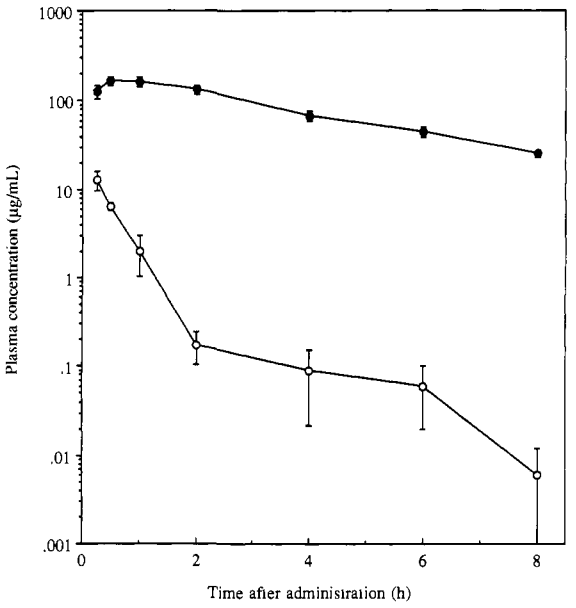

Figure 1. Plasma concentration of compounds **7** (○) and **5a** (●) after oral administration of **7** (50 mg/kg) to male F_{344} rats. Each point represents mean with SEM of three rats.

with those in the rat air-pouch model. In addition, both compounds **5a** and **5b** also inhibited the paw swelling arising from type II collagen-induced arthritis in rats (data not shown). In these models, prostaglandins are usually accepted to be the main mediator. However, compounds **5a** and **5b** exhibited no inhibitory effects on cycloxygenase activity in sheep seminal vesicles even at 100 μM. The antiinflammatory activities in these arthritic models may suggest that both the compounds **5a** and **5b** exert their effects by a novel mechanism of action.

On the basis of its inhibitory effects on IL-1 generation and the relative efficacies in animal models of inflammation, compound **5a** was selected as the most potent orally active inhibitor of IL-1 generation. However, compound **5a** was slightly unstable.[11] This physical instability of **5a** suggested that the development of a stable formulation would be difficult. The best way to achieve a stable formulation would be to protect the 4-hydroxy substituent. Fortunately, we found that the 4-acetyloxy compound **7**(E5090)[12] was quite stable. Compound **7** itself had little or no inhibitory effect on IL-1 generation in vitro (IC_{50} > 30 μM for both human monocytes and rat exudated macrophages), but orally administered **7** was rapidly absorbed and immediately transformed to the deacetylated form **5a** (Figure 1), which was pharmacologically active. The effectiveness of compound **7** was quite equal to compound **5a** in the rat air-pouch model (MED, 25 mg/kg, po)[13] and in the arthritic models of rats.[14]

(11) This compound was isomerized to the *E* form **3a** by irradiation by light (1000 lux, 1 week, ca. *Z*/*E* = 4/1) and mainly converted to the *E* form **3a** in acidic solutions or in organic solvents such as EtOH.

(12) Mp 148–150 °C; anal. ($C_{19}H_{20}O_5$) C, H. This compound was stable to heat (55 °C, 3 months, no change) and irradiation by light (1000 lux, 1 month, *Z*/*E* = 30/1).

(13) This compound dose-dependently inhibited IL-1 generation by oral administration. The inhibition percents were 41% (25 mg/kg), 56% (50 mg/kg), and 95% (200 mg/kg).

(14) Compounds **5a** and **7** were simultaneously evaluated in adjuvant arthritis of rats. These compounds inhibited the swelling of the adjuvant-untreated paw at the 18th day after adjuvant treatment. The inhibition percents of **5a** were 44% (25 mg/kg), 24% (50 mg/kg), and 64% (100 mg/kg), and those of **7** were 36% (25 mg/kg), 48% (50 mg/kg), and 66% (100 mg/kg).

