Quantitative Analysis of the Structural Requirements for Blockade of the *N*-Methyl-D-aspartate Receptor at the Phencyclidine Binding Site

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Received July 7, 1997[⊗]

Blockade of the *N*-methyl-D-aspartate receptor by uncompetitive antagonists has implications for symptomatic and neuroprotective therapy of various neuropsychiatric diseases. Since the three-dimensional (3D) structure of this ion channel is unknown, the structural requirements for uncompetitive inhibition were investigated by application of a three-step strategy: At first, K_i values were measured for a number of structurally diverse compounds at the phencyclidine (PCP) binding site in postmortem human frontal cortex. Second, a pharmacophore model was developed for this set of molecules employing a mathematical method called graph theory. The resulting pharmacophore provided a very good explanation for the ability of structurally diverse compounds to bind to the same binding site. Using the experimental data and the pharmacophore as a basis for the third step, a three-dimensional quantitative structure−activity relationship (3D-QSAR) applying comparative molecular field analysis (CoMFA) was performed. The QSAR proved to be highly consistent and showed good predictiveness for several additional molecules. The results give a deeper insight into the structural requirements for effective NMDA receptor antagonism and offer the opportunity for improved drug design. The study represents the first quantitative 3D-QSAR model for NMDA receptor blockade, and it comprises structurally very different molecules. That the alignment for a highly consistent CoMFA is based on an automated 3D pharmacophore analysis has important methodological implications.

Introduction

N-methyl-D-aspartate (NMDA) receptors belong to the group of ionotropic glutamate receptors which transmit their signal by changing membrane permeability to Na^+ and Ca^{2+} ions.[1] Pathologically enhanced glutamate receptor activity has been associated with neurotoxicity in multiple acute and chronic neurological and neuropsychiatric disorders and is related to excessive influx of Ca^{2+} into neurons.[2,3]

Uncompetitive NMDA inhibitors are known to bind at the so-called phencyclidine (PCP) binding site, located at the interior of the receptor.[4] However drugs with high affinity have strong psychotomimetic side effects.[5−8] Antagonists with a K_i value above 200 nM are tolerated clinically and are usually not associated with unacceptable psychiatric side effects.[7,9]

Thus far the three-dimensional (3D) structure of the receptor−ion channel complex is unknown. Nevertheless, several attempts have been made to obtain an idea of the structural requirements for binding at the PCP binding site, by analysis of molecules known to bind to the receptor.[10−15] However, in general these studies focused on sets of similar compounds, such as PCP derivatives[16] or MK801-like molecules.[17,18] Two main interactions were proposed: a hydrogen bond and a hydrophobic interaction.

The present study served several purposes: (1) To measure the activities of an additional set of molecules at the PCP binding site. (2) After the generation of the experimental data, to analyze the compounds systematically at a theoretical level, to derive common pharmacophore elements. This was especially interesting because of the structural diversity of the data set, ranging from "classical" PCP blockers such as ketamine and MK801 to adamantane amines, procyclidine, and alaproclate, and investigation of the ability of these molecules to fulfill the pharmacophoric requirements. (3) Combining the information of the previous steps, to correlate the experimental data with the structural knowledge generated. For this task a three-dimensional quantitative structure−activity relationship (3D-QSAR) method called comparative molecular field analysis[19] (CoMFA) was applied.

The 3D-QSAR model generated in the third step not only gave an indication of the validity of the pharmacophore but also gave a deeper insight into the structural requirements at the PCP binding site. Furthermore, it will enable us to predict the activities of additional molecules prior to synthesis and/or testing.

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⊗ Abstract published in *Advance ACS Abstracts,* December 15, 1997.

S0022-2623(97)00441-X CCC: $15.00
Published on Web 01/01/1998

Materials and Methods

Determination of Inhibition Constants. For a number of molecules, we have previously described inhibition constants at the PCP binding site (Table 1). Here, we have determined inhibition constants for additional substances: alaproclate, procyclidine, dextrorphan, dextromethorphan and MRZ substances 2/16, 2/22, 2/24, 2/138, and 2/174. To reduce variance of K_i values due to differences in methodology, all inhibition constants used for the CoMFA procedure were determined using identical methods and postmortem human brain tissue.

