Articles

Modeling of Competitive Phosphono Amino Acid NMDA Receptor Antagonists

Jeffrey P. Whitten,* Boyd L. Harrison, Herschel J. R. Weintraub, and Ian A. McDonald

Marion Merrell Dow Research Institute, 2110 East Galbraith Road, Cincinnati, Ohio 45215. *Received June 24, 1991*

A pharmacophore for the phosphono amino acid antagonists of the NMDA receptor has been developed using computer-based molecular modeling techniques. An important feature of this model is that a single binding site is proposed for the phosphonic acid moiety. All competitive antagonists we have examined incorporating amino acid and phosphonate groups in their structure fit the pharmacophore in energetically accessible conformations.

The amino acids L-glutamic acid, glycine, and L-aspartic acid play key roles in central nervous system excitatory neurotransmission. The availability of various receptor antagonists, coupled with pharmacological and electrophysiological experimentation, has enabled the nature of the receptors upon which these amino acids act to be studied in detail. In particular, this research has shown that the glutamic acid *N*-methyl-D-aspartate (NMDA) receptor complex is composed of binding sites for glutamic acid, glycine, polyamines, and zinc.[1-6] Activation of the receptor complex facilitates the opening of an ion channel to which both magnesium and phencyclidine (PCP) or 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP) can bind. One of the essential components leading to channel opening is activation of the NMDA binding site by glutamic acid. Competitive NMDA antagonists have been shown to modulate channel opening and control calcium entry into the cell.[7] The therapeutic potential of these antagonists in epilepsy and various neurodegenerative disorders has been widely recognized in recent years, leading to intense interest in the search for potent, selective, centrally acting compounds.[8,9,14]

A number of antagonists of the NMDA binding site of the glutamate receptor have been described,[2,6,10,11,12] the most interesting of these being structurally related to AP5 (2-amino-5-phosphonopentanoic acid, 1) and AP7 (2-amino-7-phosphonoheptanoic acid, 2) (Figure 1). These classes of compounds are defined by the length of the chain from the phosphorus atom up to, and including, the carboxylic acid group; AP5- and AP7-like molecules have 5- and 7-membered chains, respectively. While these D-amino acids depend, in part, upon a terminal phosphonic acid moiety for their efficacy, very similar compounds (Figure 2), such as AP6 (2-amino-6-phosphonohexanoic acid, 9), are only very weak antagonists.[13] Moreover, AP5 and AP7 have pharmacology which has been interpreted as due to action at two receptor subsites or to binding in different overlapping modes to a single binding site.[4] Recently, Monahan et al.[15] strongly argue that antagonists based upon either the AP5 or AP7 structure interact differentially with the receptor complex. This difference was attributed to partially overlapping C-5 and C-7 binding sites which lends support to a preliminary receptor model, previously put forward by Hutchison et al.,[11] in which the phosphonic acid moiety is able to occupy one of two different positions in the receptor. This "two binding site

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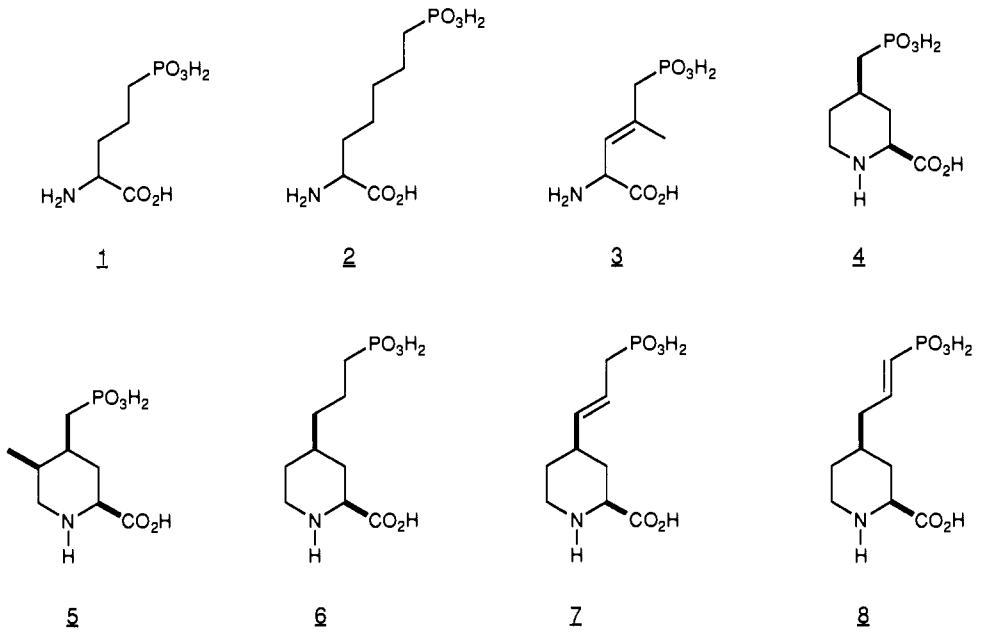