In conclusion, 3-(5-alkyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-2-propenoic acids showed potent inhibitory activity against IL-1 generation. Among the compounds evaluated, (*Z*)-3-(5-ethyl-4-hydroxy-3-methoxy-1-naphthalenyl)-2-methyl-2-propenoic acid (**5a**) was chosen as the most potent orally active inhibitor of IL-1 generation. Compound **5a** inhibited transcription of IL-1α and β mRNA of LPS-treated human monocytes.[15] But the precise details of this mechanism are unknown and are now under investigation. Further details of the medicinal chemistry of this series will be described in forthcoming publications.

Supplementary Material Available: Analytical data for compounds **3a,b**, **5a,b**, and **7** (2 pages). Ordering information is given on any current masthead page.

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Probing the Bradykinin Receptor: Mapping the Geometric Topography Using Ethers of Hydroxyproline in Novel Peptides

The approach of preparing conformationally constrained peptide analogues of a natural peptide ligand in order to obtain insight about its bioactive conformation has become widely accepted in pharmaceutical research. The rationale is particularly appropriate in those cases where neither X-ray crystallographic nor NMR data pertaining to the receptor or ligand–receptor complex are available as is the case for the nonapeptide hormone bradykinin (Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9). Since bradykinin has been implicated in such a variety of pathophysiological processes[1,2] including pain[3] and symptoms of the common cold,[4] a bradykinin-receptor antagonist could have significant therapeutic value. Despite the recent developments toward improved peptide antagonists, there are no potent and selective nonpeptide antagonists of the bradykinin receptor.[5] The challenge of deriving one ad hoc may ultimately rely on precise knowledge about the receptor-binding environment. A portion of this knowledge

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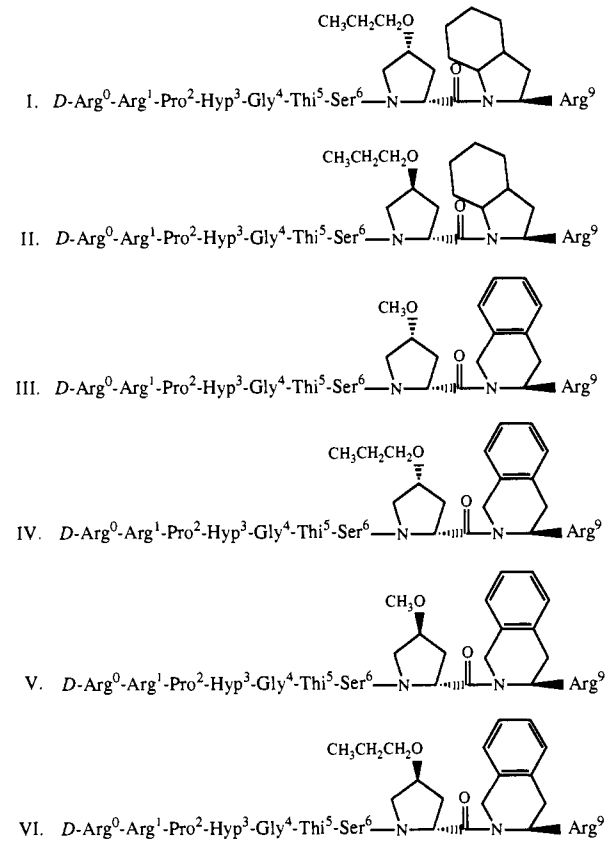

Figure 1. Constrained decapeptides prepared as probes of the bradykinin receptor.

could be obtained from conformationally constrained peptides.

Several bradykinin analogues containing conformational constraints such as *N*-methyl,[6] C^{α}-methyl,[7] and α,β-unsaturated[8] amino acids have been reported over the past several years, but until recently, there have been no antagonists credited with improved binding affinity with respect to the bradykinin antagonist NPC 567 [D-Arg⁰-Arg¹-Pro²-Hyp³-Gly⁴-Phe⁵-Ser⁶-D-Phe⁷-Phe⁸-Arg⁹].[9] Hence, information about the receptor structure deduced from a preferred conformation of a peptide ligand could not be extracted. The most recent examples of conformationally constrained bradykinin antagonist peptides with a dramatic increase in binding affinity have been described by Hock et al.[10] On the basis of conformational analyses using empirical calculations incorporating the CHARMm[11] force field, Kyle et al.[12] has suggested that

