carboxamide (2k and 2l). Beginning with **3** (R = triazolyl), the protocol described in method A was followed with the following changes. The silylation step was conducted at 65 °C for 2 days. A solution of the crude bis-silylated product (0.015 mmol) in 2 mL of dioxane was treated with 1.8 mL (0.9 mmol) of 0.5 M LiOH. After being stirred at ambient temperature for 2.5 h, the solution was concentrated in vacuo and the product was purified by flash chromatography using 15% methanol in chloroform. The resulting acid was coupled to **5** as described in method A and the diastereomeric products were separated by MPLC using 5% methanol in dichloromethane. The purified silyl ethers were converted to **2k** and **2l** as described in method A.

(4*S*,5*S*,8*R*,10*R*,11*S*)-*N*-Isobutyl-6-aza-11-[[(*tert*-butyloxy)carbonyl]amino]-5-(cyclohexylmethyl)-4,10-dihydroxy-8-methyl-12-phenyl-1-dodecene-2-carboxamide (13). A solution of 45.6 mg (0.143 mmol) of (3*R*,5*R*)-**3** (R = H)[9] in 1.5 mL of toluene was cooled under a N_2 atmosphere to −78 °C and treated dropwise with 0.6 mL (0.6 mmol) of diisobutylaluminum hydride in dichloromethane. After being allowed to stir for 15 min, the reaction was quenched with methanol, partitioned between ethyl acetate and 1 M HCl, filtered through Celite, and concentrated in vacuo to give a white solid. The crude lactol, 0.14 mmol of the hydrochloride of **5**,[7] and 28 mg of sodium acetate were combined in 10 mL of 2-propanol. The resulting mixture was treated with 18 mg (0.29 mmol) of sodium cyanoborohydride and allowed to stir for 16 h. After removal of the solvent in vacuo, the residue was taken up in ethyl acetate, washed sequentially with aqueous $NaHCO_3$ and saturated brine, dried over $MgSO_4$, and concentrated. Purification by flash chromatography using 5% methanol in chloroform gave 27 mg (32%) of **13**.

Inhibition Studies. Assays of purified human renin in maleate buffer at pH 6.0 were performed as described previously.[7]

Stability Studies. Incubation of inhibitors with bovine pancreatic chymotrypsin (Sigma) was performed as described previously.[3]

Registry No. **2a**, 123382-01-4; **2b**, 123382-02-5; **2c**, 123482-65-5; **2d**, 123482-66-6; **2e**, 118251-57-3; **2f**, 123411-30-3; **2g**, 123438-10-8; **2h**, 123411-31-4; **2i**, 123484-24-2; **2i** (TBDMS ether), 123411-33-6; **2j**, 123484-25-3; **2k**, 123382-03-6; **2l**, 123482-67-7; **3a**, 104293-43-8; **3a** (lactol), 123382-20-7; **3b**, 104293-47-2; **3c**, 104293-55-2; **3d**, 104293-51-8; **3e**, 104293-45-0; **3f**, 123382-05-8; **3g**, 123382-06-9; **3h**, 123411-06-3; **3i**, 104293-46-1; **3j**, 104293-50-7; **3k**, 123382-07-0; **3l**, 123382-08-1; **4a**, 123382-09-2; **4b**, 123482-68-8; **4c**, 123482-69-9; **4d**, 123482-70-2; **4e**, 123482-71-3; **4f**, 123382-10-5; **4g**, 123382-11-6; **4h**, 123382-12-7; **4i**, 123382-13-8; **4j**, 123482-72-4; **4k**, 123382-14-9; **4l**, 123482-73-5; **5**·HCl, 118233-41-3; **6**, 110613-34-8; **7**, 123382-15-0; (2*R*)-**8**, 123382-16-1; (2*S*)-**8**, 123535-78-4; (2*R*)-**9**, 123382-17-2; (2*S*)-**9**, 123482-74-6; **10**, 123382-18-3; **11**, 123411-32-5; **12**, 110613-45-1; **13**, 123382-19-4; (5*R*,1′*S*)-5-[1-[[(*tert*-butyloxy)-carbonyl]amino]-2-phenylethyl]-3-methylenedihydrofuran-2-(4*H*)-one, 104293-39-2; 1,2,4-triazole, 288-88-0; renin, 9015-94-5.

Relationship between Structure, Conformational Flexibility, and Biological Activity of Agonists and Antagonists at the *N*-Methyl-D-aspartic Acid Subtype of Excitatory Amino Acid Receptors

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The relationship between conformational flexibility and agonist or antagonist actions at the *N*-Methyl-D-aspartic acid (NMDA) subtype of central L-glutamic acid (GLU) receptors of a series of racemic piperidinedicarboxylic acids (PDAs) was studied. The conformational analyses were based on ^1H NMR spectroscopy and supported by computer simulations and molecular mechanics calculations. While the trans forms of 2,3-PDA and 2,4-PDA and *cis*-2,5-PDA show NMDA receptor agonist activities, *cis*-2,3-PDA and *cis*-2,4-PDA are NMDA antagonists. The compounds *trans*-2,5-PDA and *cis*-2,6-PDA did not interact with NMDA receptors. Each of the three cyclic acidic amino acids showing NMDA agonist activities was found to exist as an equilibrium mixture of two conformers in aqueous solution. In contrast, the NMDA antagonists *cis*-2,3-PDA and *cis*-2,4-PDA as well as the inactive compounds *trans*-2,5-PDA and *cis*-2,6-PDA were shown to exist predominantly in a single conformation. These results seem to indicate that a certain degree of conformational flexibility of analogues of GLU is a prerequisite for activation of, but not for binding to, the NMDA receptor.