Figure 1. Potent, competitive NMDA antagonists; relative binding affinities are less than 1 μM (versus [^{3}H]CPP or [H^{3}H]CGS 19755): **1**, D-2-amino-5-phosphonopentanoic acid (D-AP5); **2**, D-2-amino-7-phosphonoheptanoic acid (D-AP7); **3**, D-(*E*)-2-amino-4-methyl-5-phosphono-3-pentanoic acid (4-methyl APPA); **4**, D-*cis*-4-(phosphonomethyl)piperidine-2-carboxylic acid (CGS 19755); **5**, D-*cis*-4-(phosphonomethyl)-5-methylpiperidine-2-carboxylic acid; **6**, D-*cis*-(3′-phosphonopropyl)piperidine-2-carboxylic acid; **7**, D-*cis*-4-[(1*E*)]-(5-phosphonoprop-1-enyl)piperidine-2-carboxylic acid; **8**, D-*cis*-4-[(1*E*)]-(3′-phosphonoprop-2-enyl)piperidine-2-carboxylic acid.

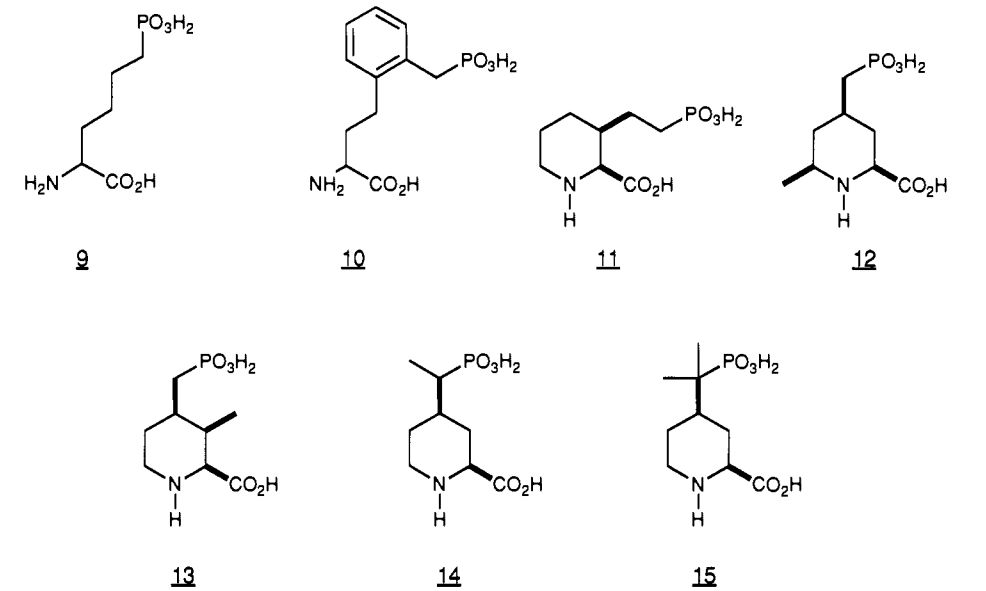

Figure 2. Weakly active NMDA antagonists; relative affinities are greater than 1 μM ([^{3}H]CPP): **9**, D-2-amino-6-phosphonohexanoic acid (D-AP6); **10**, D-3-[2-(2-phosphonoethyl)phenyl]-2-aminopropanoic acid; **11**, D-*cis*-3-(2-phosphonoethyl-1-yl)piperidine-2-carboxylic acid; **12**, D-*cis*-4-(phosphonomethyl)-6-methylpiperidine-2-carboxylic acid; **13**, D-*cis*-4-(phosphonomethyl)-3-methylpiperidine-2-carboxylic acid; **14**, D-*cis*-4-(1-phosphonoethyl)piperidine-2-carboxylic acid; **15**, D-*cis*-4-(dimethylphosphonomethyl)piperidine-2-carboxylic acid.

model" would appear to accommodate all potent, competitive NMDA antagonists (Figure 3). In light of these investigations, we wish to communicate the results of our own modeling efforts. In this paper we present evidence that the two binding site model is not an *obligatory* one and propose another pharmacophore for the NMDA receptor in which the phosphonic acid group of antagonists (**1**–**15**) bind to a single site.