Table I. Pharmacological Data Measured for Peptides II–VI and VII (NPC 567)

peptide	K_i (nM) (guinea pig ileum)	pA_2 (guinea pig ileum)	pA_2 (SV-T_2 cells)
II	0.16 ± 0.03	8.54 ± 0.02	9.6 ± 0.3
III	102 ± 19.0	5.05 ± 0.03	6.6 ± 0.4
IV	144 ± 37.0	5.24 ± 0.01	6.8 ± 0.5
V	2.12 ± 0.23	6.73 ± 0.03	8.4 ± 0.2
VI	0.77 ± 0.27	7.61 ± 0.03	8.8 ± 0.2
VII	57.9 ± 12.4	5.82 ± 0.12	8.0 ± 0.1

several of these molecules are likely to adopt a β-turn[13] in the backbone of the four C-terminal residues. This hypothesis is consistent with observations made previously from two-dimensional NMR experiments at 500 MHz in which a β-turn was reported in bradykinin and the bradykinin-receptor antagonist NPC 567.[14] These turns were reported to span the residues Ser⁶-Pro⁷-Phe⁸-Arg⁹ and Ser⁶-D-Phe⁷-Phe⁸-Arg⁹, respectively. The studies were performed in solvents including dioxane/water (90:10) and SDS micelles[15] as mimics of the amphiphilic membrane-embedded receptor environment.

In order to pursue the hypothesis that a β-turn in the four C-terminal amino acid residues of bradykinin analogues might be a prerequisite for high receptor affinity, a simple chemical surrogate was sought that would not only induce the turn in a decapeptide but could also be functionalized with a variety of groups that would serve as "probes" of the allowed steric binding environment. Such a system would be valuable in mapping the geometric and electronic topography about the β-turn accepting portion of the bradykinin receptor.

Initially, five decapeptides were prepared,[16] each having

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(16) All peptides were synthesized by the solid-phase method of Merrifield[22] using standard procedures on a MilliGen Biosearch 9600 peptide synthesizer. Protected (*tert*-butyloxycarbonyl) amino acids were purchased from Bachem Bioscience (Philadelphia, PA) with the exception of Boc-protected methyl and propyl ethers of hydroxyproline. The D-*cis*-4-hydroxyproline ethers were prepared by the method of Smith et al.[23] The D-*trans*-4-hydroxyproline ethers were prepared in the same manner with use of D-*trans*-4-hydroxyproline prepared by the method of Braish and Fox.[24] The amino acid Oic was also prepared by a modification of the literature method.[25] Boc-protected amino acid PAM (phenylacetamidomethyl) resins were purchased from Applied Biosystems (Foster City, CA). Single diisopropylcarbodiimide mediated coupling reactions were run on the automatic synthesizer with the first amino acid routinely recoupled to the resin. Peptides were cleaved from the resin by anhydrous liquid HF (10 mL/g of resin) containing 10% anisole at 0 °C for 1 h. All peptides were purified by RPHPLC on a Vydac C_{18} column using an CH_3CN/H_2O (0.1% TFA) gradient. All peptides were characterized by analytical HPLC, amino acid analysis, and FABMS.

