, $r = 0.78$; mAb C, $r = 0.30$; mAb D, $r = 0.15$; mAb E, $r = 0.05$).

Some additional IC_{50} values were generated for all five mAbs using a set of compounds whose structures are too dissimilar to that of haloperidol for inclusion in the QSAR study, but whose structures provide supporting evidence regarding the conformation and regions of the haloperidol molecule being recognized by the mAbs. This set of compounds includes the original haloperidol haptens, the isomeric semi-rigid neuroleptics R 48 455 and R 49 399, three haloperidol metabolites, and the original agonist itself, dopamine. These data are shown in Table 6. As expected, all five mAbs show high affinity for the haloperidol hapten against which they were raised. mAbs A and B, raised against the succinic acid conjugate, fail to recognize

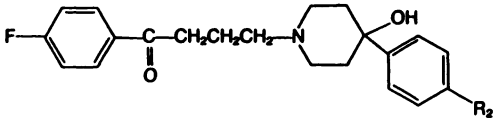
TABLE 1

IC_{50} values (nM ± standard error) of five monoclonal anti-haloperidol antibodies for 16 haloperidol analogs vs. [^{3}H]haloperidol (except mAb C, vs. [^{3}H]spiperone), followed by relevant physico-chemical parameter values for the pictured analogs

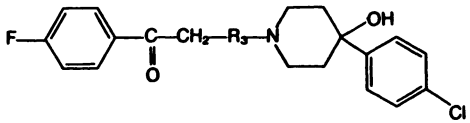
Physico-chemical parameters are defined in Tables 2, 3, and 4. R1625 = haloperidol.



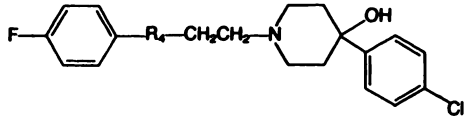
R_1	Analog	mAb A	mAb B	mAb C	mAb D	mAb E	Molar vol.	Length (Å)	Δπ
F—	R1625	0.7 ± 0.2	8.2 ± 2.0	129.2 ± 34.0	1.6 ± 0.6	12.4 ± 2.0	0.115	2.650	0.000
CH_3—	R1820	1.5 ± 0.5	25.0 ± 8.0	100.2 ± 22.0	4.2 ± 2.0	18.8 ± 3.0	0.245	3.000	0.080
CH_3O—	R1811	159.5 ± 12.0	390.7 ± 20.0	396.6 ± 79.0	1.7 ± 0.8	16.1 ± 5.0	0.304	3.980	−0.460
i-Pr—	R2693	665.2 ± 63.0	1885.3 ± 156.0	79.6 ± 28.0	10.3 ± 0.7	49.2 ± 10.0	0.553	4.110	0.840



R_2	Analog	mAb A	mAb B	mAb C	mAb D	mAb E	Dipole (μ)	σ_P	Min. radius (B_1)
—Cl	R1625	0.7 ± 0.2	8.2 ± 2.0	129.2 ± 34.0	1.6 ± 0.6	12.4 ± 2.0	−1.590	0.230	1.800
—Br	R11333	0.5 ± 0.2	5.7 ± 2.0	444.0 ± 124.0	2.2 ± 1.0	8.7 ± 2.0	−1.570	0.230	1.950
—CH_3	R1658	20.4 ± 7.0	31.5 ± 8.0	933.0 ± 127.0	69.5 ± 8.0	81.8 ± 6.0	0.360	−0.170	1.520
—OCH_3	R2842	7.3 ± 2.0	29.0 ± 6.0	116.4 ± 24.0	116.2 ± 21.0	996.7 ± 78.0	−1.300	−0.270	1.350
—*i*-Pr	R4324	63.7 ± 10.0	119.0 ± 27.0	319.6 ± 32.0	57.4 ± 6.0	10.8 ± 1.0	0.400	−0.150	2.040



R_3	Analog	mAb A	mAb B	mAb C	mAb D	mAb E	Chain-Y	Chain-X	Min. width	$\sigma_P - C = 0$	Δπ − Chain
—CH_2—	R8479	610.3 ± 97.0	3105.3 ± 259.0	11460.0 ± 1897.0	46.4 ± 13.0	31.4 ± 5.0	0.000	0.000	3.800	0.500	−0.12
—CH_2CH_2—	R1625	0.7 ± 0.2	8.2 ± 2.0	129.2 ± 34.0	1.6 ± 0.6	12.4 ± 2.0	1.000	1.000	3.800	0.500	0.00
—$CH_2CH_2CH_2$—	R8573	162.0 ± 12.0	503.3 ± 40.0	56.4 ± 5.0	4.3 ± 0.7	20.0 ± 3.0	1.000	0.000	3.800	0.500	0.45
—$CH_2CH_2CH_2CH_2$—	R8823	115.8 ± 3.0	337.1 ± 31.0	319.4 ± 106.0	2.3 ± 1.0	18.2 ± 2.0	1.000	0.000	3.800	0.500	0.80