The principal reason for the proposal[11] of the two-binding-site model was the apparent inability to overlap the critical amino acid and phosphonic acid residues of AP5 (**1**) and **7** in energetically feasible conformations. Our initial modeling efforts, however, showed that there were no serious obstacles to a single-binding-site pharmacophore. For example, an initial study using MULTIFIT (see

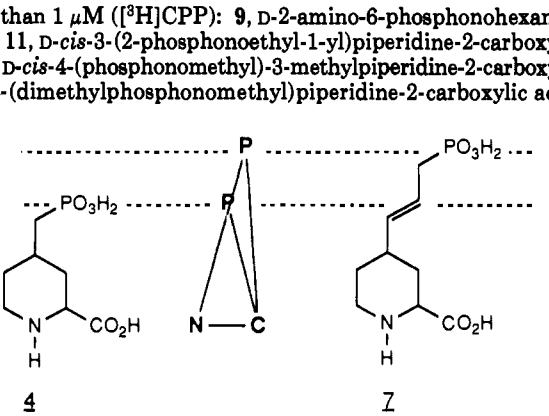

Figure 3. The two binding site model for the phosphono amino acid, competitive NMDA antagonists.[11]

below) demonstrated that the conformationally restricted analogues **4** and **7** could adopt common energetically ac-

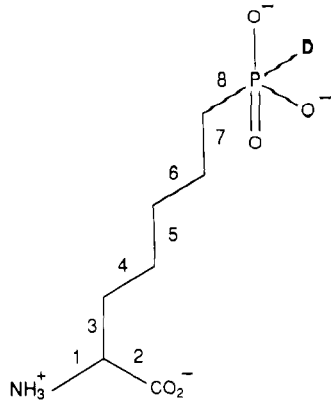

Figure 4. Conformational search of **2**. The numbers indicate the eight rotatable bonds; the phosphorus–dummy atom D bond was used as a reference to create the vector maps.

cessible conformations[16] in which the amino acid and phosphonic acid moieties of each molecule were constrained to occupy the same regions of space. Similarly, AP5 (**1**) and AP7 (**2**) can be fitted with apparent ease to **4** and **7** (data not shown). If these results were correct, all AP5- and AP7-like potent antagonists should be able to adopt a common conformation. Furthermore, this common conformation should exclude all conformations of the weakly active analogue AP6. We set out, therefore, to rigorously define a single pharmacophore to encompass potent, competitive, phosphono amino acid NMDA receptor antagonists.

Materials and Methods

Software. Tripos Associates' SYBYL molecular modeling package was used for this study.[17] SEARCH, MULTIFIT, MAXIMIN2, and MVOLUME refer to software modules which enable the search of conformational space, flexible fitting of two or more molecules with energy minimization using the SYBYL force field,[18] energetically minimizing a single compound, and various van der Waals volume interaction calculations, respectively.

SEARCH. All molecules in this study were constructed in SYBYL and minimized using the Tripos force field MAXIMIN2, initially with no electrostatic term. Inclusion of an electrostatic term for compounds **1**, **2**, **4**, and **7** had minimal effects on the results (see below) and was thus omitted from these calculations. Convergence was obtained when the energy changed by less than 10^{-6} kcal/mol. Charges were calculated with the AM1 Hamiltonian in AMPAC.[19] All molecules were considered to exist with two negative charges on the phosphonate group, a protonated amine and an ionized carboxylate at physiological pH. Using options available in SEARCH, conformations were generated for each molecule; the approach taken for AP7 (**2**) which follows was typical. All carbon–carbon, carbon–nitrogen, and carbon–phosphorus bonds (bonds 1–8, Figure 4) were defined as rotatable. Bond 2 was rotated in 120° increments, bonds 1 and 8 in 30° increments, and all others in 10° increments. A "dummy" atom was incorporated to facilitate vector mapping; the phosphorus–dummy bond length was set at either 0.2 Å for acyclic molecules (**1**, **2**, and **3**) or 1.0 Å for cyclic compounds, and the dummy–phosphorus–carbon bond angle was 180°. The dummy atom, which is chargeless and dimensionless, facilitates plotting of the SEARCH results but has no effect on these results. In order to restrict the number of conformations, two distance constraints were imposed: the nitrogen–phosphorus and carboxyl carbon–phosphorus distances were set in the range of 5–6 Å. These distances are approximately ±0.5 Å of the extended conformation of AP5; AP7 is able to adopt conformations in regions of space defined by these limitations. Finally, in order to eliminate unfavorable conformations due to severe steric interactions, the van der Waals radii scaling factors were set at 0.950, 0.870, and 0.650 (general, 1–4, hydrogen bonding). Using this method, 53 579 conformations of AP7 (**2**), clustered in three functional domains were generated and plotted as a vector map (Figure 5) in which each vector indicates the location in space of the phosphorus–dummy atom bond. Similarly, vector maps were created for the other molecules (Figure 5).

MULTIFIT. Individual compounds were constructed in SYBYL and minimized using the Tripos force field MAXIMIN2.[18] The atoms in compounds **1**–**15** selected for flexible fitting included all the heteroatoms and the carboxylic acid carbon atom. Where necessary, additional carbon atoms were added. If the heteroatoms were substituted with a hydrogen atom (e.g. OH, NH), these hydrogen atoms were also identified. Spring constants of 3 were assigned to the oxygen and hydrogen atoms; all other atoms were assigned a spring constant of 20. Two or more molecules were fitted using the default parameters. Molecules were considered to be in energetically feasible conformations if the energies were within 6 kcal/mol of the energies calculated from the initial MAXIMIN2 minimization and from subsequent MAXIMIN2 minimization of the conformation in question. These conformations were also within 6 kcal/mol of the extended conformation, assumed to be at or near the global energy minimum (see below).

