

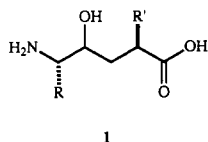
Renin Inhibitors Based on Dipeptide Analogues. Incorporation of the Hydroxyethylene Isostere at the P₂/P₃ Sites¹

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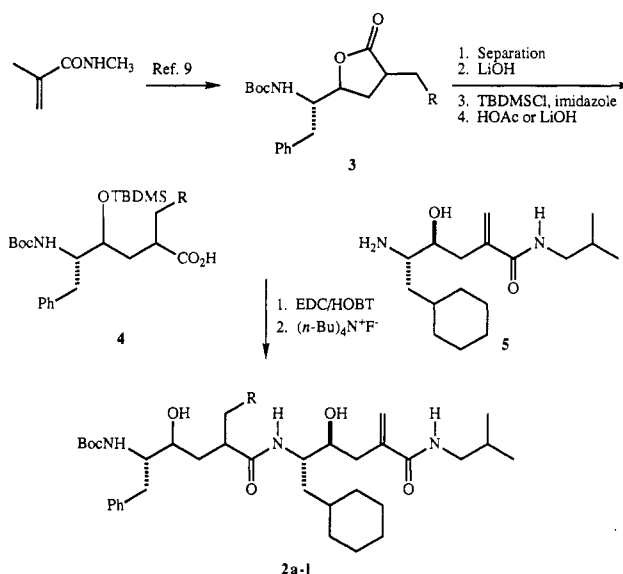
The synthesis of a series of renin inhibitors in which the P₂ and P₃ amino acids are replaced with the hydroxyethylene dipeptide isostere is reported. In vitro evaluation of the inhibitors has revealed that this isostere is an acceptable amide-bond replacement in which activity is maintained and stability is enhanced. Structure-activity relationships of this series resemble but do not parallel those of the corresponding dipeptide-containing inhibitors.

The replacement of amide bonds by peptidomimetics has become a useful technique for imparting novel characteristics to bioactive peptides.² Peptidomimetics can provide enhanced biological activity and/or metabolic stability to systems of potential therapeutic interest. The identification and alteration of peptide linkages which can be replaced without sacrifice of activity can therefore be crucial for successful manipulation of biological response and pharmacokinetic behavior. Recent results have demonstrated that the peptide bond between the P₂ and P₃ sites in inhibitors of renin, the aspartic proteinase responsible for initiation of the renin-angiotensin system, is susceptible to proteolytic cleavage by chymotrypsin.³ Various modifications which are effective in partially or completely inhibiting this cleavage without drastic loss of activity against the target enzyme renin include the use of N-methylated amino acids in the P₂ site^{4,5} and structurally modified phenylalanine analogues in the P₃ site.⁵ Recently a preliminary report described the replacement of the P₃ and P₂ amino acids by the Phe-Phe hydroxyethylene dipeptide isostere.⁶ Herein we report the details of our results¹ with this dipeptide analogue (1) with a variety of side chains (R') at the P₂ site. Incorporation of these analogues into previously described low molecular weight renin inhibitors⁷ leads to compounds of novel potency and stability.



The hydroxyethylene isostere has found wide use as a transition-state analogue of the scissile bond in inhibitors of aspartic proteinases.⁸ Use of this isostere to impart

Scheme I



proteolytic stability to a peptide bond which is not the cleavage site, however, is much less common.⁶ Thus, at the outset, the ability of 1 to successfully mimic a trigonal peptide bond rather than a tetrahedral intermediate was open to question. Structurally, 1 would be expected to be more flexible than the corresponding dipeptide. In addition, the hydrogen-bond-donating ability of the peptide NH is lost; however, the hydrogen-bond-accepting ability of the peptide carbonyl is maintained. The P₂/P₃ positions of peptide-based renin inhibitors seemed a useful site to test the incorporation of 1 since the use of N-methylated amino acids in the P₂ position had established that NH hydrogen-bond donation is not essential for binding^{4,5} and since a limited study using Phe-Phe isosteres had shown promising potency.⁶ Due to conformational differences, the structure-activity relationships of inhibitors containing the hydroxyethylene isostere would not be expected to exactly mimic that of the parent peptides. We therefore sought a method whereby we could study these relationships at the P₂ site in a series of compounds 2 and compare the in vitro activities to that of the parallel peptide-based inhibitors. Synthetically, this required a general method for the synthesis of the hydroxyethylene isostere which combined elements of stereocontrol with the greatest amount of flexibility for introduction of various functionality at the P₂ side chain.⁹

