Identification of a Key Region of Kinin B₁ Receptor for High Affinity Binding of Peptide Antagonists

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To investigate the molecular basis for the specificity of ligand recognition in human kinin B₁ (B₁R) and B₂ (B₂R) receptors, we constructed a series of chimeric receptors by progressively replacing, from the N to the C terminus, the human B₂ domains by their B₁ counterparts. The chimeric construct possessing the C-terminal tail and the transmembrane domain VII (TM VII) of the B₂ receptor (construct 6) displayed 7- and 20-fold decreased affinities for the B₁ agonist [³⁵S]desArg¹⁰-kallidin (desArg¹⁰-KD) and the B₂ antagonist [³⁵S]desArg¹⁰-[Leu⁹]-KD respectively, as compared with the wild-type B₂ receptor. Moreover, the substitution of the B₁ TM VII by its B₂ homologue TM increased the affinity for the pseudopeptide antagonists, Hoe140 and NPC 567. High affinity for desArg¹⁰-KD binding was fully regained when the B₂ residue Thr²⁹⁷ was replaced in construct 6 by the corresponding B₁ Leu²⁹⁷ residue. When the B₂ residue Tyr³⁸⁵ was exchanged with the corresponding B₁ Phe³⁹², high affinity binding for both agonist and antagonist was recovered. Moreover, the L294T and F302Y mutant B₂ receptors exhibited 69- and 6.5-fold increases, respectively, in their affinities for the B₂ receptor antagonist, Hoe140. Therefore we proposed that Leu²⁹⁴ and Phe³⁹² residues, which may not be directly involved in the binding of B₂ ligands and, hence, their Thr²⁹⁷ and Tyr³⁸⁵ B₂ counterparts, are localized in a receptor region, which plays a pivotal role in the binding selectivity of the peptide or pseudopeptide kinin ligands.

Bradykinin (BK)¹ and kallidin (Lys-bradykinin (KD)) are biologically active peptides derived from large precursors (kininogens) by the action of serine proteases named kallikreins (1). Kinins are released in response to tissue injury and activate sensory pain fibers, contract smooth muscle, cause endothelium-dependent vasodilatation, and induce plasma extravasation (1, 2). Most of the acute effects of kinins seem to be mediated by the activation of constitutive B₁ receptors (2, 3), whereas some chronic responses to kinins are mediated by B₂ receptors (4). B₁ and B₂ kinin receptors have been cloned (5, 6) and are members of the G-protein-coupled seven-transmembrane receptor superfamily. B₁ and B₂ receptors display 36% sequence identity; they of the G-protein-coupled seven-transmembrane receptor superfamily. B₁ and B₂ receptors display 36% sequence identity; they

Materials—[³⁵S]desArg¹⁰-[Leu⁹]-KD, [³⁵S]desArg¹⁰-KD, [³⁵S]BK, [³⁵S]NPC 17731 (50–120 Ci/mol), and myo-[³⁵S]inositol (10–20 Ci/mol) were obtained from NEN Life Science Products. Bradykinin and its analogs were from Bachem (Basel, Switzerland). Hoe140 was kindly provided by J. Martinez (CNRS, URA 1845, Montpellier, France). DesArg¹⁰-kallidin, desArg¹⁰-[Leu⁹]-kallidin, and desArg¹⁰-Hoe140 were from Neosystem (Strasbourg, France). All molecular biology and cell culture reagents were purchased from Life Technologies, Inc. All other chemicals were supplied by Sigma.

Construction of Chimeric and Mutant Receptors cDNA—The original B₁ and B₂ receptor cDNA were subcloned into the mammalian expression vector pCDNA3.0 (Invitrogen, Leek, The Netherlands). Chimeric receptor constructs were made using a PCR-ligation-PCR protocol. T7 and SP6 were chosen as flanking primers, and internal primers were designed according to the desired fusion point in the chimeric construct and contained a Ksp6312 site. Appropriate B₁ and B₂ fragments were amplified for 10 cycles in a 50-µl PCR reaction containing 10 ng of template DNA, 200 µM dNTPs, 100 pmol of each primer, 1.5 mM MgCl₂, 5 units of Red Goldstar polymerase, and the reaction buffer supplied by the manufacturer (Eurogentec, Angers, France). Another 10 cycles of amplification was performed in the presence of 5-methyldeoxycttosine (5dCTP) to protect already-existing internal Ksp6312 sites from subsequent cleavage by the endonuclease. The isolated PCR products were...
digested by 10 units of Ksp632I, purified using 1% agarose gel, and ligated using T4 DNA ligase (CLONTECH, Palo Alto, CA). In a second PCR, ligation products were amplified for 30 cycles, purified as described above, and subcloned into pCDNA3.0. Mutant constructs were obtained using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The identity of chimeric and mutant constructs was confirmed by cycle sequencing.