R_4	Analog	mAb A	mAb B	mAb C	mAb D	mAb E	Chain-Y	Chain-X	Min. width	$\sigma_P - C = 0$	Δπ − Chain
—$COCH_2$—	R1625	0.7 ± 0.2	8.2 ± 2.0	129.2 ± 34.0	1.6 ± 0.6	12.4 ± 2.0	1.000	1.000	3.800	0.500	0.00
—$CH(OH)CH_2$—	R2572	260.0 ± 14.0	686.1 ± 45.0	5458.1 ± 382.0	18.4 ± 5.0	27.4 ± 2.0	1.000	1.000	4.850	−0.070	−0.10
—S—CH_2—	R5986	7.7 ± 1.0	30.4 ± 3.0	842.2 ± 197.0	5.3 ± 1.0	26.7 ± 2.0	1.000	1.000	3.800	0.000	0.81
—CONH—	R30072	43.9 ± 7.0	82.7 ± 3.0	410.2 ± 65.0	15.2 ± 4.0	20.8 ± 2.0	1.000	1.000	3.200	0.360	−0.51
—C(—$O(CH_2)_2O$—)CH_2—	R29808	160.8 ± 13.0	368.7 ± 20.0	2820.7 ± 218.0	38.5 ± 5.0	72.3 ± 3.0	1.000	1.000	5.060	0.000	0.48
—$CH(CH_2)$—	R2835	5122.0 ± 261.0	18113.0 ± 2173.0	12189.0 ± 2200.0	17.8 ± 3.0	76.5 ± 6.0	0.000	0.000	4.800	−0.150	0.66

TABLE 2

Monoclonal antibodies A and B—QSAR equations

mAb A	Log $1/IC_{50}$ = 1.36(chain-X)[a] + 6.3 n[b] = 16 r = 0.54 s = 0.97 F = 5.85
	Log $1/IC_{50}$ = 1.34(chain-X) − 1.12(min. width)[c] + 10.8 n = 16 r = 0.70 s = 0.85 F = 6.48
	Log $1/IC_{50}$ = 1.66(chain-X) − 1.43(min. width) − 1.24(L-R1)[d] + 15.4 n = 16 r = 0.86 s = 0.62 F = 11.89
	* Log $1/IC_{50}$ = 1.97(chain-X) − 1.71(min. width) − 1.53(L-R1) − 0.65(μ-R2)[e] + 16.3 n = 16 r = 0.93 s = 0.47 F = 18.68
mAb B:	Log $1/IC_{50}$ = 1.09(chain-X) + 6.00 n = 16 r = 0.57 s = 0.72 F = 6.83
	Log $1/IC_{50}$ = 1.31(chain-X) − 3.40(MV-R1)[f] + 6.40 n = 16 r = 0.73 s = 0.62 F = 7.47
	* Log $1/IC_{50}$ = 1.34(chain-X) − 4.24(MV-R1) − 0.94(min. width) + 10.3 n = 16 r = 0.88 s = 0.46 F = 13.10

[a] *Chain-X*, indicator variable describing chain length: if chain is composed of 4 atoms, then chain-X = 1; if not 4, then chain-X = 0.
[b] n, number of analogs tested; r, correlation coefficient; s, standard deviation; F, F test value; *, most statistically significant equation.
[c] Min. width, the smallest dimension, in Å, of the first two chain members attached to ring 1, measured in the plane perpendicular to the ring.
[d] L-R1, the length of the substituent attached to ring 1.
[e] μ-R2, the dipole moment, in debyes, of the substituent attached to ring 2.
[f] MV-R1, the molar volume, in cubic Å, of the substituent attached to ring 1.

TABLE 3

Monoclonal antibodies D and E—QSAR equations

mAb D:	Log $1/IC_{50}$ = 2.42(σ_p − R2)[a] + 7.5 n = 16 r = 0.68 s = 0.46 F = 12.12
	Log $1/IC_{50}$ = 2.87(σ_p − R2) − 0.67(min. width)[b] + 10.2 n = 16 r = 0.83 s = 0.37 F = 14.27
	* Log $1/IC_{50}$ = 3.02(σ_p − R2) − 0.59(min. width) + 0.56(chain-Y)[c] + 9.39 n = 16 r = 0.88 s = 0.32 F = 14.33 ($p < 0.001$)
mAb E:	* Log $1/IC_{50}$ = 2.77(B1 − R2)[d] + 2.54 n = 16 r = 0.85 s = 0.26 F = 39.5 ($p < 0.001$)

[a] σ_p − R2, electron-withdrawing or -donating capacity of the substituent attached to ring 2.
[b] Min. width, see Table 2.
[c] Chain-Y, an indicator variable describing the length of the chain connecting ring 1 and the piperidine nitrogen: if 4 atoms or greater, then Chain-Y = 1; if less than 4, Chain-Y = 0.
[d] B1-R2, the smallest radius of the substituent attached to ring 2, as measured in the plane perpendicular to the ring plane.