- (1) Presented in part at the 194th Meeting of the American Chemical Society, New Orleans, LA, August, 1987.
- (2) Recent reviews: (a) Spatola, A. F. *Chem. Biochem. Amino Acids, Pept., Proteins* 1983, 7, 267. (b) Tourwe, D. *Janssen Chim. Acta* 1985, 3, 3.
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Table I. Chemical Data for Renin-Inhibiting Compounds

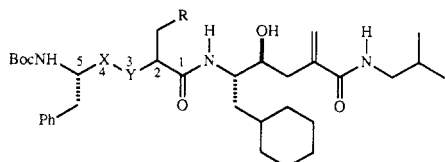
no.	% yield ^a	R _f (solvent) ^b	formula ^c
2a	20	0.20 (A)	C ₃₅ H ₅₇ N ₃ O ₆
2b	43	0.21 (A)	C ₃₅ H ₅₇ N ₃ O ₆ ·0.25H ₂ O
2c	33	0.21 (A)	C ₃₅ H ₅₇ N ₃ O ₆ ·0.25H ₂ O
2d	53	0.23 (A)	C ₃₅ H ₅₇ N ₃ O ₆ ·0.25H ₂ O
2e	27	0.20 (B)	C ₃₈ H ₆₃ N ₃ O ₆
2f	46	0.20 (B)	C ₃₈ H ₆₃ N ₃ O ₆ ·0.5H ₂ O
2g	13	0.34 (B)	C ₃₉ H ₆₅ N ₃ O ₆ ·0.25H ₂ O
2h	11	0.37 (B)	C ₄₀ H ₆₇ N ₃ O ₆ ^d
2i	41	0.22 (B)	C ₃₈ H ₆₃ N ₃ O ₆
2j	29	0.24 (B)	C ₃₉ H ₆₃ N ₃ O ₆ ·0.25H ₂ O
2k	22	0.24 (C)	C ₃₇ H ₅₈ N ₃ O ₆ ·0.75H ₂ O
2l	17	0.20 (C)	C ₃₇ H ₅₈ N ₃ O ₆ ·0.75H ₂ O
7		0.20 (B)	C ₃₇ H ₆₀ N ₄ O ₆
8		0.23 (B)	C ₃₇ H ₆₀ N ₄ O ₆
9		0.22 (B)	C ₃₈ H ₆₀ N ₄ O ₆ ·0.5H ₂ O
10	52	0.55 (B)	C ₃₆ H ₆₀ N ₂ O ₆
11	23	0.47 (B)	C ₃₅ H ₅₉ N ₂ O ₆ ·0.25H ₂ O
13	32	0.19 (C)	C ₃₅ H ₅₉ N ₃ O ₅ ^e

^a Overall yields based on lactones 3. ^b TLC solvent systems: A, 75% ethyl acetate/chloroform; B, 60% ethyl acetate/hexane; C, 7.5% methanol/chloroform. ^c Analyses for C, H, N were within $\pm 0.4\%$ of the calculated values for the formulas shown unless otherwise indicated. ^d Exact mass calcd for C₄₀H₆₇N₃O₆: 686.5108. Found: 686.5114. ^e Exact mass calcd for C₃₅H₅₉N₃O₅: 602.4533. Found: 602.4533.

Results and Discussion

The synthesis of renin inhibitors 2, detailed in Scheme I, proceeded via the key intermediate lactones 3, which contain all the appropriate functionality of 1 in a suitably protected form. The preparation of lactones 3 was accomplished by using our previously described method⁹ whereby the titanium derivative of dilithiated *N*-methylmethacrylamide was condensed with Boc-L-phenylalaninal and the resulting hydroxyamide was thermolyzed to give the α -methylenebutyrolactone. Conjugate addition of various nucleophiles led to 3. Alkyl nucleophiles were introduced via cuprous cyanide catalyzed addition of the corresponding Grignard reagents while nitrogen heterocycles added smoothly to the unsaturated lactone by simply heating in toluene.¹⁰ Separation of diastereomers and structural determination were most convenient at this stage to allow elaboration of the diastereomer with the appropriate stereochemistry at C₃ and C₅ into renin inhibitors 2. This was accomplished by using a variation of a previously described sequence^{6,11} wherein lactone 3 was hydrolyzed and protected as the bis-*t*-butyldimethylsilyl derivative. Mild hydrolysis led to acid 4, which was coupled by using the carbodiimide method to previously described⁷ transition-state analogue 5. A final deprotection step using tetra-*n*-butylammonium fluoride led to 2. The characterization of inhibitors 2a–1 and the overall yields from lactones 3 are detailed in Table I.