Tissue from the frontal cortex was taken at autopsy from male and female subjects with no apparent history of neurological or psychiatric disorders. The postmortem interval was between 4 and 44 h. The samples were placed in a freezer at −80 °C until analysis. All details of the experimental procedures were identical with those previously described.[22,23] In brief, membrane homogenates of brain tissue were prepared, and binding experiments were carried out at 21 °C in plastic microtiter plates in a total volume of 200 μL. The incubation medium consisted of 5 mM Tris-HCl (pH 7.4) containing 3 nM [^{3}H]MK801, 5 μM L-glutamate, 5 μM glycine, and 10 μM $MgCl_2$ (final concentrations). Test substances were added at increasing concentrations ranging up to 300 μM. Protein concentration was around 0.40 mg of protein/mL. After incubation for 540 min bound ligand was separated by rapid filtration through Whatman GF/B filters using a Titertek cell harvester followed by a 10-s wash with cold assay buffer. Filters were transferred into plastic vials, and upon addition of 5 mL of a toluene-based scintillation cocktail, they were monitored after 2 h for tritium in a Beckman LS 1801 counter at 54% efficiency. The binding displaced by 100 μM unlabeled MK801 was taken as specific binding. Pseudo-Hill coefficients and preliminary estimates of binding parameters from displacement experiments were provided by the EBDA program.[25] The K_i values were determined with the iterative nonlinear curve-fitting program developed by Munson and Rodbard.[26] Mean values from at least three independent experiments are given.

[^{3}H]MK801 was purchased from New England Nuclear, cold MK801 from RBI. The protein assay solutions and protein standards were purchased from Bio-Rad (München, Germany). Alaproclate was provided by Astra, Sweden. The MRZ substances 2/16, 2/22, 2/24, 2/138, and 2/174 were provided by Merz & Co., Frankfurt/M, Germany. All other compounds, including procyclidine, dextromethorphan, and dextrorphan, were obtained from Sigma.

Computation. All molecules were assumed to be protonated under physiological conditions, and their molecular structures were generated accordingly. Ketamine, alaproclate, orphenadrine, and procyclidine were tested as racemates. In these cases the same activity value was assigned to both enantiomers, respectively. Generation of molecular structures and modeling was performed with Sybyl 6.2.[27] Partial atomic charges were calculated using the semiempirical method AM1[28] implemented in MOPAC 6.0.[29]

Pharmacophore Analysis. The pharmacophore analysis was carried out using the DISCO module[30] in Sybyl 6.2, slightly modified for our own purposes. The basic theory behind this program is that each molecular structure is represented by points of potential pharmacological interest. The properties of these points could be, for example, "positively charged atom", "center of hydrophobic moiety", or "hydrogen bond acceptor/donor". Thus, each molecule is described by a number of points bearing certain properties and—as we deal with 3D structures—by the distances between these points. In mathematical terms such a description is referred to as a "graph". At first all flexible structures are submitted to a conformational search, and the resulting conformers are stored in databases. It is important that these conformers are energy-minimized, thus avoiding the consideration of high-energy structures. After the generation of the graphs for all molecules (and for all their conformers), one molecule is selected as a reference (normally one with high affinity, i.e., one assumes it contains the elements necessary for exhibiting pharmacological action). The pharmacophore model is then generated by finding the maximum subgraph common to this compound and the remaining ones in the data set. Structures known not to bind to the receptor are excluded from this step, because one has to assume that they do not contain the necessary pharmacophore elements. To find a common subgraph, a distance tolerance value has to be set, which specifies the maximum deviation in the pairwise point distances of the individual graphs still allowed to be recognized as a "match". The common subgraph then represents the 3D description of the pharmacophore elements which may be required for biological activity.[31] As this procedure is performed for each conformer of the reference compound, one arrives initially at several pharmacophore models which have to be evaluated further. An intriguing aspect of this whole procedure is that one is able to perform the analysis with very few preconceptions. The conformational space of each compound may be explored completely, and one does not need to restrict the study by taking into account only a few selected conformers.

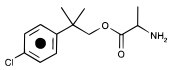
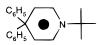
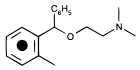
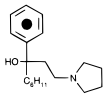
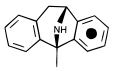
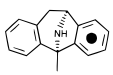
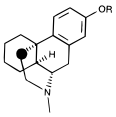
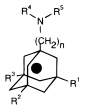
In this particular study, the pharmacophore points were assigned according to the standard library implemented in the program. The threshold for classification of atoms with respect to their partial charge was set to 0.23 atomic unit, thus classifying the protonated amines as "polar". Phencyclidine served as the reference during the analysis and was chosen for three reasons: (1) It represents one of the most active compounds in the data set, and one can therefore assume that it contains the basic pharmacophore elements required for binding. (2) It is conformationally restricted and consequently well-suited for a reference molecule. (3) As PCP-like compounds were investigated already earlier, its choice made a comparison with these studies easier.