TABLE 4

Monoclonal antibody C—QSAR equations

* Log $1/IC_{50}$ = 2.18(σ_p − C = 0)[a] + 5.54 n = 16 r = 0.80 s = 0.40 F = 26.7 ($p < 0.001$)
Log $1/IC_{50}$ = 1.94(σ_p − C = 0) + 0.62(chain-Y)[b] + 5.0 n = 16 r = 0.86 s = 0.36 F = 19.4
Log $1/IC_{50}$ = 2.10(σ_p − C = 0) + 0.66(chain-Y) − 0.25(μ-R2)[c] + 4.6 n = 16 r = 0.90 s = 0.32 F = 16.9
Log $1/IC_{50}$ = 2.05(σ_p − C = 0) + 0.65(chain-Y) − 0.23(μ-R2) + 0.51($\Delta\pi$-R1)[d] + 4.6 n = 16 r = 0.92 s = 0.30 F = 14.9

[a] σ_p − C = 0, electron-withdrawing or -donating capacity of the first two chain members attached to ring 1.
[b] Chain-Y, see Table 3.
[c] μ-R2, see Table 2.
[d] $\Delta\pi$-R1, hydrophobicity relative to fluorine of the substituent attached to ring 1.

the metabolite consisting of ring 2 and the piperidine ring, whereas this metabolite is well recognized by the anti-oxime conjugate mAbs D and E. mAb C is unusual in that it too recognizes this metabolite even though it is of the same family as mAbs A and B. mAb C is also unusual in that it possesses an affinity for the 3-hydroxy derivative of haloperidol that is higher than the original hapten. Finally, the isomeric semi-rigid compounds R 48 455 and R 49 399 (Fig. 4) provide interesting data regarding the conformation of haloperidol favored by each of the mAbs. mAbs A and B favor the axial or "folded" isomer R 49 399, whereas mAbs D and E prefer the equatorial or "extended" version, R 48 455. Once again, mAb C defies the pattern and mimics the D-2 receptor by demonstrating a dramatic preference for the equatorial isomer. Finally, it is interesting to observe that, in contrast to mAbs A and B, dopamine at high concentrations can completely inhibit [^{3}H] haloperidol binding to mAb D and [^{3}H]spiperone binding to mAb C. The neurotransmitters serotonin and (−)-epinephrine were also tested and found to inhibit binding in the millimolar range.

TABLE 5

IC_{50} values (nM ± standard error) of bovine striatal D-2 dopaminergic receptor for 16 haloperidol analogs (structures, Table 1) vs. [^{3}H]spiperone

Analog	Receptor IC_{50}
	nM
R1625	16.7 ± 6.0
R1820	256.1 ± 25.0
R1811	1906.9 ± 228.0
R2693	1705.4 ± 238.0
R11333	20.5 ± 8.0
R1658	37.0 ± 6.0
R2842	104.1 ± 30.0
R4324	118.1 ± 31.0
R8479	3243.4 ± 421.0
R8573	421.7 ± 29.0
R8823	550.5 ± 33.0
R2572	2851.1 ± 200.0
R5986	47.8 ± 12.0
R30072	3013.9 ± 301.0
R29808	881.2 ± 132.0
R2835	930.9 ± 83.0

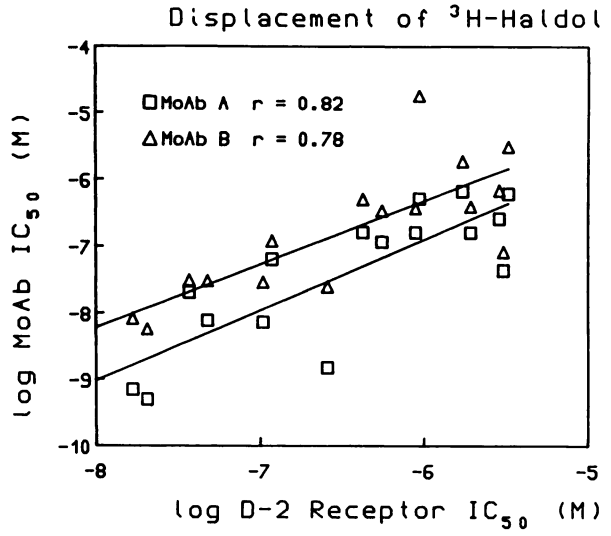


Fig. 3. Correlation between binding to mAbs A and B and the D-2 dopaminergic receptor. The inhibition of [^{3}H]haloperidol (*^{3}H-Haldol*) binding to mAb A by 16 unlabeled haloperidol analogs displayed a good correlation [$r = 0.82$, $n = 16$, $m = 1.06(\pm 0.38)$] with inhibition of [^{3}H] spiperone binding to D-2 receptor binding, suggesting that the parts of the D-2 receptor combining site which recognize butyrophenone antagonists may have molecular characteristics which are similar to those of mAb.

Discussion

The successful generation of both anti-acetylcholine receptor antibodies (18) and anti-β-adrenergic receptor antibodies (5) via intermediate anti-agonist or antagonist antibodies clearly demonstrates that the immune repertoire consists of amino acid segments which are capable of "mimicking" the molecular determinants which are responsible for binding of synthetic organic molecules (7). Presumably, such "molecular mimicry" arises from duplication of the three-dimensional arrangement of hydrophobic, electronic, and steric features of the drug by the immunoglobulin-variable region. These anti-idiotypic immunoglobulin molecules are thought to recognize chemical determinants which are internal to the combining site of the antigen and have been called the "internal image" of the antigen (2). Although the three-dimensional interactions of antigen and antibody have been studied for several systems using X-ray crystallography (19–24), no such studies have been performed for a derived anti-idiotypic/idiotypic pair in support of the internal image concept.