Inclusion of an Electrostatic Term. The common conformations of compounds **1**, **2**, **4**, and **7** (see Results and Discussion) were initially minimized using MAXIMIN2 without an electrostatic term and then repeated using a Coulomb's law function employing dielectric constants of 3.5 (lipophilic environment) and 80 (aqueous environment).

Global Minimum. Since the exceedingly large number of conformations generated by the SEARCH routine precluded calculating energies for each conformation, the global minima were estimated by the following procedure. Compounds **1**, **2**, **4**, and **7** were each built in an extended conformation, presumably within the conformational domain of the global minimum, and then minimized using MAXIMIN2, with and without an electrostatic term.

MVOLUME. The van der Waals volumes of the molecules were calculated using default parameters. These volumes were compared using logical mathematical operations to generate regions of common volume or areas in space which fall outside the common volume. This technique is referred to as volume mapping.

Results and Discussion

As discussed earlier, our initial study using MULTIFIT indicated that there may be one common energetically

(16) Molecules were considered to be in energetically accessible conformations if the energies were within 6 kcal/mol of the energies calculated from both the initial MAXIMIN2 minimization and from subsequent MAXIMIN2 minimization of the conformation in question. The energy values were calculated using the default SYBYL force field.

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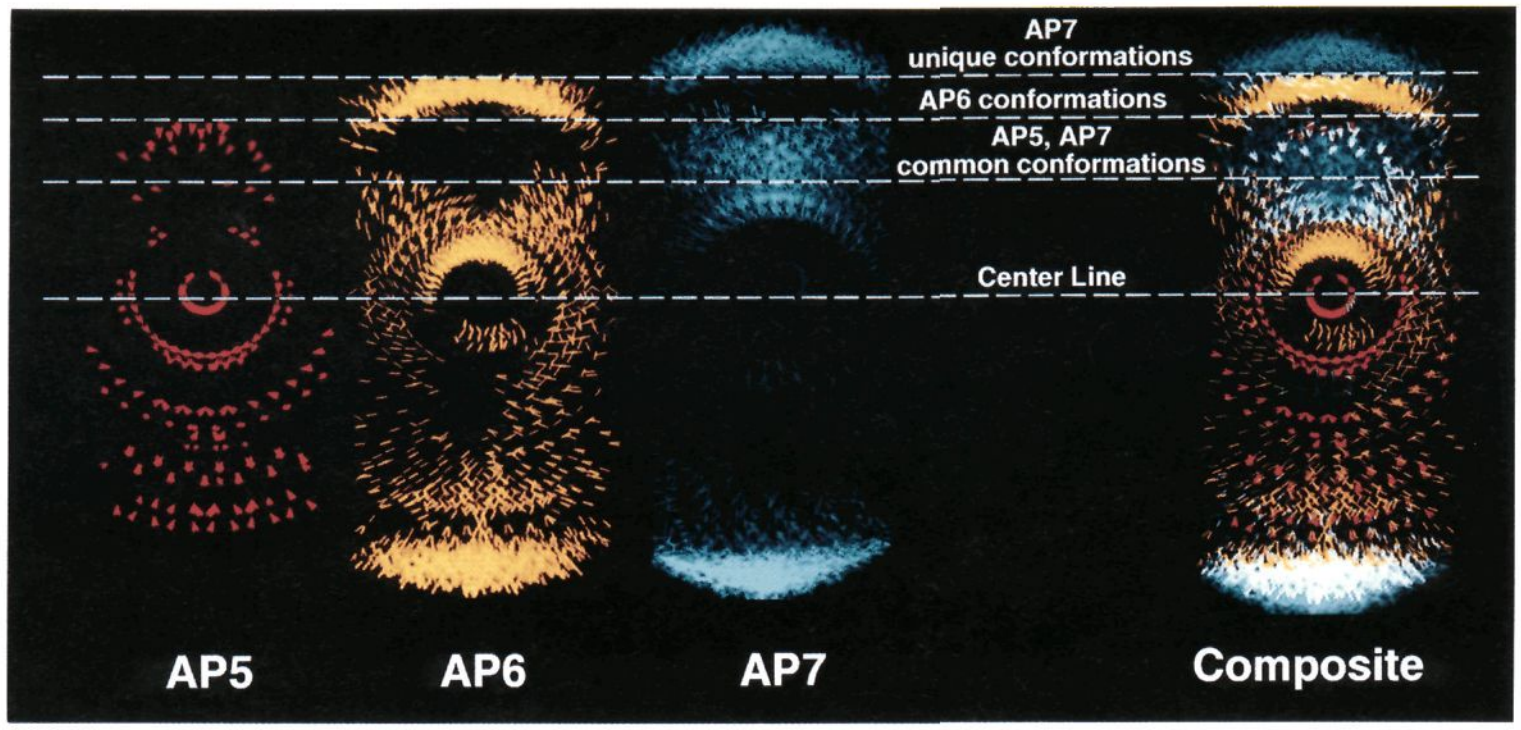

Figure 5. Vector maps of **1** (AP5, red), **2** (AP7, yellow), and **9** (AP6, blue). The maps are a collection of conformations created by tracking the phosphorus–dummy atom bond; each vector represents the position of the phosphorus–dummy atom bond in one particular conformation. The view has been taken from "above" the molecule, looking down the molecule toward the amino acid moiety. The composite map was created by overlapping the maps of the three molecules. Photographs were taken from the Evans and Sutherland PS390 screen, combined, and annotated.