It is generally accepted that L-glutamic acid (GLU), and probably also L-aspartic acid (ASP), are excitatory neurotransmitters in the central nervous system (CNS).[1-5] Other amino acids with neuroexcitatory actions have been detected in the CNS, and some of these compounds, notably L-homocysteic acid,[6] L-serine-*O*-sulfate,[6,7] and quinolinic acid,[8] may participate in central neurotransmission processes.

In analogy with other neurotransmitters, GLU and ASP operate through multiple receptors. These excitatory amino acid (EAA) receptors are at present most conveniently subdivided into four main classes:[1-5,9-12] (1) *N*-Methyl-D-aspartic acid (NMDA) receptors at which NMDA is a selective agonist and 2-amino-5-phosphonovaleric acid (AP5)[1] and 2-aminoadipic acid (2AA)[2] are selective an-

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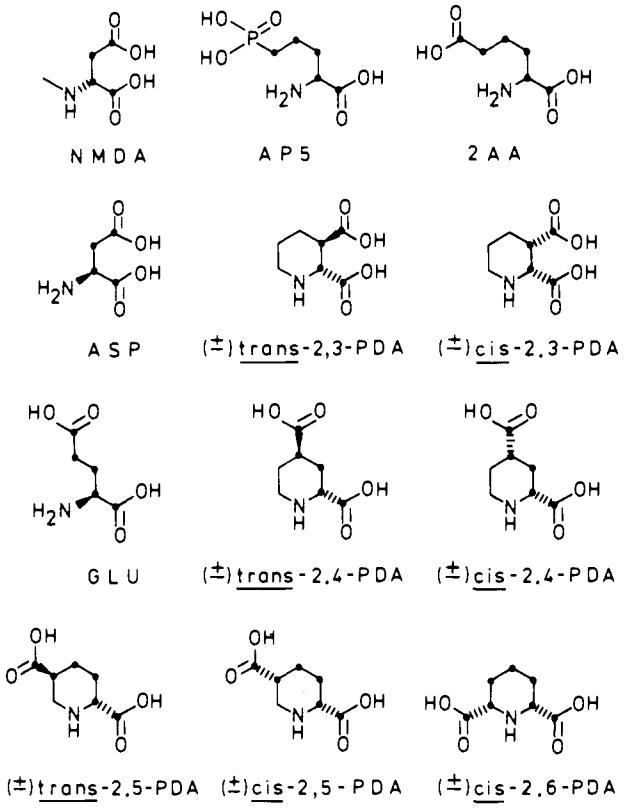
Chart I



tagonists (Chart I); (2) QUIS/AMPA receptors at which quisqualic acid (QUIS) is a nonselective agonist and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) is a highly selective agonist;[13] (3) kainic acid receptors, which are selectively activated by kainic acid;[14,15] (4) AP4 receptors,[9] at which L-2-amino-4-phosphonobutyric acid (L-AP4) antagonizes synaptic excitation.

Hyperactivity at central EAA synapses has been associated with the etiology of certain neurological disorders characterized by progressive neuronal degeneration, notably Huntington's chorea and dementia of the Alzheimer type.[4,5,16-19] Excitotoxic mechanisms are also likely to underlie the severe neuronal injury caused by ischemia, anoxia, and hypoglycemia.[4,5,16] Although the relative importance of the multiple EAA receptors as mediators of these excitotoxic processes is unknown, several lines of evidence strongly suggest an important role of the NMDA receptor(s) in neurodegenerative mechanisms, notably in Alzheimer's disease.[5,19] The NMDA receptor(s) appear to play a key role in long-term potentiation (LTP), which is of primary importance in learning and memory processes,[19-22] and hypoactivity of central EAA neuronal pathways

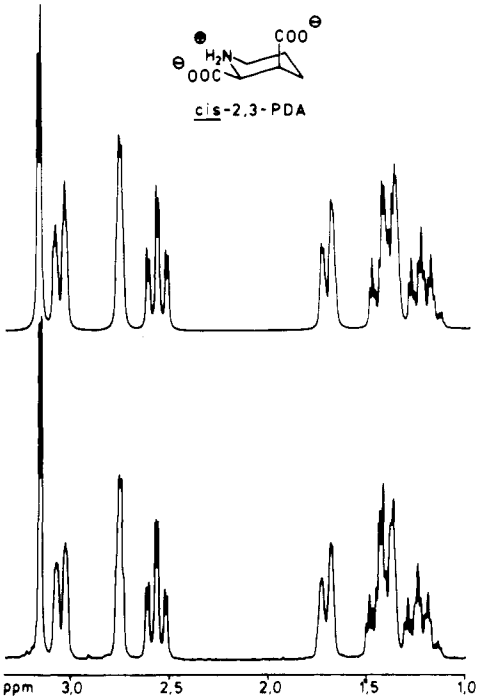


Figure 1. A 270-MHz ^{1}H NMR spectrum (bottom) and the computer-simulated spectrum (top) of *cis*-2,3-PDA.

operating through NMDA receptor(s) have recently been proposed to underlie the learning and memory impairment in Alzheimer patients.[18]

On the basis of these apparently paradoxical findings that hyperactive as well as hypoactive EAA neuronal mechanisms may be operative in Alzheimer's disease,[18] possibly in different brain regions, drugs capable of both protecting and activating NMDA receptor(s) may be of therapeutic interest. Thus, an obvious challenge is to design NMDA receptor ligands showing appropriately balanced partial agonist/antagonist profiles.