It has been argued that certain non-internal image antibodies, such as those recognizing the reverse side of amino acids forming the antigen-binding site, would be just as useful for receptor isolation as the actual internal image counterparts (7). However, the binding portion of such an anti-idiotypic antibody would have no resemblance whatsoever to the original receptor-binding ligand.

Because of our interest in development of peptide analogs of synthetic drugs, we have chosen to characterize extensively the molecular features of our anti-haloperidol mAbs via structure-activity studies so that clones possessing binding sites topographically similar to that of the receptor can be systematically selected for generation of internal image anti-receptor antibodies.

It is clear from the above binding data to the isomeric analogs R 48 455 and R 49 399 that two different conformations of haloperidol are being recognized by these mAbs: a folded conformation preferred by mAbs A and B, and an extended conformation preferred by mAbs C, D, and E and the D-2 receptor. R 48 455, the extended isomer whose tertiary nitrogen lone pair is cis to ring 2, is the biologically active member of the pair and is one of the most potent neuroleptics known.[3] This implies that mAbs C, D, and E might serve as better templates for production of anti-receptor antibodies than would mAb A or B.

Extensive X-ray crystallographic data are available for many of the butyrophenones, including haloperidol, which consistently crystallizes in the extended conformation (25). Molecular mechanics calculations also indicate that this is the most energetically favored conformer (26, 27). Although an extended conformation in the solid phase or *in vacuo* does not guarantee a similar conformation in solution, it remains puzzling why all five mAbs do not recognize the same conformer of the drug. The conformation adopted by haloperidol during B-cell and T-cell recognition is most likely to be directly related to its mode of attachment to the protein carrier. Molecular mechanics calculations indicate that the phenyl ring attached to a piperidine ring favors the equatorial position (28). However, attachment of haloperidol through the tertiary alcohol to the bulky succinyl-lysyl-protein group during conjugation to BSA might force the phenyl ring into the axial position. Indeed, a molecular mechanics study on the structurally related 4-phenylpiperidine analgesics such as meperidine (which possess amide or ester chains in place of haloperidol's alcohol function) indicates that the energy difference between the equatorial phenyl group and the axial ester chain is only 0.6 kcal/mol (29). Dreiding models indicate that forcing haloperidol's phenyl ring 2 into the axial position ideally positions it for an internal ring-stacking interaction between itself and the butyrophenone ring 1, which would further stabilize a folded conformation (Fig. 5). Thus, the production of mAbs A and B which seem to recognize the folded conformation can be explained. Those protein conjugates made by attachment through the keto group of haloperidol (oxime conjugate which produced mAbs D and E) would retain ring 2 in an equatorial conformation and would therefore be

[3] Pierre Laduron, personal communication.

TABLE 6

IC_{50} values (nM ± standard error) of five monoclonal anti-haloperidol antibodies for 10 miscellaneous compounds related to haloperidol

Analog	MoAb A	MoAb B	MoAb C	MoAb D	MoAb E	Receptor
Pip. metab.	>1 × 10^5	>1 × 10^5	25,606 ± 7,579	3,338 ± 941	338 ± 33	9657 ± 1738
R49 399	42 ± 11	160 ± 80	5,200 ± 2,028	6,900 ± 1,932	15,000 ± 4,200	337 ± 57
R48 455	1,400 ± 420	5,900 ± 1,700	170 ± 66	1,200 ± 144	8,900 ± 2,670	3 ± 1
3-OH metab.	88 ± 8	138 ± 9	62 ± 8	83 ± 8	60 ± 23	100 ± 14
3-OH reduced	884 ± 309	2,139 ± 340	2,728 ± 346	148 ± 20	83 ± 132	
Succ. conj	2 ± 0.7	4 ± 0.6	433 ± 142	426 ± 153	2,732 ± 437	
Oxime conj.	318 ± 108	646 ± 381	4,232 ± 1,176	6 ± 0.7	25 ± 3	
Dopamine	>100 mM	>100 mM	31 mM ± 15	6 mM ± 1.8	90 mM ± 90	
Serotonin			17 mM ± 16	0.52 mM ± 0.3	2.6 mM ± 0.6	
Epinephrine			22 mM ± 22	2.9 mM ± 1.5	2.6 mM ± .9	

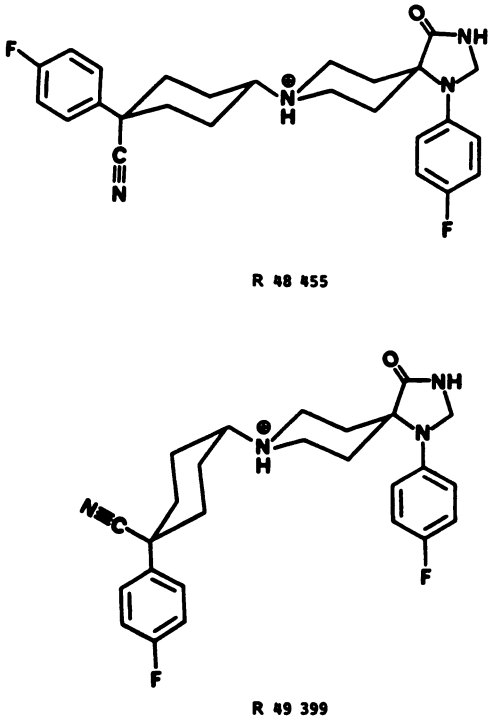


Fig. 4. Structures of semi-rigid neuroleptics R 48 455, the equitorial or "extended" isomer, and R 49 399, the axial or "folded" isomer.