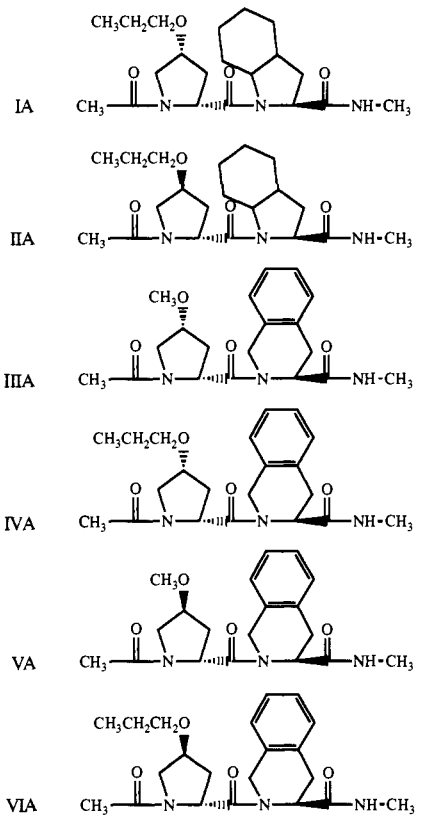

Figure 2. Model dipeptides expected to enforce β-turn conformations when incorporated as replacements for X-Y in the generic peptide D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-X^7-Y^8-Arg^9. IA is *N*-acetyl-(D-4-hydroxyproline *cis*-propyl ether)-(Oic)-*N'*-methylamide, IIA is *N*-acetyl-(D-4-hydroxyproline *trans*-propyl ether)-(Oic)-*N'*-methylamide, IIIA is *N*-acetyl-(D-4-hydroxyproline *cis*-methyl ether)-(Tic)-*N'*-methylamide, IVA is *N*-acetyl-(D-4-hydroxyproline *cis*-propyl ether)-(Tic)-*N'*-methylamide, VA is *N*-acetyl-(D-4-hydroxyproline *trans*-methyl ether)-(Tic)-*N'*-methylamide, VIA is *N*-acetyl-(D-4-hydroxyproline *trans*-propyl ether)-(Tic)-*N'*-methylamide.

the generic primary sequence D-Arg^0-Arg^1-Pro^2-Hyp^3-Gly^4-Thi^5-Ser^6-X^7-Y^8-Arg^9 as shown in Figure 1. The desired β-turn was anticipated when X was an alkyl ether of D-4-hydroxyproline in either the cis or trans geometric state and Y was either a Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid) or Oic (octahydroindole-2-carboxylic acid) residue. The B_2 receptor affinities of these decapeptides and NPC 567 are presented in Table I as determined by K_i and pA_2 values. The latter were determined in both SV-T_2 fibroblasts by bradykinin-stimulated prostaglandin synthesis[17] and in guinea pig ileal longitudinal muscle as described elsewhere.[18] Both the SV-T_2 cells and guinea pig ileum express only B_2 bradykinin receptors.[19] At concentrations up to 10 μM, none of the compounds showed any agonist activity either on tissues or in the cells. The results indicate that, although they are competitive antagonists, compounds III and IV

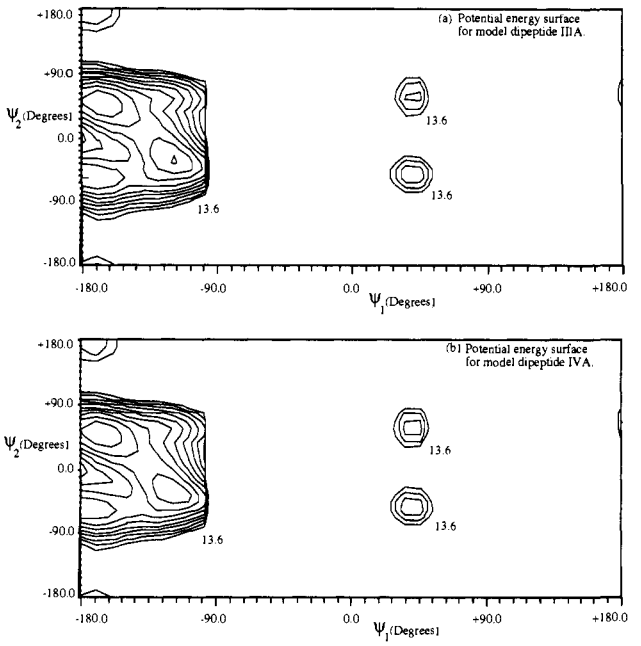

Figure 3. Potential energy contour plots corresponding to the two respective conformational searches done on *cis*-hydroxyproline ether containing model compounds (a) IIIA, (b) IVA. Energy units are kilocalories per mole, and the highest value contour intervals are shown on the plots. The contour interval is 0.5 kcal mol^{-1}.

have only very weak receptor affinities, having K_is in ileal muscle of 102 and 144 nM, respectively. However, II, V, and VI are significantly more potent (respective K_i values of 0.16, 2.12, 0.77 nM) than the reference antagonist NPC 567 (K_i = 57.9 nM) and are also competitive antagonists. It is worth noting that these compounds are the first examples of bradykinin antagonists to be reported that do not contain a D-aromatic amino acid at position 7 of the primary sequence as had previously been considered essential.[20]

To quantify the conformational impact of these dipeptides at the C-terminus of their parent decapeptides, a systematic grid search[21] was performed on model di-

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(18) Tissue strips were prepared as described elsewhere.[26] Cumulative dose–response curves were constructed to bradykinin in the absence and in the presence of increasing concentrations of bradykinin antagonists (0.1–0.3 μM). The EC_{50} of bradykinin was ca. 20 nM.