3D-QSAR (CoMFA). An advantage of the procedure described above is that its result—the common subgraph—can be used to superimpose the different molecular structures, a prerequisite for performing the next step, the 3D-QSAR. In the method applied (CoMFA), steric and electrostatic interaction energies between a probe atom and a set of superimposed molecules are calculated at the surrounding points of a predefined grid.[19] The rationale behind this is that the probe atom corresponds to a receptor atom and scans the superimposed molecules for favorable and unfavorable interactions with a putative receptor. Furthermore, it is known that the noncovalent interactions between a ligand and its receptor are predominantly of steric and electrostatic nature, with H-bonding being regarded as a special case of the latter interactions.[32] The calculated energy values are then correlated with some property of the compounds (usually biological activity). Once a reliable correlation has been found, the QSAR model can be used for predicting the activities of novel compounds. Very useful results of the CoMFA procedure are also the 3D coefficient contour maps which indicate regions of favorable steric and electrostatic interaction around the set of aligned molecules.

In the present investigation standard settings for the CoMFA procedure were applied, as implemented in Sybyl 6.2. The CoMFA grid extended the superimposed molecules by at least 4 Å in all directions of a Cartesian coordinate system. An sp^3 carbon with a probe charge of +1 served as the probe atom. The steric and electrostatic interaction energies between this probe and each molecule were calculated separately at the grid points using the Lennard−Jones and Coulombic potential function of the Tripos force field,[33] respectively. The steric and electrostatic cutoff values were set to ±30 kcal/mol. At lattice intersections "inside" the molecules (as determined by a corresponding steric interaction energy value of 30.0 kcal/mol) no electrostatic energies were calculated. Equal weights for the steric and electrostatic descriptors were assigned using the CoMFA scaling option. In the statistical procedure biological activity was expressed as log K_i.

Linear equations were derived by means of the partial least-squares (PLS) algorithm.[34] The overall quality of the analyses was expressed by the corresponding cross-validated r^2 value

Table 1. Structures and Activities of the Molecules Investigated

compd	structure[a]	$K_{i\,(\mu M)}$ measured[b]	pred CV[c]	pred[d]
(±)-alaproclate		4.10 (2.27)[e]	*R*: 7.57 *S*: 5.86	
budipine		11.70 (0.20)[f]	2.96	
(±)-orphenadrine		6.00 (0.70)[g]	*R*: 2.27 *S*: 2.46	
(±)-procyclidine		1.70 (0.33)[e]	*R*: 6.89 *S*: 1.37	
(±)-ketamine		0.42 (0.045)[h]	*R*: 0.14 *S*: 0.12	
(+)-MK801		0.0012 (0.0002)[h]	0.02	
(−)-MK801		0.0068 (0.0005)[h]	0.01	
phencyclidine		0.023 (0.0086)[h]	0.64	
dextromethorphan (R = Me)		1.68 (0.22)[e]		0.9
dextrorphan (R = H)		0.22 (0.03)[e]		0.7
adamantane amines:				

	R^1	R^2	R^3	R^4	R^5	*n*			
MRZ 2/16	H	H	H	H	H	1	33.3 (6.35)[e]		7.5
MRZ 2/23	H	H	H	H	H	3	9.50 (0.48)[e]		6.0
MRZ 2/24	H	H	H	H	H	4	17.4 (3.56)[e]		13.8
MRZ 2/138	Me	Me	H	H	H	2	0.79 (0.02)[e]		8.1
MRZ 2/150	Et	Et	H	H	H	0	0.19 (0.06)[i]	1.29	
MRZ 2/169	Me	Me	H	Me	H	0	1.61 (1.17)[i]	3.62	
MRZ 2/170	Me	Me	H	Me	Me	0	4.44 (2.18)[i]	3.47	
MRZ 2/171	Me	Me	H	Et	H	0	1.72 (0.43)[i]	2.20	
MRZ 2/174	H	H	H	H	H	2	3.80 (0.64)[e]		5.9
MRZ 2/175	Et	H	H	H	H	0	0.60 (0.27)[i]	1.41	
MRZ 2/177	i-Pr	H	H	H	H	0	7.2 1(9.54)[i]	0.70	
MRZ 2/180	C_6H_5	H	H	H	H	0	15.2 (0.87)[i]	14.9	
MRZ 2/184	C_6H_5	Et	H	H	H	0	4.08 (0.78)[i]	3.35	
MRZ 2/187	H	H	H	Me	Me	0	20.8 (2.14)[i]	8.39	
MRZ 2/188	H	H	H	Me	H	0	21.7 (1.63)[i]	11.6	
memantine	Me	Me	H	H	H	0	0.54 (0.23)[i,j]	0.64	
amantadine	H	H	H	H	H	0	10.5 (6.10)[i]	6.89	