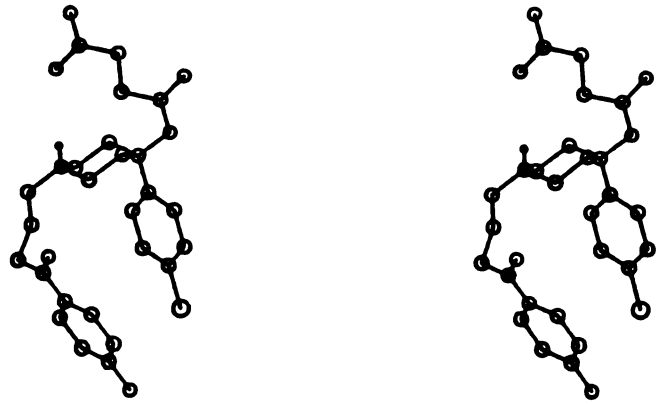


Fig. 5. Stereo-representation of a folded conformation of the haloperidol-succinic acid hapten. Three-dimensional cartesian coordinates of the conjugates were calculated from internal coordinates, displayed, and plotted with an IBM PC/XT using software developed by M. B. Bolger for the IBM Advanced Education Project.

expected to remain in the energetically favored extended conformation.

Envisioning a folded conformation for the succinic acid-conjugated haloperidol also explains why mAbs A and B strongly prefer analogs which bear narrow sp^2 hybridized substitutions in the keto position of the butyrophenone chain. Dreiding models indicate that wide, bulky substituents inhibit the folding of the molecule found necessary for binding to these mAbs. Folding also explains the extreme dependency on butyrophenone chain length displayed by mAbs A and B. The haloperidol analog which is short by one methylene group is one of the worst binding analogs in the series, most probably because its chain is too short to permit the stacking of its phenyl rings which stabilizes a folded conformer. Those analogs which bear extra methylene groups in the chain possess enough flexibility to align the phenyl rings in the proper orientation and, thus, display better binding. These features are schematically displayed in Fig. 6. Finally, it can be seen that the analog with the highest affinity for mAbs A and B is the free succinic acid hapten iteslf. Since our data indicate that the alcohol position of the molecule is only weakly recognized by the antibody, the high binding affinity displayed for this analog most likely arises from the folded conformation that it most likely adopts as a result of conjugation through the tertiary alcohol.

mAb C is unique in many respects, as already discussed, especially with regard to its high affinity for spiperone. Even though this antibody was raised against the succinic acid con-

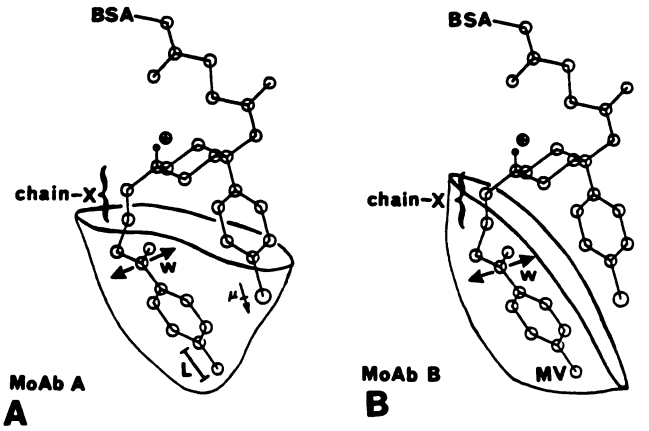


Fig. 6. Schematic representation of the molecular interactions between mAbs A and B and the haloperidol-succinic acid conjugate. A. The primary substituent parameters effecting the binding affinity of mAb A are: *chain-X* = indicator variable describing chain length; *w* = minimum width of carbonyl; *L* = length of substituent attached to ring 1; and μ = dipole moment of substituent attached to ring 2. B. The primary substituent parameters effecting the binding affinity of mAb B are: chain-X, *w*, and *MV* = molar volume of the substituent attached to ring 1.