The compounds 2a–1 were evaluated for their ability to inhibit purified human renal renin in vitro by radioimmunoassay for angiotensin I production.⁷ Inhibitory potencies, expressed as IC₅₀ values, are shown in Table II. In order to gain insight on the ability of the hydroxyethylene isostere to mimic the local conformation of the P₂/P₃ peptide linkage, Phe-Ala analogues 2a–d, derived from each of the four possible diastereomers of 3 (R = H),⁹ were evaluated. Fortunately, both 2a and 2c, which retain

Table II. Inhibition of Human Renin by Compounds 2a–1


no.	X–Y	R	confign	IC ₅₀ , nM
2a	CH(OH)–CH ₂	H	2R,4R	20
2b	CH(OH)–CH ₂	H	2S,4R	100
2c	CH(OH)–CH ₂	H	2R,4S	20
2d	CH(OH)–CH ₂	H	2S,4S	3000
2e	CH(OH)–CH ₂	CH(CH ₃) ₂	2R,4R	25
2f	CH(OH)–CH ₂	CH ₂ CH ₂ CH ₃	2R,4R	5.5
2g	CH(OH)–CH ₂	CH ₂ (CH ₂) ₂ CH ₃	2R,4R	19
2h	CH(OH)–CH ₂	CH ₂ (CH ₂) ₃ CH ₃	2R,4R	70
2i	CH(OH)–CH ₂	CH ₂ CH ₂ CH=CH ₂	2R,4R	1
2j	CH(OH)–CH ₂	CH ₂ CH ₂ CH=CH ₂	2S,4R	8
2k	CH(OH)–CH ₂	1-triazolyl	2S,4R	95
2l	CH(OH)–CH ₂	1-triazolyl	2R,4R	2700
6 ^a	CO–NH	H	2S	10
7	CO–NH	CH(CH ₃) ₂	2S	6
8	CO–NH	CH ₂ CH ₂ CH ₃	2RS	2
9	CO–NH	CH ₂ CH ₂ CH=CH ₂	2RS	18
12 ^a	CO–NH	4-imidazolyl	2S	2

^a See reference 7.

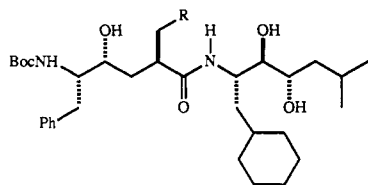
the natural stereochemistry of the C₂ side chain, display significantly greater inhibitory potency than “unnatural” isomers 2b and 2d. It was thus our expectation that the structure–activity relationships in this series might resemble, although not necessarily parallel, those of the parent peptides. Interestingly, the stereochemistry at C₄ has little effect on activity since 2a and 2c were equipotent (20 nM). This observation parallels that previously reported for Phe-Phe analogues.⁶

For synthetic convenience, the 2R,4R isomers were chosen to pursue structure–activity relationships. The effect of extending the P₂ side chain is demonstrated with inhibitors 2e–j. Surprisingly, Phe-Leu analogue 2e (25 nM) is slightly less active than Phe-Ala analogue 2a. This is apparently a result of differences in conformation between the peptidomimetic and the native dipeptide since the corresponding Phe-Leu containing inhibitor 7 is about twice as potent as 6, which contains Phe-Ala (6 and 10 nM, respectively). Phe-Nle analogue 2f, on the other hand, is four times more potent than 2a, a trend which closely parallels the native dipeptide series (compare 8 to 6). Increasing the length of the linear side chain produces 2g and 2h with diminished potency (loss of 4 and 14 times, respectively, over 2f), apparently a result of overcrowding the P₂ binding site. The most striking result is provided by 2i, which has an IC₅₀ value of 1 nM. Thus, simple introduction of unsaturation into the end of the P₂ side chain of 2g to give 2i results in a nearly 20-fold boost in activity. The origin of this effect is not well understood, and appears to be a unique property of the hydroxyethylene isostere in conjunction with the particular transition-state mimic which is attached. The corresponding dipeptide-containing inhibitor 9 (tested as 1:1 mixture at the P₂ site) does not show the same enhanced potency over 8. Moreover, inhibitor 10 (IC₅₀ = 12 nM), which results from attachment of the same isostere to a different transition-state mimic,¹² fails to show improved binding over

(10) In addition to the conjugate addition of 1,2,4-triazole to an α -methylenebutyrolactone to produce 3 reported here, we have found that imidazole, pyrazole, and tetrazole react equally as well to produce novel dipeptide analogues.