[a] The black-filled circles indicate the location of the hydrophobic pharmacophore point used in this study. As there is only one nitrogen atom in each molecule, the other three pharmacophore points can be assigned unambiguously to this atom. [b] Mean of at least three experiments. Standard deviation given in parentheses. [c] Predicted values from cross-validation (CV) with the leave-one-out method. For racemic compounds the values for both enantiomers (*R* and *S*) are listed separately. [d] These compounds were not included in the set of molecules analyzed initially (the "training set"), and their activity was predicted afterward, i.e., they constitute the "test set". [e–j] Data taken from: [e]Kornhuber et al., manuscript in preparation; [f]ref 20; [g]ref 21; [h]ref 22; [i]ref 23; [j]ref 24.

(r^2_{cv}) which is defined as

$$r^2_{cv} = \frac{SD - PRESS}{SD} \quad (1)$$

where "SD" is the variance of the biological activities of the molecules around the mean value.[19,35] "PRESS" represents the sum of the squared differences between the predicted and actual target property values for every compound. By definition, the r^2_{cv} can take up values in the range from $-\infty$ to 1.0. The ideal value of 1.0 is reached when "PRESS" becomes 0.0 (i.e., the internal prediction is perfect). Therefore, the r^2_{cv} is considered to be a very critical indicator for the internal consistency of the analyses, and r^2_{cv} values of 0.5 or higher indicate a successful and internally consistent QSAR.[19] In this study cross-validation was carried out by excluding each molecule once from the data set and predicting its activity by the QSAR model derived from the remaining ones, also referred to as the "leave-one-out" method.[35]

Results

Inhibition Constants. The results for the newly determined inhibition constants in human postmortem frontal cortex are given in Table 1. Dextrorphan and MRZ 2/138 had K_i values in the submicromolar range (0.22 and 0.79 μM); all other substances had values in the low-micromolar range. The lowest affinity was found with MRZ 2/16 (33.3 μM).

Pharmacophore. During the pharmacophore analysis of the molecules for which inhibition constants had been determined (Table 1), several different models were generated initially. These varied in their size (3−4 pharmacophore points included), the composition of points included, and the tolerance within which at least one conformer of each molecule was able to fit the model. Inspection of the 3-point models revealed that they were essentially contained also in the 4-point ones, with the difference being slightly lower tolerance values for the graph matching in the 3-point models. As we were interested in finding a pharmacophore model having as many pharmacophore points as possible, only the 4-point models were investigated further.

In the group of these models it was obvious that some of them could be generated only by allowing a high tolerance in the pairwise point distances (>3 Å). Therefore, these ones were discarded as well. Of the remaining two models one had a significant lower tolerance value than the other one (1.2 versus 1.8 Å). Furthermore, after superimposition of the molecules according to these two models, a calculation of the van der Waals volume overlap of the compounds indicated that the model with the lower distance tolerance also showed the better overlap. Based on the assumption that the molecules have to fit into the receptor and to fulfill some pharmacophore requirements (i.e., their orientation within the receptor cannot be arbitrary), the overlap of the molecules with one of the most active ones is an important indicator for the quality of the pharmacophore model generated. Application of all these selection criteria indicated that this model was superior to the other ones, and it was therefore selected.

A graphical representation of the resulting pharmacophore is presented in Figure 1, where PCP—the reference compound—is shown. Features common to all molecules investigated can be divided essentially into two elements. The first one consists of the protonated amine which is represented by three points: the nitrogen ("donor atom"), a positive partial charge at the hydrogen ("positive"), and the putative interaction site at the receptor ("acceptor site"). The distance between this interaction site and the amine hydrogen is approximately 2 Å. The second part of the pharmacophore is represented by a centroid at the cyclohexyl moiety of PCP and indicates a hydrophobic region.