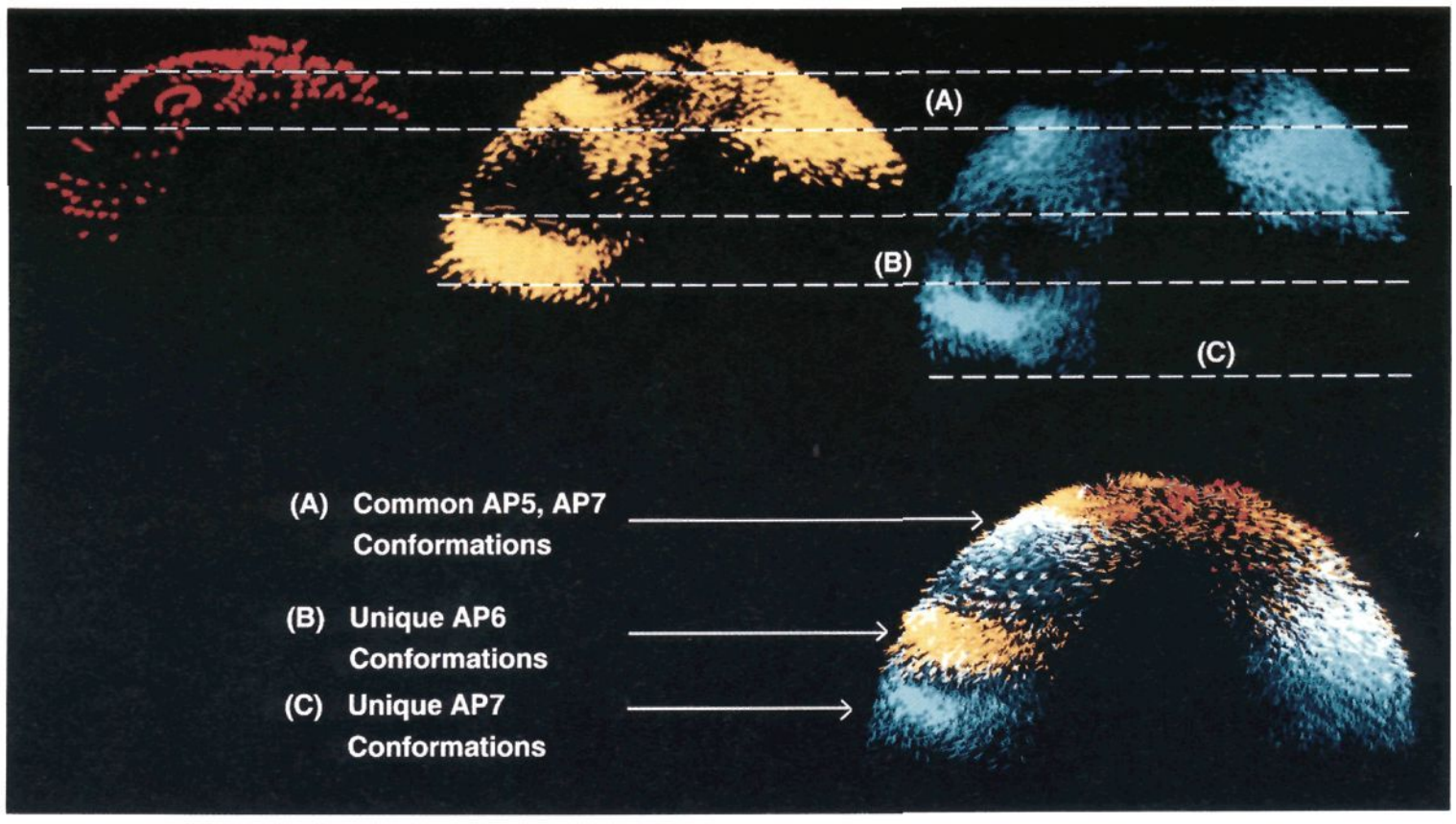

Figure 6. Vector maps of **1** (AP5, red), **2** (AP7, yellow), and **9** (AP6, blue). These views have been taken from the "side" with the molecules presented in a way similar to the structures seen in Figure 1. In the top portion, from left to right, are the maps of AP5, AP6, and AP7. The major band of conformations of AP6, as represented by the positions of the phosphorus–dummy atom bond, is shared by AP7, but not by AP5. In contrast, the common conformations of AP5 and AP7 can not be attained by AP6.

accessible conformation for phosphono amino acids. To explore this further, a series of phosphono amino acids was examined using the systematic conformation analysis routine SEARCH.[23] We sought conformations which were common to the potent antagonists, and to which the weakly active antagonists would not readily fit. By using the SEARCH routine, this was accomplished without assuming a prejudicial starting geometry.