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(21) All energy calculations were performed using the program CHARMm,[11] version 21 on a Silicon Graphics 4D120GTXB workstation. In each case all amide bonds were assumed to exist in the trans geometry in conformity with the observations made in previous NMR experiments.[14] Since the dihedral angles corresponding to ϕ_1, ϕ_2 in each model peptide are incorporated into either a five- or a six-membered ring thereby limiting their rotational degrees of freedom, the grid search was performed on those angles corresponding to ψ_1 and ψ_2 which dominate the overall backbone conformational states. At each grid point the ψ_1, ψ_2 dihedral angles were constrained to the specific grid value and 500 cycles of conjugate gradients energy minimization were performed. For each tetrahydroisoquinoline carboxylic acid (Tic) residue, both endo- and exo-boat forms of the saturated ring were considered explicitly.

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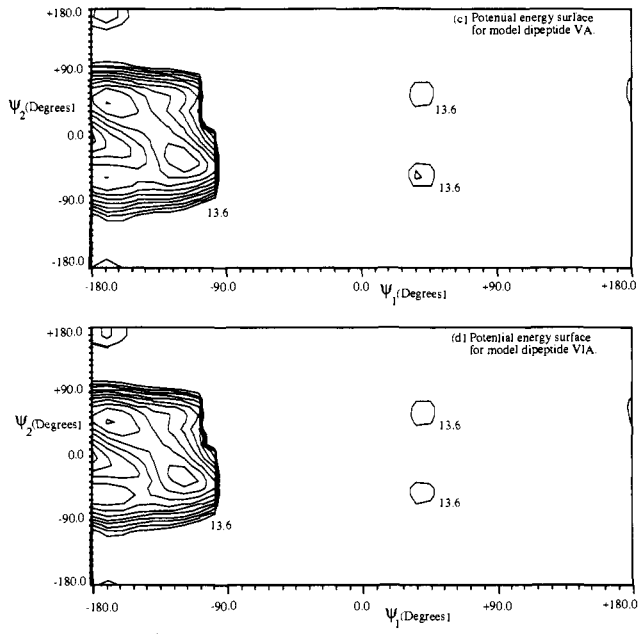

Figure 4. Potential energy contour plots corresponding to the two respective conformational searches done on *trans*-hydroxyproline ether containing model compounds (c) VA, (d) VIA. Energy units are kilocalories per mole, and the highest value contour intervals are shown on the plots. The contour interval is 0.5 kcal mol^{-1}.

peptides corresponding to each. These model peptides are shown in Figure 2. Contour plots corresponding to not more than 5 kcal mol^{-1} above the global minimum were plotted for each model dipeptide with a 0.5 kcal mol^{-1} contour interval. Each conformational search was repeated twice. In one case, the electrostatic contribution in the overall potential energy was neglected by assigning no partial charges to any of the atoms. In the other case, the default charges assigned by the program CHARMm were used such that the overall potential energy included an electrostatic contribution. In each case the two respective contour plots were identical, suggesting that the conformational preferences were being driven by steric interactions, not by poorly represented partial charges assigned to each atom. For those dipeptides containing the Tic residue, the ψ_1, ψ_2 (where ψ_i corresponds to the backbone dihedral angle for residue i defined by the four adjacent amino acid backbone atoms N_i-C^{α}_i-C_i-N_{i+1}) coordinate values corresponding to the local minima were the same regardless of the Tic residue being in the endo- or exo-boat form. Hence, where appropriate, contour plots shown in this report were derived from the endo conformation of this residue, but for each local minimum it was assumed that either an endo- or exo-boat Tic conformation is possible. Shown in Figures 3 and 4 are the four respective contour plots corresponding to conformational searches done on Tic-containing model compounds. Figure 3 depicts the *cis*-hydroxyproline ether containing peptides IIIA and IVA and Figure 4 depicts the *trans*-hydroxyproline ether containing peptides VA and VIA.