As part of a systematic mapping of the structural parameters of importance for activation or blockade of EAA receptors,[23,24] the present ^{1}H NMR spectroscopic studies and molecular mechanics calculations on certain piperidinedicarboxylic acids (PDAs) have been performed in order to elucidate the relationship between conformational properties and NMDA agonist or antagonist effects of these compounds.

The 2,3-PDAs are cyclic analogues of NMDA and ASP, whereas the 2,4-PDAs and the 2,5-PDAs are analogues of GLU and 2AA, respectively (Chart I). The trans forms of 2,3-PDA and 2,4-PDA are reported to be NMDA receptor agonists, whereas *cis*-2,3-PDA and *cis*-2,4-PDA have NMDA antagonist profiles.[25-27] Different effects on NMDA receptors of commercially available *cis*-2,5-PDA, which actually is an approximately 3:2 mixture of *cis*-2,5-PDA and *trans*-2,5-PDA,[28] have been reported.[25-27,29]

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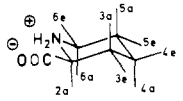
Table I. Computer-Simulated Chemical Shifts (ppm, DOH at δ 4.7) and Proton–Proton Coupling Constants (Hz) for the PDAs



	cis-2,3-PDA	*trans*-2,3-PDA	*cis*-2,4-PDA	*trans*-2,4-PDA	*cis*-2,5-PDA	*trans*-2,5-PDA	*cis*-2,6-PDA
H_{2a}	3.19	3.77	3.18	3.80	3.64	3.28	3.16
H_{3a}		2.66	1.21	2.05	1.83	1.42	1.16
H_{3e}	2.78		2.02	2.14	2.01	2.03	1.81
H_{4a}	1.45	1.68	2.07		1.81	1.35	1.22
H_{4e}	1.73	1.78		2.44	1.82	1.89	1.60
H_{5a}	1.24	1.60	1.26	1.86		2.29	1.16
H_{5e}	1.40	1.70	1.67	1.88	2.61		1.81
H_{6a}	2.59	3.00	2.59	3.13	3.09	2.71	3.16
H_{6e}	3.08	3.25	3.09	3.23	3.41	3.27	
J_{2a3a}		7.7	13.1	7.3	9.0	12.2	11.9
J_{2a3e}	3.4		3.1	4.5	4.0	3.4	3.0
J_{3a3e}			13.8	14.3	14.0	13.7	13.8
J_{3a4a}		7.8	12.5		9.5	11.6	13.0
J_{3a4e}		4.2		4.4	4.0	4.0	4.0
J_{3e4a}	4.0		3.6		4.5	4.0	4.0
J_{3e4e}	2.5			7.4	5.0	3.1	2.5
J_{4a4e}	13.3	14.0			13.0	13.0	13.0
J_{4a5a}	13.7	9.5	12.2			11.7	13.0
J_{4a5e}	4.0	4.0	3.8		4.5		4.0
J_{4e5a}	4.0	4.0		4.3		3.6	4.0
J_{4e5e}	2.5	8.0		7.5	4.5		2.5
J_{5a5e}	14.1	14.5	14.4	14.5			13.8
J_{5a6a}	13.4	8.7	13.3	7.3		12.2	11.9
J_{5a6e}	4.0	4.0	4.0	3.5		4.0	
J_{5e6a}	3.2	3.8	3.2	3.5	3.3		3.0
J_{5e6e}	2.5	6.7	2.0	7.5	4.8		
J_{6a6e}	12.8	12.6	13.1	12.5	12.7	12.5	
Frequency, MHz	270	500	270	250	250	270	270

We have synthesized these compounds in isomerically pure form and studied their NMDA receptor pharmacology.

Results

^{1}H NMR Spectroscopy. High-resolution ^{1}H NMR spectra were obtained of the seven PDAs in a phosphate buffer (pH 7.4). Selective decoupling experiments were used in the assignment of the NMR spectra, which subsequently were computer simulated with the program MIMER.[30,31]

Analysis of the ^{1}H NMR spectra of the PDAs under study were carried out by this computer simulation and all proton–proton coupling constants were determined (±0.1 Hz). The chemical shift values and coupling constants are shown in Table I, and on the basis of these analyses, the compounds were divided into two groups; one group of compounds, which were found to exist primarily in one conformation in aqueous solution, and another group of compounds found to exist as an equilibrium mixture of two conformations under the same conditions. In the lower part of Figure 1 the recorded ^{1}H NMR spectrum of *cis*-2,3-PDA is illustrated, whereas the upper part of this figure shows the computer-simulated spectrum. Interpretation of this spectrum revealed that *cis*-2,3-PDA exists preferentially in the chair conformation with the 2-carboxylate group in an equatorial position as illustrated. Similarly, three other compounds, namely *cis*-2,4-PDA, *trans*-2,5-PDA, and *cis*-2,6-PDA, were shown to exist almost exclusively in one conformation in aqueous solutions.