Five molecules were chosen for this part of the study; these being the potent antagonists AP5 (**1**, K_i = 260 nM versus [^{3}H]CPP[20]), AP7 (**2**, K_i = 912 nM, [^{3}H]CGS 19755[11]), **4** (K_i = 130 nM, [^{3}H]CPP[20]), **7** (K_i = 14 nM, [^{3}H]CGS 19755[11]), and the weakly active analogue AP6 (**9**, K_i = 42 μM, [^{3}H]CPP[21]). As these highly flexible molecules may exist in many possible conformations, there are a

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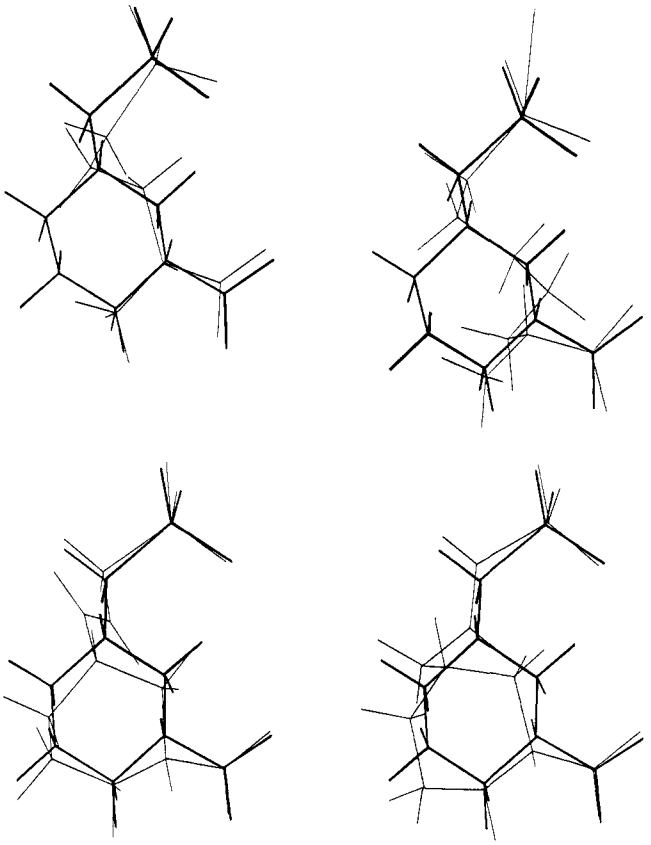

Figure 7. Results of flexible fitting experiments. Common conformations of 1, 2, 4, 7, and 8 were selected from the vector maps and pairs of molecules were flexibly fitted and minimized. Top left, 1 (AP5) and 4; top right, 2 (AP7) and 4; bottom left, 4 and 7; bottom right, 4 and 8.

correspondingly large number of local energy minima. To avoid the problem of comparing fortuitous local energy minima, this study was designed to explore as many conformations as possible for each molecule by independently rotating all flexible bonds. The number of resulting conformations (e.g., 2.5×10^{10} for AP7) effectively prohibits the calculation of energies at this stage; therefore, restrictive van der Waals nonbonded interactions were imposed in order to exclude most of the high-energy conformations. Vector maps, generated as described in Methods, clearly showed that AP5 (1), AP7 (2), 4, and 7 can adopt similar conformations, whereas the weakly active analogue AP6 (9) is unable to adopt these conformations (Figures 5 and 6). Common conformations were sought from the vector maps of 1, 2, 4, and 7 in the following way. Of the four molecules, the relatively rigid compound 7 generated the fewest conformations—131. The four vector maps were overlaid on the screen together with the first conformation of 7, subsequently all the conformations of 7 were manually scanned while care was taken to identify conformations of 7 which superimposed conformations of 1, 2, and 4. Only one common conformation for each of the four molecules was identified; each of these conformations was retrieved and further minimized using MULTIFIT.[16] The results shown in Figure 7 clearly indicate that competitive NMDA receptor antagonists can be understood in terms of a single pharmacophore. The recently described[20] alkene 3, which is one of the most potent antagonists reported to date (K_i = 35 nM as the racemate, [^{3}H]CPP[20]), was fitted to this model and has been taken to define the molecular pharmacophore (Figure 8) for all subsequent flexible fitting routines. In simplest terms, the pharmacophore is defined by the triangle formed by the