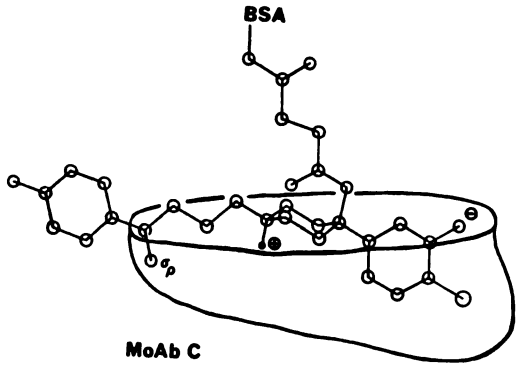


Fig. 7. Schematic representation of the molecular interactions between mAb C and the haloperidol-succinic acid conjugate. The primary substituent parameter effecting the binding affinity of mAb C is σ_p = electron-withdrawing or -donating capacity of the first two chain members attached to ring 1.

jugate, it prefers the extended isomer R 48 455 over the folded isomer by a ratio of 40 to 1. Additionally, it displays its highest binding affinity for a metabolite of haloperidol which bears an hydroxyl substitution ortho to the chlorine atom on ring 2. These observations strongly suggest that the original haloperidol-BSA conjugate molecule against which mAb C formed had been metabolized to the 3-hydroxy derivative. An additional phenolic group on ring 2, ionized at physiological pH, would totally alter the electronic properties of ring 2, thus modifying the ability of this hapten to stabilize itself through a folded, ring-stacked conformation (Fig. 7). The addition of the charged phenoxide moiety on ring 2 might also explain the affinity of this mAb for spiperone whose spirodecanone ring system possesses several polar atoms in this region.

Much controversy exists as to which portion of the butyrophenone structure represents the pharmacophore, i.e., the portion which mimics the natural agonist dopamine. Early investigators using molecular models believed that dopamine overlapped with haloperidol's ring 1 and its piperidinyl-charged nitrogen (30–32), whereas recent molecular mechanics studies have suggested overlap with ring 2 (26, 27). Dopamine binding is observed for all of the mAbs that prefer the extended conformation of the butyrophenone and that are sensitive to changes in ring 2 of haloperidol (mAbs C, D, and E, Figs. 7 and 8). This supports the assignment of ring 2 as the proposed pharmacophore. Since these same mAbs also bind the positively charged phenyl-piperidine metabolite of haloperidol, it could be argued that the weak affinity for dopamine is simply reflecting an affinity for any small molecule with a positively charged nitrogen and a phenyl ring. However, the affinity for dopamine in no way correlates with the affinity for this metabolite (see Table 6). These data alone indicate that it may be possible to use a library of mAbs raised against different portions of a drug molecule to define its pharmacophore.

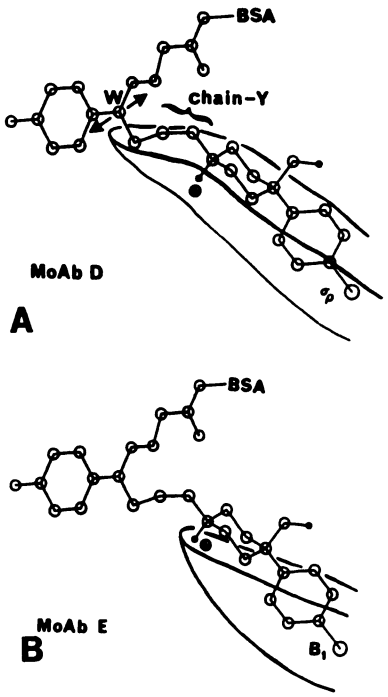


Fig. 8. Schematic representation of the molecular interactions between mAbs D and E and the haloperidol-oxime conjugate. A. The primary substituent parameters effecting the binding affinity of mAb D are: σ_p = electron-withdrawing or -donating capacity of the substituent attached to ring 2; *W* = minimum width of the first two chain members attached to ring 1; and *chain Y* = indicator variable describing the aliphatic chain length. B. The primary substituent parameter which effects the binding affinity of mAb E is: B_1 = the smallest radius of the substituent attached to ring 2 as measured in the plane perpendicular to the ring plane.

Finally, it is appropriate to predict which of the five mAbs characterized in this study is most likely to serve as a template for the production of anti-receptor antibodies. Several structure-activity relationship studies for the binding of butyrophenones to the D-2 dopaminergic receptor have been reported (31, 33, 34), and several models of this receptor have recently been proposed based on the activity of conformationally rigid analogs (35–38). However, most of the butyrophenone studies have been qualitative in approach, with activity evaluated through various behavioral tests routinely used to evaluate dopaminergic activity (30). In order to eliminate possible pharmacokinetic and metabolic influences on binding affinity, we have chosen to compare our antibody binding data to a comparable set of data using the same haloperidol analogs to displace [^{3}H]spiperone from bovine striatal D-2 dopaminergic receptors. Our correlation of the receptor data with antibody data (16 haloperidol analogs) indicates that the binding site of mAb A most closely resembles that of the receptor (r = 0.85).

However, the correlation fails when one examines binding data for the semi-rigid isomers R 48 455 and R 49 399. The receptor prefers the extended isomer by a factor of 200, whereas mAb A prefers the folded isomer 33:1. Both binding sites are sensitive to the size of the substituent on ring 1, the width of the function in the keto position, and the number of atoms in the butyrophenone chain. However, the conformational change in the haloperidol molecule most likely induced by coupling it to a protein through its tertiary alcohol has created an antibody binding site which is conformationally unrelated to that of the receptor. Although mAbs C, D, and E bind the agonist dopamine, they also display an affinity for the neurotransmitters serotonin and (−)-epinephrine, in the relative order serotonin > epinephrine > dopamine. This relative order is uncharacteristic of binding to the D-2 receptor, which binds the neurotransmitters in the order dopamine > epinephrine > serotonin (39).