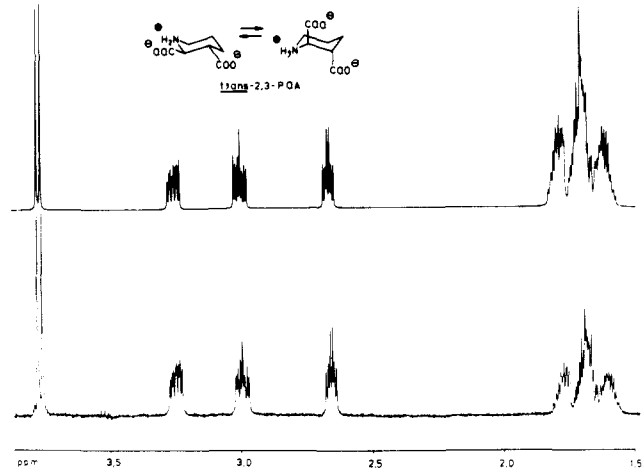


Figure 2. A 500-MHz ^{1}H NMR spectrum (bottom) and the computer-simulated spectrum (top) of *trans*-2,3-PDA.

In contrast, *trans*-2,3-PDA, *trans*-2,4-PDA, and *cis*-2,5-PDA were found to exist as a mixture of two conformers. The spectrum of one of these compounds, *trans*-2,3-PDA, is shown in Figure 2. An analysis of this spectrum led to the conclusion that *trans*-2,3-PDA exists in aqueous solution as an equilibrium mixture of the two chair conformations shown, with a slight preference for the diequatorial form. The ring inversion between the two conformers is fast as compared to the NMR time scale, leading to an NMR spectrum representing an average spectrum for two conformations.

Proton–proton torsion angles in the four compounds existing predominantly in one conformation were calculated from the coupling constants determined by computer simulation of the spectra. The calculations were carried out with a parametrization[32] of the Karplus equation,

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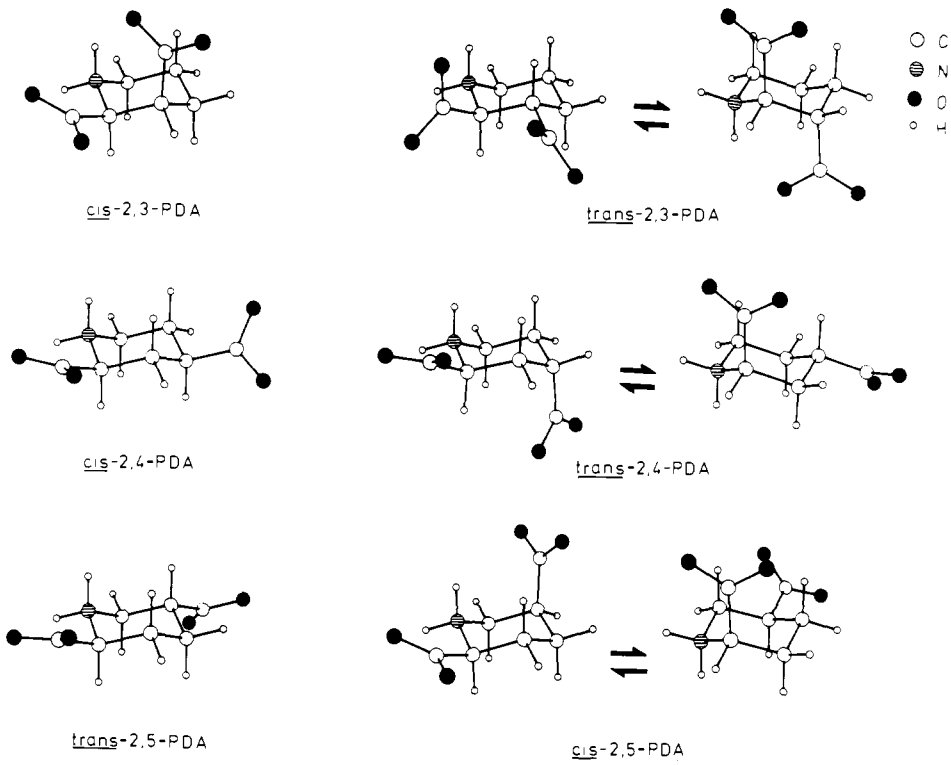

Figure 3. Illustrations of the conformations of the fully charged molecules of *cis*- and *trans*-2,3-PDA, *cis*- and *trans*-2,4-PDA, and *cis*- and *trans*-2,5-PDA measured in aqueous solutions at pH 7.4 by using ^{1}H NMR spectroscopic techniques.

which correlates torsion angles with gauche coupling constants, electronegativity of substituents, and orientation of these on the ethane fragment, on which the involved protons are placed. The equations made it possible to calculate torsion angles for all protons placed either diequatorially or axial–equatorially (gauche orientations) on the piperidine rings in question. These proton–proton torsion angles in the molecules give a good estimation of the conformation of the piperidine rings.