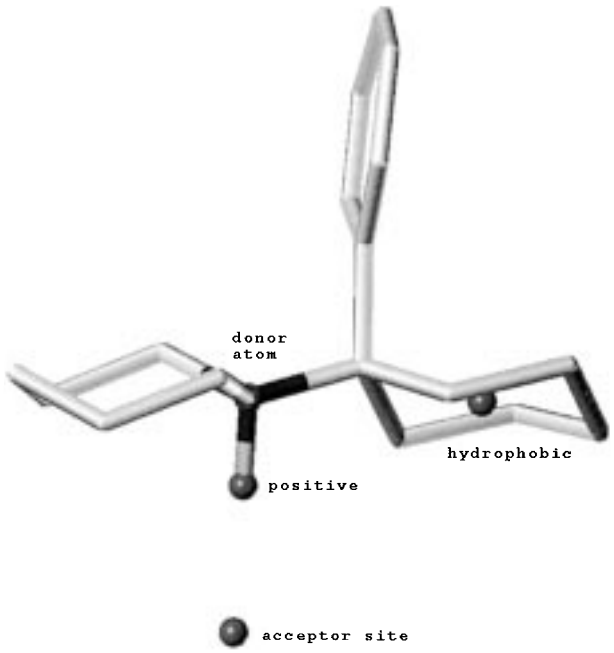


Figure 1. Graphical representation of the pharmacophore model generated by application of graph theory to a set of PCP binding site blockers. PCP is shown as a reference.

Table 2. Summary of the CoMFA

r^2_{cv}	k^a	$r^{2\,b}$	standard error of prediction	steric contribution[c]
0.72	2	0.94	0.608	0.819

[a] Optimum number of PLS components extracted. [b] Conventional r^2 determined with the optimum number of components. [c] The sum of steric and electrostatic contributions equals 1.0.

CoMFA. After having performed the pharmacophore analysis, the structures were aligned according to the selected model and a 3D-QSAR was performed using the CoMFA method. In this analysis, the measured inhibition constants were correlated with the corresponding structural data (the molecules' steric and electrostatic fields). The resulting CoMFA model (Table 2) shows a very high degree of internal consistency, as indicated by a cross-validated r^2 value (r^2_{cv}) of 0.72. This high r^2_{cv} value also supports the validity of the pharmacophore model. Apparently, alignment of the molecules according to the pharmacophore leads to a QSAR analysis which is highly self-consistent.

Very useful results of such a QSAR analysis are the "standard-deviation-times-coefficient" fields ("std*coeff" fields; Figure 2). These fields combine information about the regions around the aligned molecules with respect to the variance of interaction energies and the impact of putative modifications on biological activity. The steric "std*coeff" fields (Figure 2A) reveal several features influencing the binding abilities of the molecules. The green contours around the phenyl moiety of PCP (which is shown as a reference) indicate that in this region steric interaction with the receptor leads to

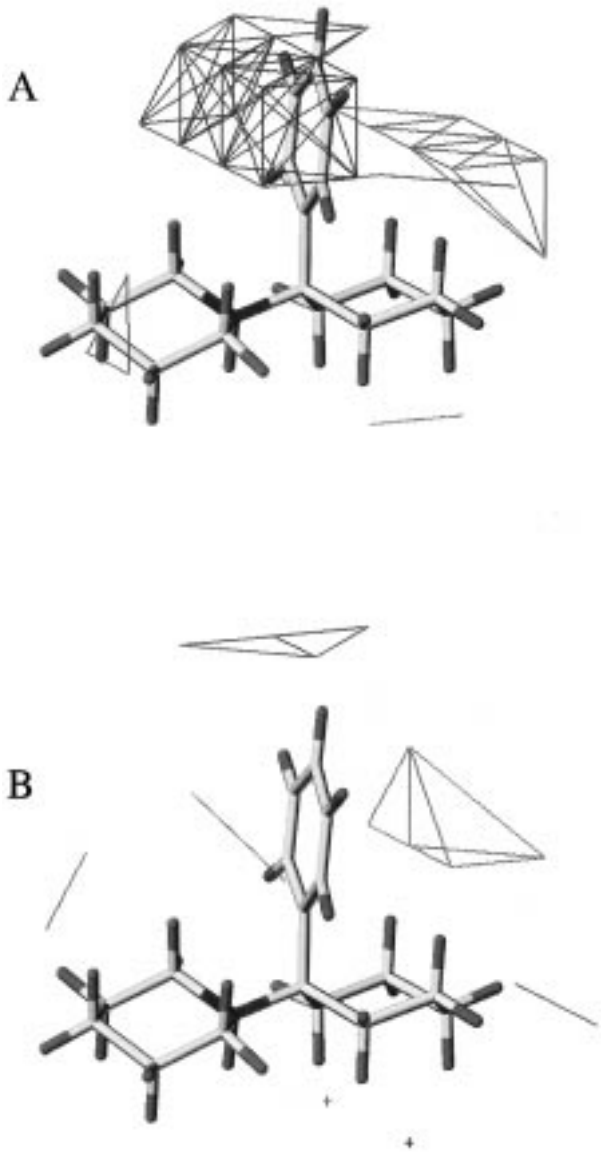


Figure 2. Std*coeff contour plots for the K_i CoMFA. PCP is shown as a reference. (A) Sterically favored areas are green, and disfavored areas are red. (B) Areas where negatively charged substituents are favored are orange, and areas where positively charged substituents are advantageous are blue.