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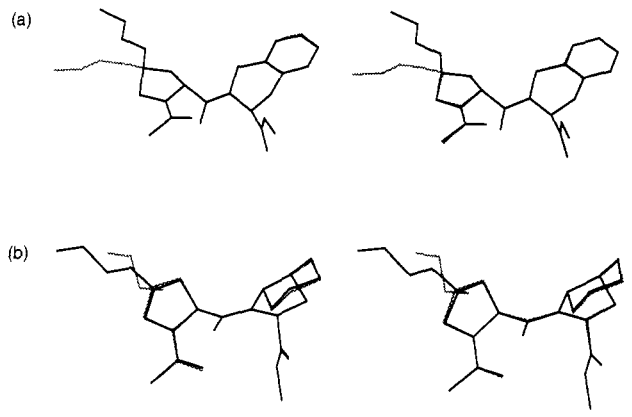

Figure 5. (a) Representative conformations extracted from the potential energy wells corresponding to the preferred β-turn in model peptides IVA (gray) and VIA (black). (b) Representative conformations extracted from the potential energy wells corresponding to the preferred β-turn in model peptides IA (gray) and IIA (black).

Most striking in Figures 3 and 4 is that all plots are identical, regardless of whether the alkyl ether is methyl or propyl or, more importantly, in the cis or trans geometry with respect to the carbonyl group. This suggests that, for an ether of D-4-hydroxyproline adjacent to an L-Tic amino acid, the preferred backbone conformational state is the same, regardless of the ether being cis or trans. More significant is the allowed range for the dihedral angle ψ_1, which lies between −90° and −170° for each Tic-containing model. With the exception of another small local energy minimum centered about $\psi_1 = +36°$, $\psi_2 = \pm 48°$, there are no other allowed states for this dihedral angle. The inherent conformational constraints on the backbone dihedral angles ϕ_1 and ϕ_2 in conjunction with this relatively narrow, preferred range of values for the dihedral angle ψ_1 leads to the conclusion that a β-turn-like structure is highly favored in the backbone of these dipeptide models. Analysis of the binding affinities for the decapeptides containing these amino acid pairs at position 7–8 reveals that the receptor has a zone of steric intolerance adjacent to the backbone at position 7 and cis to the carbonyl group. In contrast, there is a zone of steric tolerance on the opposite side of the backbone at position 7 (trans to the carbonyl group). This conclusion is based on the relative low affinity of cis ethers versus the very high affinity of the trans ethers. Furthermore, the size of the zone of steric tolerance is better approximated by the propyl group rather than the smaller methyl group based on their relative affinities. Currently in our laboratories, a variety of ether "probes" are being tested to more accurately map these two zones. Representative conformations ($\psi_1 = -140°$, $\psi_2 = -60°$) taken from within the region of broad local energy minimum for both the cis propyl ether of hydroxyproline-Tic, and the trans propyl ether of hydroxyproline-Tic dipeptide models are shown in Figure 5a.

Shown in Figure 6 are the contour plots corresponding to conformational searches done on model compounds IA and IIA, wherein the second residue of the dipeptide corresponds to Oic. As was observed for the Tic-containing models, these are highly constrained with similar regions of local minima. However, in these examples, the allowed range for the dihedral angle ψ_1 is between −120° and −170°, slightly compressed from the allowed range in the Tic-containing analogues. Furthermore, in contrast to the broad minimum observed for the Tic-containing models, there are two well-defined local minima, both having $\psi_1 = -140°$. These contour plots indicate that the propyl