Conformational data relying heavily on parametrized equations are always questionable. Therefore, molecular mechanics calculations were carried out in order to obtain an independent determination of the preferred conformations. The results obtained from calculations of torsion angles are listed in the next paragraph in context with the description of the molecular mechanics calculations.

Molecular Mechanics. Each of the seven PDAs under study was built in four different conformations by using the SYBYL molecular modeling system,[33] two chair conformations (as the two shown for *trans*-2,3-PDA in Figure 2), one boat conformation, and one twist-boat conformation. Calculations have been performed on uncharged molecules, having the geometries of carboxylate and ammonium groups. All of the conformations were energy minimized by using the MAXIMIN program,[34] which showed that for each compound each pair of chair conformations had approximately the same energies (±0.5 kcal/mol), while the corresponding boat and twist-boat conformation had energies typically 4–5 kcal/mol higher. From these energy calculations, it was not possible to determine which compounds exist preferentially in one conformation or whether two conformations would be equally favored in aqueous solution. No calculations of energy barriers for ring inversion were carried out. Torsion angles calculated for the energy-minimized molecules of *cis*-2,3-PDA, *cis*-2,4-PDA, *trans*-2,5-PDA, and *cis*-2,6-PDA and the corresponding angles in the single conformations found by the ^{1}H NMR analysis are compared in Table II. It is seen that there is a very good agreement between the conformations measured by the NMR method and those derived from the molecular mechanics calculations.

Table II. Torsion Angles (deg) for Some PDAs Based on Molecular Mechanics (MM) Calculations on Energy-Minimized Molecules and Calculated on the Basis of ^{1}H NMR Data

	cis-2,3-PDA		*cis*-2,4-PDA		*trans*-2,5-PDA		*cis*-2,6-PDA	
	NMR	MM	NMR	MM	NMR	MM	NMR	MM
φ_{2a3e}	56	57	60	60	59	59	61	59
φ_{3a4e}					59	58	59	58
φ_{3e4a}	57	55	61	59	59	58	59	59
φ_{3e4e}	64	64			60	62	63	62
φ_{4a5e}	59	58	61	59			59	59
φ_{4e5a}	59	58			61	59	59	58
φ_{4e5e}	63	62					63	62
φ_{5a6e}	60	58	60	59	61	61		
φ_{5e6a}	59	58	59	59			61	59
φ_{5e6e}	60	62	63	61				

Biological Testing. A modified version[35] of the rat cortical slice preparation described by Harrison and Simmonds[36] was used for the determination of pharmacological effects on EAA receptors. As standard agonists were used NMDA, AMPA, and kainic acid, and AP5 was used as an NMDA receptor antagonist. Contradictory activities for commercially available *cis*-2,5-PDA, which actually is a 3:2 mixture of *cis*- and *trans*-2,5-PDA (see above), are reported in the literature.[25-27,29] We have synthesized isomerically pure samples of these two compounds and studied their effects on EAA receptors in the rat cortical slice prepa-

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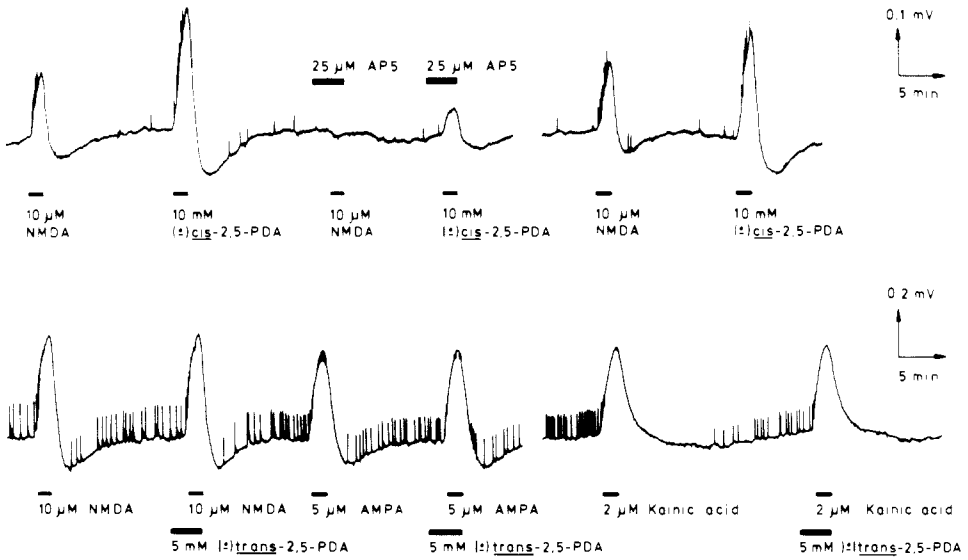

Figure 4. Comparison of excitatory effects of NMDA and (±)-*cis*-2,5-PDA and the antagonistic effect toward these by AP5 (top) and illustration of the lack of agonistic as well as antagonistic effects of (±)-*trans*-2,5-PDA toward NMDA, AMPA, and kainic acid (bottom).