increased binding. A brief inspection of the aligned molecules in the data set confirms this. All molecules exposing a substituent in this position, such as the aromatic rings of PCP and MK801, have increased binding affinity compared to the other ones. The red contours in Figure 2A mark areas where substitution will lead to a repulsive interaction with the (putative) receptor, hence reduced activity. This explains also why the adamantane amines, despite fulfilling the basic pharmacophoric requirements, are less active than, for example, PCP: Their adamantane structure is too bulky for the receptor, and they do not expose a substituent in that region which is important for binding.

Another interesting finding is that the QSAR analysis is mainly dominated by the steric descriptors (Table 2). Considering the type of molecules in the data set, this is actually no surprise: the majority contains one polar moiety (the protonated amine), with the remaining part being apolar. This polar moiety is always aligned, thus keeping the electrostatic variance low. Therefore this result does not necessarily imply that electrostatic interactions are not important, but it could be an indication that the training set is not diverse enough with respect to electrostatics. The electrostatic std*coeff fields are displayed in Figure 2B. The blue region represents an area where a positive partial charge of the molecule would lead to increased activity, whereas the orange area indicates favorable interaction of atoms with a negative partial charge. Such a charge distribution is actually provided by the phenyl moiety of compounds such as PCP, where the hydrogens are slightly positively charged, in contrast to the carbon atoms, which bear a negative partial charge. However, one should not overinterpret this result. It is more likely that this finding is due to a pure statistical treatment of the electrostatic fields. In fact it is known that a phenyl ring is an electron-rich moiety with respect to the ring atoms, which can function as a hydrogen bond acceptor.

The best test for the general validity of a QSAR analysis is to predict the activity of molecules which were not members of the training set. In the present study, this was done for seven additional compounds (Table 1, last column). Compared to the training set, some of these molecules were structurally unique. MRZ compounds 2/23 and 2/24 were the only ones with exceptionally long "linkers" between the protonated amine and the adamantane moiety (three and four methylene groups, respectively). Dextrorphan and dextromethorphan were novel in that they are bridged tricyclics. Nevertheless, despite the fact that some of these molecules were unique compared to the training set, the CoMFA model was able to predict the activities of these compounds rather accurately, indicating a high general predictiveness of the CoMFA. To quantify the quality of the predictions, we compared the root-mean-square errors in the training set and the test set of the QSAR analysis. It was an encouraging result to find that the magnitudes of the errors were very similar (0.57 for the training set, 0.52 for the test set). That one could still improve on these predictions is indicated by the fact that not all test set compounds were assigned successfully to the top or bottom half of the range. However, the K_i value for dextrorphan which is the most active molecule in this series was properly predicted to be the lowest. Also none of the less active molecules were predicted to have high activity.

Discussion

Inhibition Constants. The K_i values for procyclidine, dextromethorphan, dextrorphan, and MRZ 2/138 in human postmortem frontal cortex were in good agreement with those previously published for the rodent brain.[36−38] While alaproclate has previously been characterized as an uncompetitive NMDA receptor antagonist in electrophysiological and neurotoxicity studies,[39,40] binding studies have not been performed. The newly investigated MRZ compounds are structurally related to amantadine and memantine but have a spacer between the nitrogen and the bulky hydrophobic cage structure. These compounds exhibited moderate (MRZ 2/138) to low (MRZ 2/16) affinity to the PCP binding site.

Pharmacophore. A comparison of the pharmacophore model generated with previous investigations re-