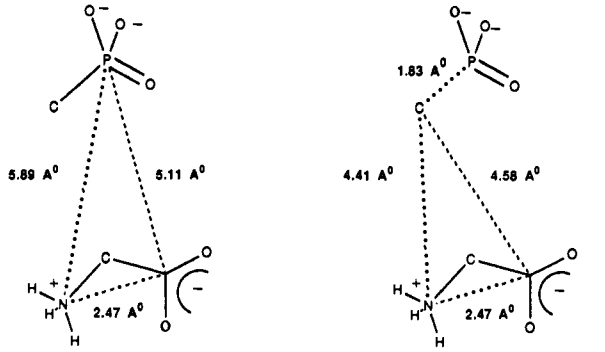

Figure 8. Pharmacophore for competitive, phosphono amino acid NMDA antagonists. The left hand side schematic representation describes the spacial arrangement of the key nitrogen, carboxylic acid carbon, and phosphorus atoms. The relationship of the phosphonic acid moiety to the rest of the molecule is additionally described by the right hand side figure.

phosphorus, nitrogen, and carboxylic acid carbon atoms (Figure 8, left-hand side). However, the angle of the vector formed between the phosphorus atom and the adjacent atom (Figure 8) and the plane of the triangle also appears to be critical; this is represented by triangulating the atoms shown in Figure 8, right-hand side.

We next turned out attention to the other alkene 8 (K_i = 72 nM versus [^{3}H]CGS 19755[11]). Following the same flexible fitting procedure, a low-energy conformation[16] for this molecule was found that was readily accommodated by the above pharmacophore (Figure 7). In these molecules (7 and 8) the piperidine ring adopts a twist-boat conformation rather than the usual chair conformation. Hutchinson et al.[11] similarly reported that the trans forms of 7 and 8 adopted the twist-boat conformation when fitted to the two-binding-site model. Apparently, these compounds can adopt this conformation with the expenditure of only a few kilocalories/mole and are able to interact favorably with the receptor.[24]

To complete this study, all the compounds (Figures 1 and 2) were flexibly fitted to the pharmacophore (data not shown) with the constraints outlined in Material and Methods (MULTIFIT). With the exception of AP6 (9), all compounds fitted to the pharmacophore in energetically accessible conformations[16] and were used in the subsequent volume mapping studies. Employing the above spring constants, it was not possible to fit AP6 to the pharmacophore. For example, the nitrogen, carboxyl carbon, and phosphorus atoms were offset by 0.5, 0.19, and 0.46 Å, respectively. If the spring constants were uniformly increased to 100, a good fit could be obtained only at the expense of considerable energy cost (13 kcal/mol).

We next turned to validate the assumptions that the electrostatic term can be omitted in the calculations and that the common conformations of 1, 2, 4, and 7 are within a few kilocalories/mole of their global minima. Since it was impractical to attempt to calculate energies for all conformations of these compounds, each molecule was rebuilt in an extended conformation and then subjected to minimization. The rationale behind this approach is that the global minimum conformation should be in the same conformational domain as the extended conformation. As the molecules were assumed to be fully ionized at physiological pH and had an overall charge of −2, the

(24) The trans isomer of 7 (D-*trans*-4-[(1*E*)]-5-phosphonoprop-1-enyl)piperidine-2-carboxylic acid) also fits the pharmacophore in a chair conformation with an ΔE (common-extended) of 5.9 kcal/mol. Coordinates are available in the supplementary material.

Table I. Effect of the Electrostatic Term in the Energy Calculations[a]

molecule	description	electrostatic term: Coulomb's law dielectric constant: none	3.5	80
1 (AP5)	common conformation[b]	4.50	5.27	4.54
	independently minimized[c]	2.81	1.13	2.54
	extended conformation[d]	1.54	−0.08	1.85
	ΔE (common-extended)	2.96	5.35	2.68
2 (AP7)	common conformation[b]	6.14	1.88	5.95
	independently minimized[c]	2.03	−2.54	1.84
	extended conformation[d]	1.10	−1.54	0.88
	ΔE (common-extended)	5.04	3.42	4.07
4	common conformation[b]	2.91	−0.15	2.78
	independently minimized[c]	1.00	−2.48	0.86
	extended conformation[d]	1.09	−2.76	0.93
	ΔE (common-extended)	1.82	2.61	1.85
7	common conformation[b]	11.59	7.06	11.39
	independently minimized[c]	10.36	4.93	10.13
	extended conformation[d]	10.39	1.55	9.50
	ΔE (common-extended)	1.20	5.51	1.89

[a] Energies (kilocalories/mole) calculated using the SYBYL force field in MAXIMIN2 for the competitive glutamate antagonists 1, 2, 4, and 7. [b] This conformation was obtained from the MULTIFIT experiment and is common to the four molecules. [c] These values were obtained when the common conformations were allowed to independently relax using MAXIMIN2. [d] The molecules were constructed in their extended conformations and then independently minimized using MAXIMIN2.

calculations were performed with either no electrostatic term or using a Coulomb's law term employing dielectric constants of 3.5 or 80, which should approximate any receptor environment from a nonaqueous lipophilic one to one which is extensively hydrated.