ration (Figure 4). The trans isomer of 2,5-PDA was totally devoid of EAA agonist activity at concentrations up to 5 mM, and at this maximal concentration tested no significant antagonist effects toward the EAA agonists, NMDA, AMPA, or kainic acid, were detectable. In contrast, *cis*-2,5-PDA was shown to have a weak excitatory action, which could be substantially reduced by the NMDA antagonist AP5 (Figure 4). It is a general observation that very weak agonist effects are difficult to block completely by antagonists. The NMDA agonistic effect of *cis*-2,5-PDA was approximately 3 orders of magnitude weaker than that of NMDA. At concentrations of *cis*-2,5-PDA up to 1 mM, where only marginal excitatory actions were observed, no antagonistic effects against NMDA, AMPA, or kainic acid could be detected.

The other five PDAs (*cis*-2,3-PDA, *trans*-2,3-PDA, *cis*-2,4-PDA, *trans*-2,4-PDA, and *cis*-2,6-PDA) were tested in a similar manner with results in good agreement with those reported in the literature.[26,27,29] For NMDA and the PDAs showing NMDA agonist profiles, the following ED_{50} values were determined: NMDA, 15 μm; *trans*-2,3-PDA, 35 μM; *trans*-2,4-PDA, 60 μM. *cis*-2,3-PDA was shown to be a partial agonist with a maximal response of approximately $^1/_4$ that of NMDA. This effect of *cis*-2,3-PDA, which was reached at concentrations above 100 μM, could be antagonized by AP5. At a concentration of 1 mM, *cis*-2,3-PDA was capable of reducing the excitatory effect of NMDA (10 μM) by approximately 50%. *cis*-2,4-PDA showed an antagonist profile very similar to that of *cis*-2,3-PDA, but in contrast to the latter compound, *cis*-2,4-PDA did not reveal significant excitatory effects at concentrations up to 10 mM. *cis*-2,6-PDA did not show detectable EAA agonist or antagonist effects at concentrations up to 5 mM.

Discussion

Compounds with partial NMDA agonist properties may, in principle, have neuroprotective as well as learning- and memory-improving therapeutic effects in Alzheimer patients. A prerequisite for the design of such compounds on a rational basis is information about the relative importance of different structural parameters for activation and blockade of NMDA receptors.

The α-amino-ω-phosphono carboxylic acids, including AP5 and [3-(2-carboxypiperazin-4-yl)propyl]-1-phosphonic acid (CPP),[1,4,5,37-39] are the most potent and selective competitive NMDA antagonists so far described. Although the molecular mechanisms underlying the interaction of these compounds with NMDA receptors are not fully understood, it has been proposed that the phosphono group binds to a site at the receptor complex different from that which binds the ω-carboxylic acid group of NMDA agonists.[10,25,40] The acidic amino carboxylic acids 2,3-PDA, 2,4-PDA, and 2,5-PDA are cyclic analogues of ASP/NMDA, GLU, and 2AA, respectively. This structurally homogeneous class of cyclic amino acids, which comprises NMDA agonists as well as antagonists and is assumed to interact with the same site of the NMDA receptor complex, has been selected as model compounds for studies of the conformational requirements for activation or blockade of NMDA receptor(s).

The present studies have disclosed a conspicuous correlation between the conformational flexibility of the PDA model compounds and their ability to activate or block NMDA receptors. The trans isomers of 2,3-PDA and 2,4-PDA are selective and fairly potent NMDA agonists.[1,10,11] Both of these compounds were shown to exist as an equilibrium mixture of two conformers in aqueous solution at pH 7.4, reflecting conformational flexibility of the fully ionized forms of these molecules (Figure 2 and 3). It is somewhat surprising that the diequatorial and the diaxial conformations of *trans*-2,3-PDA can be detected in aqueous solution in similar concentrations. The cis forms of 2,3-PDA and 2,4-PDA show NMDA antagonist effects, and at pH 7.4 both of these compounds exist predominantly in a single conformation in aqueous solution (Figure 1 and 3). Whereas *trans*-2,5-PDA turned out to be devoid of affinity for NMDA receptors, *cis*-2,5-PDA was shown to be a weak agonist at NMDA receptors (Figure 4). In agreement with the findings for the trans isomers of 2,3- and 2,4-PDA, *cis*-2,5-PDA was detected as an

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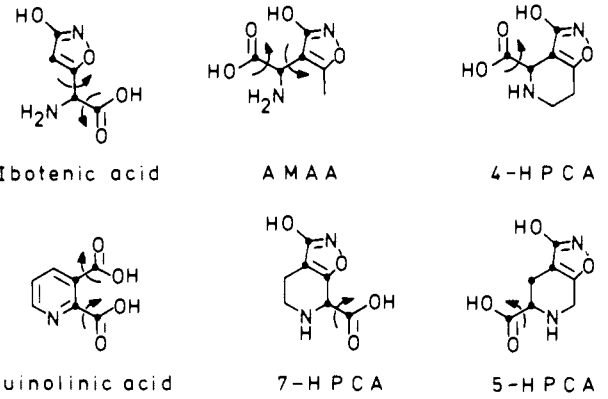
Chart II



equilibrium mixture of two conformers in aqueous solution, whereas only a single conformer of the inactive isomer *trans*-2,5-PDA could be identified.