**Epitope Tagging—**A 9-amino acid sequence (hemagglutinin (HA) tag) derived from the influenza virus HA protein was inserted at the extra-cellular N terminus of the chimeric constructs and the wild-type B1 receptor. Two reverse complementary oligonucleotides were designed to include the HA-tag sequence followed by the first eight codons encoding the N-terminal part of the B1 receptor and to generate a HindIII restriction site at the 5’ end and an XhoI site at the 3’ end. Both nucleotides were annealed and inserted in the wild-type pcDNA3 vector. The cDNA encoding for the B1 receptor and for all chimeric constructs were then subcloned into the pcDNA3-HA vector.

**Cell Culture and Transfection—**COS-7 cells (ATCC, Bethesda, MD) were grown in Dulbecco’s modified Eagle’s medium containing 4.5 g/liter glucose, 1% Glutamax (v/v), 1% nonessential amino acid (v/v), 1 mM sodium pyruvate, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum. For transient cell expression, COS-7 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. For transient cell expression, COS-7 cells were cultured overnight.

**Western Blot Analysis—**Membrane preparations were fractionated using 10% Tris-glycine precast gel (Novex, Frankfurt, Germany) and were assayed by Western blotting (20). HA-tagged chimeric constructs were detected using a monoclonal anti-HA antibody (Roche Molecular Biochemicals). The secondary antibody horseradish peroxidase-conjugated rabbit anti-rat IgG (Southern Biotechnology Associates, Montgomery, AL) was used in 1:50,000 dilution. Non-tagged mutant receptors were detected using the polyclonal antibody raised against the N-terminal part of the B1 receptor as a primary antibody. The specific secondary antibody (goat anti-rabbit IgG, Caltag, Tebu, Le Perray en Yvelines, France) was conjugated to horseradish peroxidase. The chemiluminescence immunoblot assay was performed with ECL kit (Amersham Pharmacia Biotech).

**Data Analysis—**Binding experiments and concentration-response curves for PI hydrolysis were analyzed using GraphPADInPlot (Graph-PAD Software, San Diego, CA). Statistical analysis were performed using Stastview (Abacus Concept, Palo Alto, CA). A one-way analysis of variance followed by a Student’s t test was used to establish significant differences. A P value less than 0.05 was considered as statistically significant.

**RESULTS**

**Construction and Characterization of Chimeric B1/B2 Receptors in COS-7 Cells—**Fig. 1 schematically represents the eight chimeric B1/B2 receptors corresponding to the progressive replacement of the human B2 receptor TM by their B1 counterparts, starting from the N terminus and ending at the C-terminal tail. The binding properties of B2R ([3H]BK) and B1R ligands ([3H]desArg10-KD, [3H]desArg10-Leu)-KD) to wild-type and chimeric receptors transiently transfected in COS-7 cells are collected in Fig. 2A; their functional properties are represented in Fig. 2B. Binding and functional properties of the human B2 receptor were not affected when the N-terminal part was substituted with the corresponding region of the human B1 receptor (Construct 0; Fig. 2, A and B). All other modifications of the wild-type receptor completely prevented high affinity binding of the B2-selective agonist [3H]BK (Fig. 2A) and the ability of BK (1 μM) to stimulate inositol phospholipid hydrolysis (Fig. 2B).

All chimeric receptors possessing the B2 TM VI (Construct 0 to 5) recognized neither the B1 agonist [3H]desArg10-KD nor the B2 antagonist [3H]desArg10-Leu)-KD (Fig. 2A) and failed to induce inositol phosphate formation upon desArg10-KD treatment (Fig. 2B). We assessed that the observed losses in
ligand recognition were not the result of impaired receptor expression; for this purpose, Western blot analysis of membrane proteins of COS-7 cells transfected with the human B1 receptor and all chimeric constructs, using an antibody raised against the N-terminal part of the human B1 receptor, showed bands with similar intensity with an apparent molecular mass of approximately 40 kDa (data not shown). This molecular weight is close to the calculated one of the human B1 receptor (6). To confirm our results, all chimeric receptors were tagged at their N termini with a nine-amino acid HA epitope after checking that the tagged HA-B1 receptor displayed the same affinity for [3H]desArg10-KD and [3H]desArg10-[Leu9]-KD as the wild-type receptor (data not shown). Receptor immunodetection through Western blotting analysis of plasma membrane extracts from transfected COS-7 cells revealed that all tagged chimeric constructs were adequately expressed and confirmed that there were similar levels of receptor antigen expression (data not shown).