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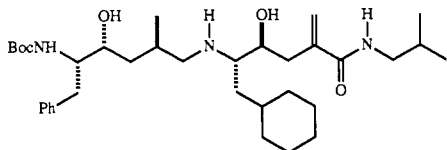


10, R = CH₂CH₂CH=CH₂
11, R = CH(CH₃)₂

the corresponding Phe-Leu analogue 11 (6 nM). Finally, 2*S*,4*R* diastereomer 2j is active at a level (8 nM) much more in line with the peptide-based inhibitor 9.

In an attempt to mimic the Phe-His dipeptide, inhibitors 2k and 2l were prepared. In this case separation of diastereomeric lactones 3 was not feasible, and the assignment of stereochemistry at C₂ in 2k and 2l (chromatographically separated at that stage) is based only on inhibitory potency. We anticipated that the N-alkylated triazole in 2k might provide a suitable isostere for the imidazole ring in histidine since previous results have shown that the free NH in histidine is not important for binding to renin.³ Unfortunately, the potency of 2k (95 nM) is nearly 50 times less than that of the corresponding dipeptide inhibitor 12 (2 nM).⁷ This difference may in part be a further manifestation of the conformational differences between the hydroxyethylene isostere and the native dipeptide since the other branched-chain analogue 2e is also less potent than anticipated.

The availability of lactones 3 presented the possibility of preparing inhibitors in which the remaining amide bond between the P₁ and P₂ sites is also isosterically modified. Thus, reduction of 3 (R = H) with diisobutylaluminum hydride followed by reductive amination of the resulting lactol with amine 5 led in 68% yield to reduced amide 13.



13

The inhibitory potency of 13 fell off dramatically (>10 000 nM), indicating the apparent critical importance of the carbonyl group between P₁ and P₂. Indeed, to our knowledge no inhibitors which lack this amide bond have been reported.

The potential benefit for use of the hydroxyethylene isostere at the P₂/P₃ position is illustrated by the in vitro stability of this series of inhibitors toward the action of chymotrypsin. Incubation of 6 with purified chymotrypsin resulted in rapid cleavage with a half-life of 3.5 min. Compound 2c, however, showed no evidence of degradation after 4 h. Although the relationship of proteolytic stability in vitro to bioavailability in vivo remains to be clearly established, we believe that one of the keys to high intestinal absorption and oral activity lies in reducing the proteolytic lability of the P₂/P₃ bond.

These results demonstrate the feasibility of successfully employing the hydroxyethylene isostere as a general amide-bond replacement to provide stability against proteolytic degradation. In the present case, the potency of renin inhibitors containing this isostere at the P₂/P₃ position ranges from 4-fold less active (in the case of 2e) to 9-fold more active (in the case of 2i) in comparison to that of the corresponding dipeptide inhibitors. The fact that the structure-activity relationships of both series resemble each other indicates that the hydroxyethylene isostere is

able to successfully mimic the conformation as well as the hydrogen-bond-accepting ability of the peptide bond and argues for its utility in providing unique characteristics to a variety of bioactive peptides.

Experimental Section

Solvents and other reagents were of reagent grade or better. Proton magnetic resonance spectra were measured on a Nicolet QE-300 (300 MHz) instrument using tetramethylsilane as an internal standard. NMR spectra, mass spectra, and elemental analyses were performed by the Analytical Research Department, Abbott Laboratories. Flash column chromatography¹³ and medium-pressure column chromatography (MPLC) were performed on silica gel 60, 0.04–0.063 mm (E. Merck). Thin-layer chromatography was performed on precoated silica gel F-254 plates (0.25 mm; E. Merck) and was visualized with phosphomolybdic acid.