These observations have been interpreted in terms of a certain degree of conformational flexibility of GLU analogues being a necessary condition for agonist activity at NMDA receptors. Alternatively, the active NMDA agonist conformation(s) is (are) not accessible to the inactive PDAs (*trans*-2,5-PDA and *cis*-2,6-PDA) or to the PDAs showing NMDA antagonist profiles (*cis*-2,3-PDA and *cis*-2,4-PDA). This possibility can not be ruled out on the basis of the present studies. The cis and trans isomers of 2,5-PDA are cyclic analogues of the NMDA antagonist 2AA (Chart I). On the basis of the present studies, it can be concluded that neither analogue reflects the conformation adopted by 2AA during its binding to the NMDA receptor.

Ideally, these conformation–activity studies should have been carried out on the enantiomers of the PDA model compounds. However, so far only one of the compounds has been resolved, namely *cis*-2,3-PDA, and the NMDA antagonist effect of this compound has been shown to reside exclusively in the (-)-isomer.[25,27]

The apparent correlation between conformational flexibility of GLU or ASP analogues and their agonist activity at NMDA receptors demonstrated in these studies are in agreement with the results of earlier conformation–activity studies on a series of 3-isoxazolol bioisosteres of GLU and ASP[23,41,42] (Chart II). Thus, the amino acid side chain of the potent NMDA receptor agonist ibotenic acid[13] is characterized by a high degree of conformational flexibility. Similarly, the structurally related ASP analogue α-amino-3-hydroxy-5-methyl-isoxazoleacetic acid (AMAA)[43] is an NMDA agonist, although weaker than ibotenic acid.[13] The degree of rotational freedom of the amino acid side chain of AMAA has not been studied yet, but it has been shown that the conformationally restricted analogue of AMAA, 3-hydroxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]-pyridine-4-carboxylic acid (4-HPCA), is completely devoid of agonist or antagonist effects at NMDA receptors.[42] 4-HPCA has been shown to exist in a single conformation in aqueous solution.[41] A comparison of the preferred conformations in aqueous solution of 4-HPCA and the NMDA antagonist *cis*-2,3-PDA reveals marked differences, which may explain why the NMDA receptors, which are

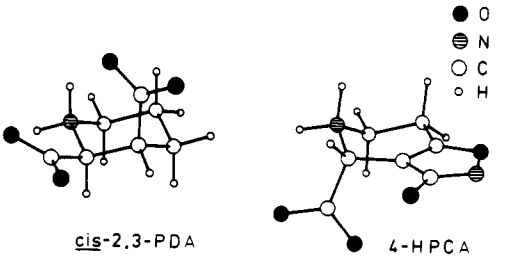


Figure 5. A comparison of the preferred conformations in aqueous solutions of 4-HPCA[41] and *cis*-2,3-PDA as determined by ^{1}H NMR spectroscopy.

capable of binding the former compound, do not recognize 4-HPCA (Figure 5). Similarly, the cyclized analogue of ibotenic acid, 3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-7-carboxylic acid (7-HPCA) (Chart II), does not show any effect on NMDA receptors.[44] Like the isomeric compound 3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]-pyridine-5-carboxylic acid (5-HPCA),[45] 7-HPCA is a potent and specific agonist at QUIS/AMPA receptors.[44] Whether the lack of effect of 7-HPCA at NMDA receptors is the result of its low degree of conformational flexibility or an inability to adopt the NMDA receptor-active conformation(s) of ibotenic acid is not known. In any case, whereas a certain degree of conformational mobility of GLU analogues appears to be a prerequisite for activation of NMDA receptor(s), this structural parameter obviously is not of critical importance for activation of QUIS/AMPA receptors.

The endogenous compound quinolinic acid is capable of exciting neurones in an AP5-sensitive manner.[8] The mechanism underlying this effect is not clear. At physiological pH, quinolinic acid exists almost completely in the dianionic form, and the excitatory effects of this compound actually are shared by phthalic acid and a number of other dicarboxylic acids.[46] It is possible that neuronal excitation induced by quinolinic acid, the structural and physicochemical properties of which are distinctly different from those of NMDA, the PDAs, and other NMDA receptor ligands, are mediated by a modulatory site or a subtype of AP5-sensitive EAA receptors.[8,47-49]

Experimental Section

^{1}H NMR spectra were obtained on Bruker AM 250, Bruker HX 270 S, and Bruker 500 at 303 K using DOH (δ 4.7) as an internal standard. Computer simulation of ^{1}H NMR spectra was carried out on an IBM AT PC connected to a Roland DXY-980 Plotter using the program MIMER.[30,31]

Molecular mechanics calculations were performed on a VAX 11/750 computer with the SYBYL modeling system,[33] minimizations were carried out with the MAXIMIN program,[34] and handling of the molecules was done on an Evans and Sutherland PS 330 system.