In conclusion, this study suggests that neither of the haloperidol-protein conjugates used in this study is able to produce antibodies which mimic the D-2 receptor in terms of analog binding specificity for a broad range of pharmacologically active compounds. However, it is worth noting that the mAbs produced against the oxime conjugate do bind dopamine, suggesting that ring 2 of haloperidol may be the pharmacophore. We are currently raising monoclonal anti-idiotypic antibodies to further test the hypotheses set forth in this paper.

Acknowledgments

We wish to thank P. Laduron for helpful discussions and for providing unlabeled and tritiated ligands for this study. We also wish to thank Minh Dinh and Richard Hatakeyama for technical support.

References

1. Abraham, G. E. *Handbook of Radioimmunoassay.* Marcel Dekker, New York (1977).
2. Bona, C. A., and H. Kohler. Anti-idiotypic antibodies and internal images, in *Monoclonal and Anti-Idiotypic Antibodies: Probes for Receptor Structure and Function* (J. C. Venter, C. M. Fraser, and J. Lindstrom, eds.). Alan R. Liss, Inc., New York, 141–149 (1984).
3. Venter J. C., C. M. Fraser, and J. Lindstrom. *Monoclonal and Anti-idiotypic Antibodies: Probes for Receptor Structure and Function.* Vol. 4. Alan R. Liss, Inc., New York (1984).
4. Co, M. S., G. N. Gaulton, A. Tominaga, C. J. Homcy, B. N. Fields, and M. I. Greene. Structural similarities between the mammalian β-adrenergic and reovirus type 3 receptors. *Proc. Natl. Acad. Sci. USA* **82**:5315–5318 (1985).
5. Guillet, J. G., S. V. Kaveri, O. Durieu, C. Delavier, J. Hoebeke, and A. D. Strosberg. β-Adrenergic agonist activity of a monoclonal anti-idiotypic antibody. *Proc. Natl. Acad. Sci. USA* **82**:1781–1784 (1985).
6. Flurkey, K., M. B. Bolger, and D. S. Linthicum. Preparation and characterization of antisera and monoclonal antibodies to serotonergic and dopaminergic ligands. *J. Neuroimmumol.* **8**:115–127 (1985).
7. Erlanger, B. F. Anti-idiotypic antibodies: what do they recognize? *Immunol. Today* **6**:10–11 (1985).
8. Bolger, M. B., K. Flurkey, R. D. Simmons, D. S. Linthicum, P. Laduron, and M. Michiels. Preperation and characterization of antisera and monoclonal antibodies to haloperidol. *Immunol. Invest.* **14**:523–540 (1985).
9. Kohler, G., and C. Milstein. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (Lond.)* **256**:495–497 (1975).
10. Kuno, T., K. Saijoh, and C. Tanaka. Solubilization of D-2 dopamine receptor coupled to guanine nucleotide regulatory protein from bovine striatum. *J. Neurochem.* **41**:841–847 (1983).
11. Hansch, C., and A. Leo. *Substituent Constants for Correlation Analysis in Chemistry and Biology.* John Wiley & Sons, New York (1979).
12. Lien, E. J., Z. Guo, R. Li, and C. Su. Use of dipole moment as a parameter in drug-receptor interaction and quantitative structure-activity relationship studies. *J. Pharm. Sci.* **71**:641–655 (1982).
13. Moriguchi, I., Y. Kanada, and K. Katsuichiro. Van der Waals volume and the related parameters for hydrophobicity in structure-activity studies. Chem. *Pharm. Bull. (Tokyo)* **24**:1799–1806 (1976)
14. Verloop, A., W. Hoogenstraaten, and J. Tipker. Development and application of new steric substituent parameters in drug design, in *Drug Design* (E. J. Ariens, ed.), Vol. 7. Academic Press, New York, 165–207 (1976).
15. Hansch, C., and Fujita, T. ρ-σ-π analysis. A method for the correlation of biological activity and chemical structure. *J. Am. Chem. Soc.* **86**:1616–1626 (1964).
16. Fujita, T., J. Iwasa, and C. Hansch. A new substituent constant, π, derived from partition coefficients. *J. Am. Chem. Soc.* **86**:5175–5180 (1964).
17. Leysen, J.E. Receptors for neuroleptic drugs. *Adv. Human Psychopharmacol.* **3**:315–356 (1984).
18. Wasserman, N. H., A. S. Penn, P. I. Freimuth, N. Treptow, S. Wentzel, W. L. Cleveland, and B. F. Erlanger. Anti-idiotypic route to anti-acetylcholine receptor antibodies and experimental myasthenia gravis. *Proc. Natl. Acad. Sci. USA* **79**:4810–4814 (1982).
19. Feldmann, R. J., M. Potter, and C. P. J. Glaudemans. A hypothetical space-filling model of the V-regions of the galactan-binding myeloma immunoglobulin J539. *Mol. Immunol.* **18**:683–698 (1981).