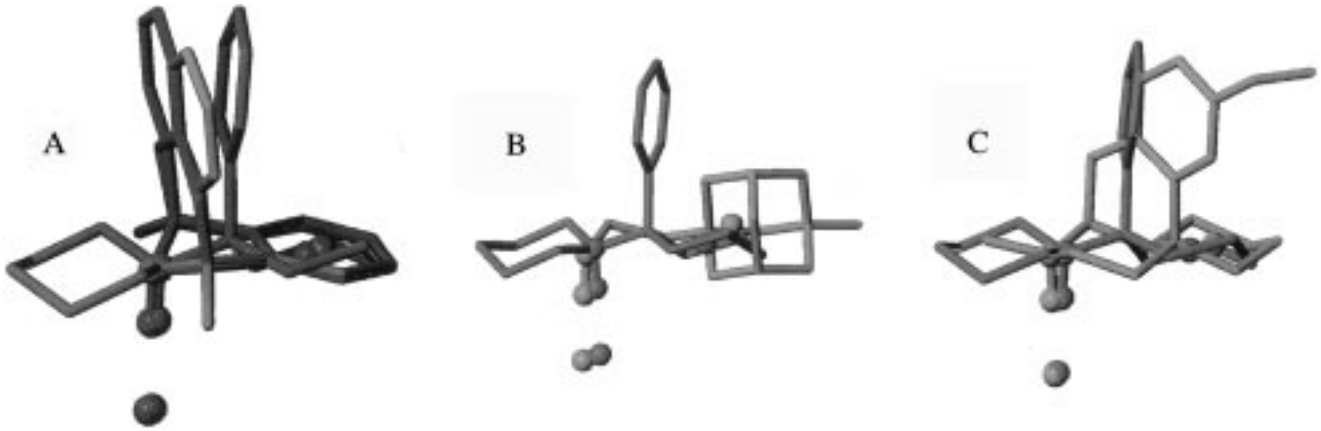


Figure 3. Illustration of the alignment of different compounds according to the pharmacophore. The molecules are displayed without hydrogens, with the reference structure PCP in cyan: (A) (+)-MK801, green; (−)-MK801, red; (B) MRZ 2/15, red; (C) dextromethorphan, red.

veals some interesting features in common, but also some differences. In the previous studies the interaction of the protonated amine with the receptor was proposed as well. However, a difference between the present model and the previous ones is that we predict the region of hydrophobic interaction common to all compounds to be centered at the cyclohexyl moiety of PCP (provided it is used as a reference) and not at its aromatic ring. There are two reasons for this prediction: First, molecules such as the series of adamantane amines give a significantly worse fit to PCP when fitted with their adamantyl moiety to the phenyl ring. Second, the prediction is supported not only by the root-mean-square fit of the corresponding pharmacophore points but also by a calculation of the van der Waals overlap of the respective moieties. The volume of a phenyl ring is much smaller than that of a cyclohexyl moiety (73.5 versus 98.8 $Å^3$), and the adamantane structure (volume 143.5 $Å^3$) overlaps much better with the latter one. Of course one could argue that poor overlay is actually the reason why even the most potent adamantane amines are still much less active than PCP, for example. However, one can assume that given the "choice" between two receptor pockets of different size the bulky substituent will be accommodated in the larger one.

The superimpositions of several different molecules according to the pharmacophore are shown in Figure 3. Apparently, the present model gives a very good explanation why several structurally different compounds are able to bind to the same site. For example, the pharmacophore indicates how the two enantiomers of MK801 could fit in an essentially similar fashion—also in comparison with PCP—into the receptor (Figure 3A; (+)-MK801, green, (−)-MK801, red). Both enantiomers lie with one of their aromatic rings in the common hydrophobic region while the amine proton points into the same direction. The other aromatic ring of each enantiomer occupies a similar region in space and also overlaps fairly well with the phenyl moiety of PCP. An adamantane with an ethylene linker between amine and polycycle (MRZ 2/15, red; Figure 3B) is able to fulfill the basic pharmacophoric requirements as well. In this case the flexible linker takes up such a conformation that there is still considerable fit to the pharmacophore, although the general overlap is reduced. The last example (dextromethorphan, red; Figure 3C) demonstrates convincingly how even a molecule, which is at first glance very different from compounds such as PCP or MK801, is able to occupy the binding site in a similar fashion. In this case the good fit of hydrophobic and polar parts of the molecule with the pharmacophore is striking, and even the aromatic ring occupies roughly the same region in space as the phenyl moiety of PCP.

CoMFA. Comparing PCP and MK801, two minor differences between the molecules become apparent. A part of the piperidine moiety of PCP protrudes into a small "forbidden" (red) region (Figure 2). MK801 does not have a substituent in this area, which provides an explanation for its higher activity. The other difference concerns the "allowed" (green) region near the phenyl ring of PCP. Detailed inspection of the alignment with MK801 (Figure 3A) showed that the aromatic ring of the latter molecule is located exactly at the center of this favorable area. The distance between the aromatic ring planes of PCP and MK801 in this region is approximately 1.5 Å. One may therefore hypothesize that these relatively subtle differences in the geometry of PCP and MK801 account for the difference in their activities. Another possible reason for the activity difference between these two compounds is not related to enthalpic data as calculated in CoMFA but to the fact that MK801 is more rigid than PCP. Therefore, the entropy loss upon binding to the receptor is less for MK801 compared to PCP and could account for the difference in activity.