A B1-type binding and functional behavior (Fig. 2, A and B) was restored for chimeric receptors possessing the six first B1 TM5s (Constructs 6 and 7). Saturation binding experiments were carried out on membrane preparation from COS-7 cells transfected with Constructs 6 and 7. \( B_{\text{max}} \) values were within the same range as those obtained for the wild-type B1 receptor, indicating a normal chimeric receptor expression (data not shown).

The B1R bound the agonist \([\text{H}]\text{desArg}^{10-}\text{KD}\) and the antagonist \([\text{H}]\text{desArg}^{10-}[\text{Leu9}]-\text{KD}\) with \( K_D \) values of 0.46 and 0.68 nM, respectively (Table I). As expected, these binding affinities values were not changed when the C-terminal tail of the B2R was substituted with the corresponding region of the B1R (Construct 7). The \( B_{\text{max}} \) values were carrying both the C-terminal tail and the transmembrane domain VII of the B2R bound \([\text{H}]\text{desArg}^{10-}\text{KD}\) and \([\text{H}]\text{desArg}^{10-}[\text{Leu9}]-\text{KD}\) with a 7- and 20-fold lower affinity, respectively, compared with the wild type B1R (Table II). The EC50 values relative to desArg10-KD-induced inositol phosphate accumulation were 37- and 14-fold higher for Constructs 6 and 7, respectively, than for the wild-type B1R (Table IV). In addition, the maximal response to
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TABLE II

| Ligands       | B1R Kᵢ | B₂R Kᵢ | Construct 7 Kᵢ | Construct 6 Kᵢ | B₂R Kᵢ |
|---------------|---------|---------|----------------|----------------|---------|
| Agonists      | nM  | nM  | nM  | nM  | nM  | nM  | nM  | nM  | nM  | nM  |
| BK            | NSB   | 6,280 ± 1,620 | NSB   | >10,000 | NSB   | 9,750 ± 192 | 1.18 ± 0.11 | NSB   | >10,000 | >10,000 |
| desArg¹⁰-KD  | 0.46 ± 0.09 | 0.64 ± 0.06 | 2.95 ± 0.43 | 5,810 ± 1,680 | NSB   | >10,000 | >10,000 |
| desArg¹⁰-BK  | 2,600 ± 603 | 4,620 ± 460 |                       |                       | NSB   | >10,000 | >10,000 |
| Antagonists   |       |         |               |                |       |       |       |       |       |
| desArg¹⁰-[Leu⁹]-KD | 0.68 ± 0.08 | 0.47 ± 0.08 | 13.78 ± 3.31 | NSB   | >10,000 | >10,000 | >10,000 | >10,000 | >10,000 |
| desArg²-[Leu⁹]-BK | 794 ± 28 | 474 ± 66 | >10,000 | 849 ± 281 | 0.12 ± 0.01 |
| NPC 567       | 145 ± 33 | 324 ± 46 |                       |                       |       |       |       |       |       |
| NPC 17721     | NSB   | NSB    |                       |                       |       |       |       |       |       |
| desArg¹⁰-Hoe140 | 21.9 ± 2.7 | 28.7 ± 11.6 | 10.6 ± 2.0 | 139 ± 0.61 | 14 ± 8.2 |
| Hoe140        | 1,810 ± 930 | 2,980 ± 1,090 | 138 ± 50     | 0.16 ± 0.07 |
| FR 173657     | >10,000 | >10,000 |                       |                       |       |       |       |       |       |

The Kᵢ values were determined in saturation binding experiments and are presented as the mean ± S.E. of at least four experiments in duplicate.

The Kᵢ values were determined in competition binding experiments using Kᵢ concentrations of either [²H]BK (B₁R) or [²H]desArg¹⁰-KD (B₁R, Construct 7 and Construct 6) and are presented as the mean ± S.E. of at least four experiments in duplicate.

*Significantly different than wild-type B₁ receptor, with p < 0.05.

desArg¹⁰-KD was significantly reduced by 70% for Construct 6 compared with Construct 7 and B₁R (Fig. 3).

Therefore, the ability of Constructs 6 and 7 to bind B₁ and B₂-specific kinin analogues (structures depicted in Table I) was determined through competition binding assays using [²H]desArg¹⁰-KD as a radioligand.