All piperidinedicarboxylic acids, except *cis*-2,5-PDA and *trans*-2,5-PDA, were purchased from Cambridge Research Biochemicals Ltd., England.

cis-2,5-PDA and *trans*-2,5-PDA were synthesized by catalytic reduction (Raney nickel, 2 M NaOH, 100 atm of H_2, 100 °C) of

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pyridine-2,5-dicarboxylic acid, giving a mixture of the two compounds, which could be separated (as zwitterions) by fractional crystallizations (from water). Physicochemical constants and spectroscopic data were identical with those reported for *cis*- and *trans*-2,5-PDA.[50]

The rat cortical slice preparation for testing of excitatory amino acids described by Harrison and Simmonds[36] was used in a modified version.[35] Wedges (500 μM thick) of rat brain containing cerebral cortex and corpus callosum were placed with the cortex part between two layers of nappy liner and constantly perfused with a Mg^{2+}-free, oxygenated Krebs solution (at room temperature), while the corpus callosum was placed on the wick of an Ag/AgCl electrode electrically insulated from the cortex part. A reference electrode was placed in contact with the nappy liner and the potential difference between the electrodes was recorded directly on a Servogor 330 recorder. Standard compounds and test compounds were dissolved in the superfusion medium.

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Registry No. *cis*-2,3-PDA, 82949-15-3; *trans*-2,3-PDA, 84229-42-5; *cis*-2,4-PDA, 84229-40-3; *trans*-2,4-PDA, 84229-43-6; *cis*-2,5-PDA, 84229-41-4; *trans*-2,5-PDA, 123099-49-0; *cis*-2,6-PDA, 59234-40-1; pyridine-2,5-dicarboxylic acid, 100-26-5.

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6-Benzoxazinylpyridazin-3-ones: Potent, Long-Acting Positive Inotrope and Peripheral Vasodilator Agents

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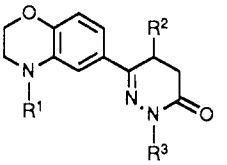
A series of 6-benzoxazinylpyridazin-3-ones was prepared and evaluated for inhibition of cardiac phosphodiesterase (PDE) fraction III in vitro and for positive inotropic activity in vivo. 6-[3,4-Dihydro-3-oxo-1,4(2*H*)-benzoxazin-7-yl]-2,3,4,5-tetrahydro-5-methylpyridazin-3-one (bemoradan) was found to be an extremely potent and selective inhibitor of canine PDE fraction III and a long-acting, potent, orally active inotropic vasodilator agent in various canine models. Additional benzoxazin-6-yl and -8-yl compounds were also prepared. Altering the pyridazinone substitution from the 6-position to the 7-position produced a 14-fold increase in the iv cardiotonic potency (ED_{50}) from 77 to 5.4 μg/kg while substitution at the 8-position reduced potency. Methyl substitution at various sites in the molecule was also examined. Positive inotropic activity was maintained for between 8 and 24 h after a single oral dose (100 μg/kg) of bemoradan in dogs, thus making it one of the most potent and long-acting orally effective inotropes yet described. Bemoradan is currently under development as a cardiotonic agent for use in the management of congestive heart failure.

The most widely used orally active inotropic agent currently available to manage congestive heart failure (CHF) is digoxin.[1] The low therapeutic ratio of digitalis, with its marginal efficacy and propensity to cause serious ventricular arrhythmias,[2] has prompted the search for new oral nonglycoside, noncatecholamine cardiotonic agents useful in the treatment of chronic CHF. A class of agents possessing both positive inotropic and systemic vasodilator activity (beneficial in reducing cardiac pre- and afterload[3]) with a much broader therapeutic ratio than digitalis has emerged recently. These agents, which may act via selective inhibition of cyclic AMP phosphodiesterase fraction III, include milrinone,[4] enoximone,[5] indolidan,[6] bemarinone (ORF 16600),[7] and others. In long-term clinical trials, milrinone has been shown to produce sustained symptomatic and hemodynamic improvement in CHF patients.[8] However, milrinone's short duration of action and inability to alter the high rate of mortality suggest that opportu-

Table I. Synthetic Routes and Physiochemical Data for 6-[4-Substituted-3,4-dihydro-1,4(2*H*)-benzoxazin-6-yl]-2-substituted-pyridazin-3-ones (4)



no.	R^1	R^2	R^3	mp, °C	formula[a]	method
4a	SO_2Me	H	H	244-245	$C_{13}H_{15}N_3O_4S$	A, F
b	SO_2Me	H	Me	162-165	$C_{14}H_{17}N_3O_4S$	A, F
c	SO_2Me	H	allyl	153-155	$C_{16}H_{19}N_3O_4S$	A, F, B
d	SO_2Me	H	pentyl	138-139	$C_{18}H_{25}N_3O_4S$	A, F, B
e	SO_2Me	H	phenyl	199-201	$C_{19}H_{19}N_3O_4S$	A, F
f	H	H	H	198-199	$C_{12}H_{13}N_3O_2$	A, F
g	acetyl	H	H	156-158	$C_{14}H_{15}N_3O_3$	A, F, C
h	H	Me	H	166-168	$C_{13}H_{15}N_3O_2$	A, D, F
i	acetyl	Me	H	185-188	$C_{15}H_{17}N_3O_3$	A, D, F, C
j	SO_2Me	Me	H	207-212	$C_{14}H_{17}N_3O_4S$	A, D, F

[a] Satisfactory elemental analyses (C, H, N) were obtained for all compounds.

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