In general, the 3D-QSAR augments and refines the basic pharmacophore model and gives rise to a complete picture of the structural requirements at the PCP binding site (Figure 4). Three areas of interaction between a molecule and the receptor can be specified. The first one is a hydrogen bond between a protonated amine in the ligand and a suitable receptor residue and results from the pharmacophore analysis. The second interaction, also suggested by the pharmacophore analysis, is the hydrophobic center common to all molecules and corresponds to the cyclohexyl moiety of PCP. From the 3D-QSAR further information about the requirements in this area can be extracted. Molecules should not be too bulky in order to interact favorably with the receptor. The third interaction is a result from the 3D-QSAR and represents another hydrophobic interaction

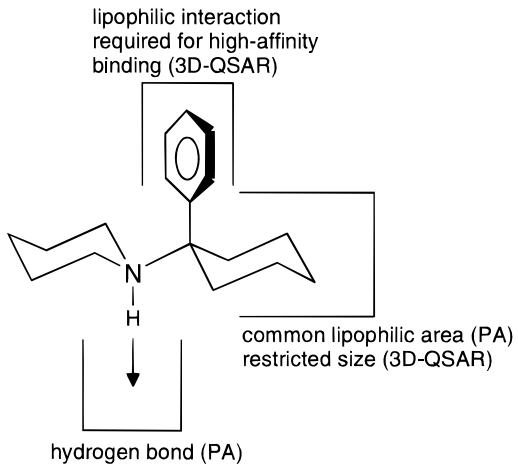


Figure 4. Combination of the results of the pharmacophore analysis (PA) and the 3D-QSAR, indicating the main structural requirements for PCP binding site blockers.

with the receptor, corresponding to the aromatic ring of PCP, and is apparently required for high-affinity binding.

An implicit assumption for a CoMFA study is that the molecules bind in a comparable fashion to the same receptor site and that the affinity differences are due to variations in the number and/or strength of interactions with the receptor. It has been shown that there might be an additional binding site for uncompetitive NMDA receptor antagonists. Mutation analysis of subunits of the NMDA receptor revealed that MK801 might also interact with the so-called M3 transmembrane element. For PCP, however, it has been shown that it essentially interacts only with the M2 segment.[41] Nevertheless, it has been pointed out that the M2 element appears to be the essential structural component, whereas the M3 segment seems to play a modulatory role with respect to pore function. That channel function and blockade might be more complicated than initially assumed has been also indicated in another study.[42] However, the high consistency of the CoMFA derived indicates that the entire series of molecules interacts with the same binding site, although further, possibly modulatory, interactions for some members of the data set cannot be ruled out.

Conclusions

In this study the requirements for blocking at the PCP binding site of the NMDA receptor were investigated by application of a three-step strategy. The steps included experimental determination of biological activities followed by mathematical analysis of pharmacophore properties using as few preconceptions (such as the choice of certain reference conformations) as possible. Finally a correlation of activity data with 3D structures was performed. This strategy proved successful, as confirmed by the resulting pharmacophore, the inner consistency of the 3D-QSAR, and its predictive quality for additional molecules. The fact that an automated 3D pharmacophore analysis was used successfully as a basis for an alignment in CoMFA represents an interesting methodological aspect of this investigation.

The combination of pharmacophore analysis, carried out on a set of structurally very diverse molecules, and 3D-QSAR yielded great insights into the requirements for binding and their relative importance: A first lipophilic region corresponds to the area where the cyclohexyl moiety of PCP interacts with the receptor. Compounds with only one but bulky hydrophobic moiety such as the adamantane amines appear to fit better into the receptor in this area, which may be designated as the common lipophilic region. However, this region appears to be of limited size, as shown by the 3D-QSAR. Adamantane amines are too bulky in this area and display reduced binding affinities and potentially higher K_{off} values. The second lipophilic area appears to be important for high-affinity binding and corresponds to the phenyl ring of PCP. Ideally, a compound should be able to interact with the receptor in both regions. The difference between the present results and earlier studies is that the common lipophilic area does not correspond to the aromatic ring of PCP but to its cyclohexyl moiety. The phenyl ring of PCP (or a corresponding element in another molecule), however, is important for enhanced affinity.

We believe that the results will prove helpful in the future for the design of better uncompetitive NMDA receptor antagonists,[43] being devoid of undesirable side effects. The establishment of a predictive 3D-QSAR model, which can be expanded further by inclusion of additional and more diverse compounds, will provide a very useful tool in this process, especially if there is no experimental structure of the receptor available.

Acknowledgment. This research was supported by the Bundesministerium für Bildung, Wissenschaft und Technologie (J.K., 01 EB 9410).

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JM9704412