The Kᵢ values were not changed when the C-terminal tail of the B₁R was substituted with the corresponding region of the B₂R (Table I). The affinities of Construct 6 for the B₁R agonist, desArg¹⁰-KD, and of the B₁R antagonists desArg²-[Leu⁹]-BK and desArg¹⁰-[Leu⁹]-KD were 6-, 50-, and 20-fold lower, respectively, than those for B₁R and Construct 7. Interestingly, chimeric B₁ receptors possessing the B₂ TM VII significantly increased the affinity of the pseudopeptide antagonists, Hoe140 and desArg¹⁰-Hoe140, by 13- and 2-fold, respectively, without affecting the binding of BK and desArg²-BK (Table II).

Role of Specific TM VII Amino Acids in the Selectivity of Kinin Ligand Recognition—To identify specific residues involved in discrimination of kinin peptides, four nonconserved amino acid residues (Thr²⁸⁷, Ser²⁹², Met²⁹³, and Tyr²⁹⁵) located in the upper portion of the TMVII of Construct 6 were mutated to their homologous B₂ amino acids (Leu²⁹⁴, Asn²⁹⁸, Phe³⁰⁰, and Phe³⁰⁵) as shown in Fig. 4. Scatchard analysis of saturation binding experiments revealed that Construct 6 [T²⁸⁷L], Construct 6 [S²⁹¹N], Construct 6 [M²⁹³F], and Construct 6 [Y²⁹⁵F] exhibited a specific saturable binding for [²H]desArg¹⁰-KD and [²H]desArg¹⁰-[Leu⁹]-KD (Table III). Bₘ values were within the same range as for the wild-type B₁ receptor (data not shown). When Thr²⁸⁷ was replaced by the corresponding Leu²⁹⁴ residue (Construct 6 [T²⁸⁷L]), high affinity for desArg¹⁰-KD binding was fully restored (Kᵢ = 0.61 ± 0.16 nM) compared with the wild-type B₁ receptor (Table III). When Tyr²⁹⁵ was exchanged with the corresponding Phe³⁰⁵ (Construct 6 [Y²⁹⁵F]), high affinity binding for both [²H]desArg¹⁰-KD and [²H]desArg¹⁰-[Leu⁹]-KD was fully recovered (Kᵢ = 0.66 ± 0.29 nM and 1.25 ± 0.52 nM, respectively) (Table III). The four mutants retained functional activity as shown by the capacity of desArg¹⁰-KD to induce phosphoinositide hydrolysis (Table IV). Consistent with binding data, the desArg¹⁰-KD potency was partially recovered in cells expressing Construct 6 [T²⁸⁷L] and Construct 6 [Y²⁹⁵F], whereas EC₅₀ values relative to desArg¹⁰-KD activation of other Constructs remained 6- to 250-fold higher than those obtained with the wild type B₁R.

The maximal response to desArg¹⁰-KD was significantly reduced for all mutant receptors (Table IV).

To confirm the involvement of Leu²⁸⁴ and Phe³⁰⁵ in the high affinity binding of desArg¹⁰-KD and desArg¹⁰-[Leu⁹]-KD, mutations of Leu²⁸⁴ to Thr and Phe³⁰⁵ to Tyr were introduced in the TM VII of the wild-type B₁ receptor (Fig. 5). The binding properties of the mutated receptors were compared with those of the wild-type B₁ and B₂ receptors. The B₁R [L²⁹⁴T]R displayed a pharmacological profile similar to the wild-type B₁ receptor (Table III and IV) with respect to ligand binding and activation of inositol phosphate production. In contrast, replacement of the Phe³⁰⁵ to the corresponding Tyr²⁹⁵ of the B₂ receptor induced a significant decrease of the binding affinities for both [²H]desArg¹⁰-KD and [²H]desArg¹⁰-[Leu⁹]-KD (Table III) and a 33-fold decrease in the desArg¹⁰-KD potency to induce inositol phosphate (Table IV). The binding properties of the double mutant B₁R[L²⁹⁴T,F³⁰⁵Y] could not be verified since it was poorly expressed, as assessed by Western blot analysis of plasma membrane extracts (data not shown).

To further characterize B₁R[L²⁹⁴T]R and B₂R[F³⁰⁵Y]R, we analyzed the binding properties of seven B₁ and B₂ agonists and antagonists (Kᵢ values are collected in Table V). BK bound...
Interestingly, the mutation of Leu294 to Thr had a profound effect on the binding affinity of pseudopeptide B2 and B1/B2 receptor antagonists. This single amino acid exchange increased the affinity of Hoe140, desArg10-Hoe140, and NPC 567, respectively, by 70-, 12-, and 32-fold (Table V). Substitution of Phe102 by Tyr also resulted in an increase of binding affinity for the aforementioned antagonists (Fig. 6). In contrast, the non-peptide B2 antagonist, FR 173657, selectively bound to the B2 receptor and had no affinity for the wild-type B1 receptor as well as for the various chimeric or mutated receptors (Table V).