Preparation of Lactones 3. With the exception of 3 (R = 1,2,4-triazolyl), all lactones 3 were prepared as previously reported.⁹

Preparation of 3 (R = 1,2,4-triazolyl). A mixture of 173 mg (0.547 mmol) of (5*R*,1'*S*)-5-[1-[(*tert*-butoxy)carbonyl]amino]-2-phenylethyl]-3-methylenedihydrofuran-2(4*H*)-one⁹ and 80 mg (1.16 mmol) of 1,2,4-triazole in 1.5 mL of toluene was heated in a sealed vial at 100 °C for 16 h. After removal of the solvent in vacuo, purification by MPLC using 3:1 ethyl acetate/chloroform gave 3 (R = triazolyl) as a 1.5:1 mixture of diastereomers.

General Procedure for Preparation of Renin Inhibitors. The preparation of peptide-based inhibitors 7–9 was accomplished by the previously described method.⁷ The general method of conversion of lactones 3 to inhibitors 2a–1, 10, and 11 followed the modified procedure of Evans et al.¹¹

Method A. (4*S*,5*S*,8*R*,2'*R*,3'*S*)-*N*-Isobutyl-6-aza-8-[3-[(*tert*-butoxy)carbonyl]amino]-2-hydroxy-4-phenylbutyl]-5-(cyclohexylmethyl)-4-hydroxy-7-oxo-1,12-tridecadiene-2-carboxamide (2i). A solution of 40.6 mg (0.109 mmol) of (3*R*,5*R*,1'*S*)-3 (R = CH₂CH₂CH=CH₂)⁹ in 1 mL of 4:1 dioxane/water was treated with 5 mg (0.1 mmol) of lithium hydroxide monohydrate. After being allowed to stir overnight at ambient temperature, the solution was concentrated in vacuo. The crude residue was taken up in 1 mL of dimethylformamide, and treated with 60 mg (0.4 mmol) of *tert*-butyldimethylsilyl chloride and 45 mg (0.66 mmol) of imidazole. After being allowed to stand at ambient temperature for 3 days, the solution was concentrated in vacuo, diluted with ethyl acetate, washed with two portions of water and one portion of saturated brine, dried over Na₂SO₄, and concentrated to a colorless oil. The residue was taken up in 1 mL of tetrahydrofuran, 1 mL of acetic acid, and 0.3 mL of water and allowed to stand at ambient temperature for 8 h. After concentration in vacuo, the residue was partially purified by flash chromatography using 2% methanol in chloroform. A solution of the resulting acid, 0.1 mmol of the hydrochloride of 5,7 15 mg (0.11 mmol) of 1-hydroxybenzotriazole monohydrate, and 0.022 mL (0.2 mmol) of 4-methylmorpholine in 1 mL of dimethylformamide was cooled under N₂ atmosphere to –23 °C and treated with 19 mg (0.1 mmol) of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride. The resulting solution was stirred at –23 °C for 2 h and slowly allowed to warm to ambient temperature overnight. After removal of the solvent in vacuo, the residue was partitioned between ethyl acetate and water, washed with additional water followed by saturated brine, dried over Na₂SO₄, and concentrated. Purification by MPLC using 2:1 hexane/ethyl acetate gave 40.4 mg (47%) of the corresponding silyl ether of 2i. This compound was dissolved in 1 mL of tetrahydrofuran and treated with 0.16 mL (0.16 mmol) of tetra-*n*-butylammonium fluoride (1 M in tetrahydrofuran). After being stirred for 20 h at ambient temperature, the solution was concentrated in vacuo and purified by MPLC using 2:1 ethyl acetate/hexane to give 30.4 mg (88%, 41% overall from 3) of pure 2i.

Method B. (4*S*,5*S*,8*S*,10*R*,11*S*)- and (4*S*,5*S*,8*R*,10*R*,11*S*)-*N*-Isobutyl-6-aza-11-[(*tert*-butoxy)carbonyl]amino]-5-(cyclohexylmethyl)-4,10-dihydroxy-7-oxo-12-phenyl-8-(1,2,4-triazol-1-ylmethyl)-1-dodecene-2-

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.

(4S,5S,8R,10R,11S)-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 (3R,5R)-3 (R = H)⁹ in 1.5 mL of toluene was cooled under a N₂ 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 NaHCO₃ and saturated brine, dried over MgSO₄, 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; 2j (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; (2R)-8, 123382-16-1; (2S)-8, 123535-78-4; (2R)-9, 123382-17-2; (2S)-9, 123482-74-6; 10, 123382-18-3; 11, 123411-32-5; 12, 110613-45-1; 13, 123382-19-4; (5R,1'S)-5-[1-[[[(*tert*-butyloxy)carbonyl]amino]-2-phenylethyl]-3-methylenedihydrofuran-2-(4H)-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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