The results of these calculations are shown in Table I together with the energies calculated for the common conformations (from the MULTIFIT experiments) of 1, 2, 4, and 7 and those obtained when each of these conformations were allowed to relax independently (using MAXMIN2). It is apparent from these results that the common conformations of 1, 2, 4, and 7 are energetically accessible and that the omission of the electrostatic term in the initial part of the study had no effect on the outcome.[18]

Mapping of the common (intersecting) van der Waals volumes of the potent antagonists (Figures 1 and 9a) was undertaken and contrasted with the exclusion volume map created from the regions of space occupied by the less active or inactive analogues (Figure 2, excluding AP6 (9), which did not fit the pharmacophore, and Figure 9b). These volume maps show that substitution on the carbon adjacent to the phosphorus atom and in the vicinity of the nitrogen atom is subject to considerable steric constraints. However, as indicated by the exclusion map, there appears to be a number of less restricted regions which could offer potential for the design of new antagonists and lead to a better understanding of the receptor topology. For example, substitution around the 5-position of the piperidine-2-carboxylic acid nucleus has been largely unexplored except for methylation (5). A limitation of this study is the relatively small number of compounds available for comparison. In particular, some of the "forbidden" regions (Figure 9b) appear somewhat exaggerated compared to other regions for which there is a paucity of information.

In conclusion, a pharmacophore for the phosphono amino acid antagonists of the NMDA receptor has been developed using computer-based molecular modeling techniques. An important feature of this model is that only a single binding site for the phosphonic acid moiety is required. All competitive antagonists incorporating amino acid and phosphonate groups in their structure which we have examined fit the pharmacophore in energetically accessible conformations (data not shown). There is no need to invoke overlapping binding sites, nor are two binding sites needed for the phosphonic acid group to accommodate both the smaller AP5 analogues, 3 and 4, for example, and the larger alkenes 7 and 8. Our model does not eliminate the possibility of more than one binding mode for AP5 and AP7 analogues. While it is clear from the vector maps that multiple binding sites for the phosphonate moiety are not automatically excluded, it is equally clear that there is a plausible alternative to the two-binding-site model and that a single pharmacophore can be developed to account for the activities of AP5- and AP7-like molecules and the relative inactivity of AP6. Finally, why, using a very similar data set of molecules, have Hutchinson et al.[11] and we arrived at such different conclusions? The answer may lie in our approach of systematically generating as many conformations as practical for each molecule using conformational searching and flexible fitting (with energy minimization) routines. We were able to discover molecular geometries that at first might appear intuitively unlikely to the chemist, but upon closer examination were proven to be feasible and provided insight into the receptor pharmacophore.

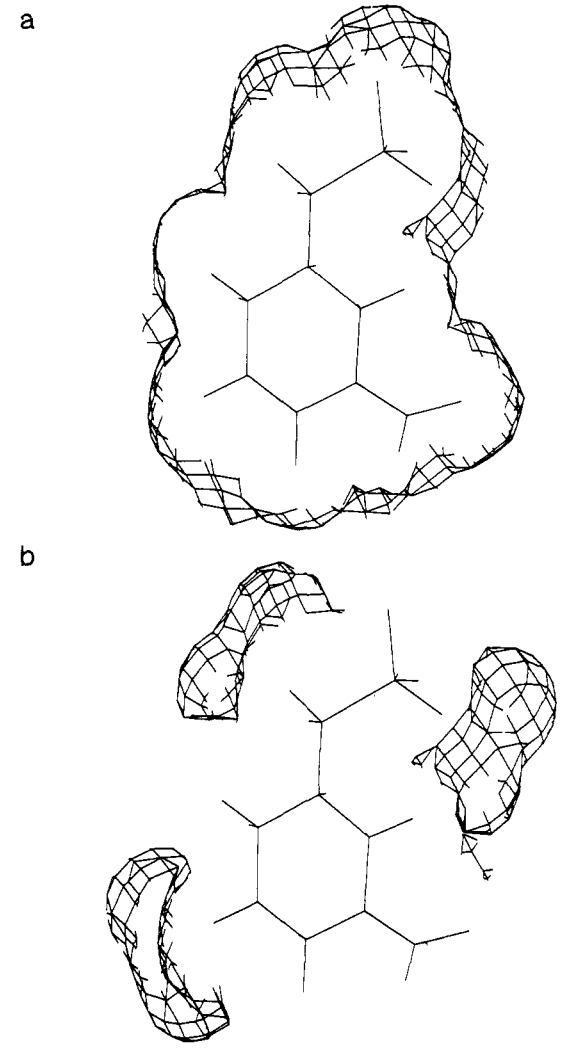

Figure 9. NMDA receptor volume maps of the allowed space (a), disallowed space (b); 4 has been included for illustrative purposes.

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Supplementary Material Available: Coordinates of 1, 2, 4, 7, and the model (6 pages). Ordering information is available on any current masthead page.