20. Edmundson, A. B., K. R. Ely, and J. N. Herron. A search for site-filling ligands in the Mcg Bence-Jones dimer: crystal binding studies of fluorescent compounds. *Mol. Immunol.* **21**:561–576 (1984).
21. Segal, D. M., E. A. Padlan, G. H. Cohen, S. Rudikoff, M. Potter, and D. R. Davies. The three-dimensional structure of a phosphorylcholine-binding mouse immunoglobulin Fab and the nature of the antigen binding site. *Proc. Natl. Acad. Sci. USA* **71**:4298–4302 (1974).
22. Dwek, R. A., S. Wain-Hobson, S. Dower, P. Gettins, B. Sutton, S. J. Perkins, and D. Givol. Structure of an antibody combining site by magnetic resonance. *Nature (Lond.)* **266**:31–37 (1977).
23. Mariuzza, R. A., A. G. Amit, G. Boulot, P. Saludjian, F. A. Saul, P. Tougard, R. J. Poljak, J. Conger, E. Lamoyi, and A. Nisonoff. Crystallization of the Fab fragments of monoclonal anti-*p*-azophenylarsonate antibodies and their complexes with haptens. *J. Biol. Chem.* **259**:5954–5958 (1984).
24. Amzel, L. M., R. J. Poljak, F. Saul, J. M. Varga, and F. F. Richards. The three dimensional structure of a combining region-ligand complex of immunoglobulin NEW at 3.5-A resolution. *Proc. Natl. Acad. Sci. USA* **71**:1427–1430 (1974).
25. Tollenaere, J. P., H. Moereels, and L. A. Raymaekers. *Atlas of the Three-Dimensional Stucture of Drugs.* Elsevier/North Holland Publishing Co., New York (1979).
26. Koch, M. H. J. The conformation of neuroleptic drugs. *Mol. Pharmacol.* **10**:425–437 (1974).
27. Tollenaere, J. P., H. Moereels, and M. H. J. Koch. On the conformation of neuroleptic drugs in the three aggregation states and their conformational resemblance to dopamine. *Eur. J. Med. Chem.* **12**:199–211 (1977).
28. Tollenaere, J. P., H. Moereels, and L. A. Raymaekers. Molecular modelling by computer techniques and pharmacophore identification, in *X-ray Crystallography and Drug Action* (A. S. Horn and C. J. De Ranter, eds.). Clarendon Press, Oxford, 461–483 (1984).
29. Froimowitz, M. Conformation-activity study of 4-phenylpiperidine analgesics. *J. Med. Chem.* **25**:1127–1133 (1982).
30. Seeman, P. Brain dopamine receptors. *Pharmacol. Rev.* **32**:229–313 (1981).
31. Jain, P. C., and N. Kumar. Agents acting on central dopamine receptors. *Prog Drug Res* **21**:409–465 (1977).
32. Horn. A. S., and S. H. Snyder. Chlorpromazine and dopamine: conformational similarities that correlate with the antischizophrenic activity of phenothiazine drugs. *Proc. Natl. Acad. Sci. USA* **68**:2325–2328 (1971).
33. Janssen, P. A. J., and W. F. M. van Bever. Structure-activity relationships of the butyrophenones and diphenylbutylpiperidines, in *Handbook of Psychopharmacology,* (L. L. Iversen, S. D. Iversen, and S. H. Snyder, eds.), Vol. 10. Plenum Press, New York, 1–35 (1978).
34. Horn, A. S. Structure-activity relations for neurotransmitter receptor agonists and antagonists, in *Handbook of Psychopharmacology* (L. L. Iversen, S. D. Iversen, and S. H. Snyder, eds.), Vol. 2. Plenum Press, New York, 179–243 (1975).
35. Humber, L. G., F. T. Bruderlein, A. H. Philipp, and M. Gotz. Mapping the dopamine receptor. 1. Features derived from modification in ring E of the neuroleptic butaclamol. *J. Med. Chem.* **22**:761–767 (1979).
36. Philipp, A. H., and L. G. Humber. Mapping the dopamine receptor. 2. Features derived from modifications in the rings A/B region of the neuroleptic butaclamol. *J. Med. Chem.* **22**:768–773 (1979).
37. Olson, G. L, H. Cheung, K. D., Morgan, J. F. Blount, L. Todaro, L. Berger, A. B. Davidson, and E. Boff. A dopamine receptor model and its application in the design of a new class of rigid pyrrolo[2,3-g]isoquinoline antipsychotics. *J. Med. Chem.* **24**:1026–1034 (1981).
38. Carnmalm, B., L. Johansson, and S. Ramsby. Absolute configuration of potentially neuroleptic rigid spiro amines: a study on the topography of the neuroleptic receptor. *Acta Pharm. Suec.* **16**:239–246 (1979).
39. Creese, I., R. Schneider, and S. H. Snyder. [^{3}H]-Spiroperidol labels dopamine receptors in pituitary and brain. *Eur. J. Pharmacol.* **46**:377–381 (1977).

Send reprint requests to: Dr. Michael B. Bolger, Division of Biomedicinal Chemistry, School of Pharmacy, University of Southern California, Los Angeles, CA 90033.