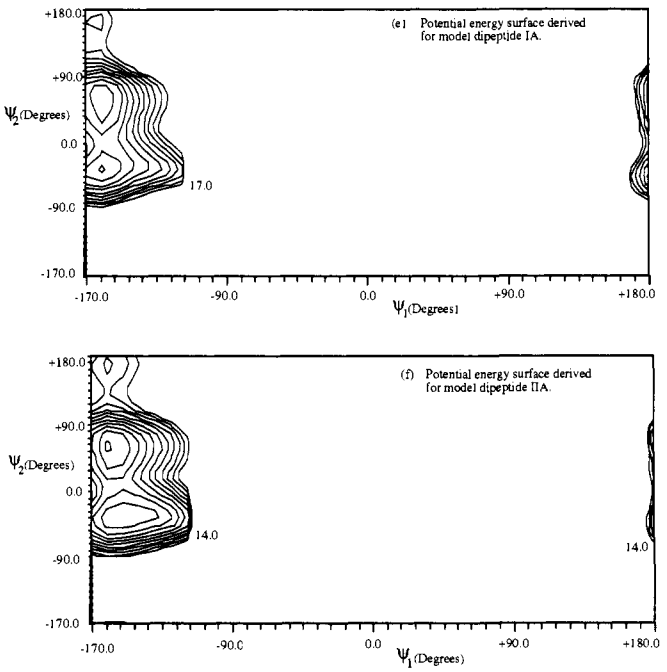

Figure 6. Potential energy contour plots corresponding to the two respective conformational searches done on model compounds IA (e) and IIA (f). Energy units are kilocalories per mole and the highest value contour intervals are shown on the plots. The contour interval is 0.5 kcal mol^{-1}.

ether of the hydroxyproline-Oic dipeptide will adopt, with higher probability than did the Tic-containing analogues, the β-turn proposed to be required for optimal receptor binding to occur. This backbone state is independent of the geometry, cis or trans, of the ether itself since the overall contour plots are identical. Shown in Figure 5b are representative conformations extracted from the potential energy well centered about $\psi_1 = -140°$, $\psi_2 = -40°$.

On the basis of the structure–activity relationship developed from the Tic-containing decapeptides wherein alkyl ethers cis to the carbonyl had poor affinities while trans ethers had high affinities, only a trans propyl ether of hydroxyproline was coupled to an Oic residue in a complete decapeptide. As shown in Table I, this combination of amino acids at the C-terminus, which greatly enhances the probability of forming a β-turn results in a peptide with a K_i measured in guinea pig ileum of 0.16 nM, making it amonst the most potent bradykinin antagonists yet reported. In addition, it has increased potency over the Tic-containing analogue, which is consistent with its increased probability of adopting the β-turn in its backbone.

In summary, several novel decapeptides were designed to energetically favor a β-turn about the four C-terminal amino acid residues, while providing an available functional group useful for attaching different sized alkyl groups as probes of the receptor topography. Two different steric zones at the receptor-binding site, one on either side of the amino acid backbone at position 7, were discovered. The zone cis to the carbonyl at position 7 apparently has only slight steric tolerance since methyl and propyl ether probes of that zone exhibited very weak binding affinity. The zone trans to the carbonyl, however, is proposed to be more readily able to accommodate the steric bulk, of at least a propyl group, since the peptide containing the trans propyl ether was so highly potent. Previously reported peptides containing a D-Tic-Oic pair in positions "X" and "Y" respectively of the primary sequence also represent highly potent B_2 receptor antagonists. These compounds, also proposed to adopt a C-terminal β-turn, indicate that the zone trans to the carbonyl could be large enough to accommodate a phenyl ring and that the D-Tic binds in the exo ring-flipped conformation.

Finally, it is generally accepted that the conformation adopted by a small dipeptide will be different from when incorporated into a larger peptide due to changes in the local chemical environment. On the basis of the calculated conformational preferences dictated by steric factors and preliminary (unpublished) NMR results, the aforementioned generality does not appear to be valid for these highly constrained dipeptides which adopt a limited number of discrete conformations despite the local environment. In this regard we have demonstrated the utility of this novel amino acid pair for forcing the backbone of a peptide into a β-turn, while providing an available hydroxyl group which lends itself to synthetic modification. Ultimate verification of the geometric states proposed in this report resides in either an X-ray crystallographic study or a high-field NMR investigation of the peptides; the latter is underway in our laboratories. However, these preliminary results on such highly potent peptides are of significant value in our ongoing efforts to understand the, as yet unknown, topographical features of the bradykinin receptor.

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