**DISCUSSION**

Human kinin B1 and B2 receptors, which display 36% identity in their amino acid sequence, are recognized by specific endogenous peptide ligands, which differ solely by the presence or absence of an arginine at the C-terminal position. Little is known regarding the domains or individual residues involved in determining subtype-specific ligand binding. However, it has been recently proposed that residues important for the binding of the B1 receptor agonist desArg10-KD may be located in TM VI, since the construction produced by substitution of B2 TM VI into the B1 receptor did not support high affinity binding of desArg10-KD (12). Fathy et al. (13) also report the complete loss of high affinity for desArg10-KD binding following the substitution of the Lys118 by a residue Ser in the TM III of the human B1 receptor, a result leading to the suggestion that Lys118 provides a counter-ion for the C terminus of B1-selective desArg peptides.

To investigate epitopes of the human kinin B1 receptor involved in the selective ligand recognition, we constructed a series of chimeric receptors by progressively replacing transmembrane, N- and C-terminal domains of the B2 receptor by their corresponding B1 counterparts. We first demonstrated that N-terminal parts of the B1R and B2R, although markedly different in amino acid composition, are not essential in determining ligand selectivity, since the pharmacological properties were unchanged upon B2/B1 N terminus exchange. This is in contrast with some other G-protein-coupled receptors such as chemokine receptors in which the N-terminal region markedly influences ligand binding (21, 22).
The major finding of this study concerns the involvement of TM VII in the specificity of ligand recognition by the human B_1 receptor. Not only was the affinity of desArg^{10}-KD to stimulate inositol phosphate production in COS-7 cells expressing Constructs 6 and 7 could at least partly be ascribed to the presence of the cytoplasmic carboxyl tail of the B_2 receptor, which is involved in internalization and signal transduction (26–28). In agreement with binding data, the functional potency of desArg^{10}-KD was partially recovered when Thr^{287} and Tyr^{295} were replaced by the corresponding Leu and Phe in the TMVII of the Construct 6. Moreover, the converse mutation of Phe^{302} to Tyr in the wild-type B_1 receptor resulted in a loss of desArg^{10}-KD-activating properties. Taken together, our results support a role of TM VII and, more specifically, of Leu^{294} and Phe^{302} in inducing a B_1 receptor conformation appropriate for ligand recognition and signal transduction.

We also evidenced that TMVII is a key region for the recognition of pseudopeptide kinin antagonists. Indeed, the replacement of the B_1 receptor TM VII by the corresponding B_2 receptor significantly increased the binding of both pseudopeptide antagonists Hoe140 and desArg^{10}-Hoe140. In addition, the replacement of Leu^{294} or Phe^{302} in the wild-type B_1 receptor by the corresponding Thr or Tyr of the B_2 receptor, respectively, was found to be sufficient to confer high affinity binding of pseudopeptide antagonist: NPC567, representative of the first generation of peptide B_2 receptor antagonists (29) exhibiting a very low selectivity between the receptor subtypes; Hoe140, belonging to the second generation of B_2 receptor antagonists and displaying increased affinity and selectivity for the B_2 receptor (30); desArg^{10}-Hoe140, possessing a drastically decreased affinity for the B_2 receptor and increased affinity for the B_1 receptor (31). Even if their pharmacological profile is somewhat different, these three ligands share the common d-Arg^{2}-[Hyp^{6}] motif and a replacement of l-Pro^{7} by an aromatic structure. It might constitute the basis for some common features, suggested by our results, in the interaction of NPC567, Hoe140, and desArg^{10}-Hoe140 with the B_1 receptor. As the binding of the synthetic non-peptide B_2 receptor antagonist, FR 173657, was not affected by the described point mutations in the B_1 receptor, the recognition of this new class of ligands should involve a different specific mechanism.

The delineation of amino acids located in TM VII and conferring high affinity binding for kinin pseudopeptide receptor antagonists is consistent with previous data showing that mutations in this domain can drastically change the selectivity of several G-protein-coupled receptors: binding of SMS 201–995, a somatostatin peptide analogue, to the human somatostatin receptors SSTR1 and SSTR2 (32); species adenosine A_{1} receptor differences in the binding of various ligands (33); subtype-specific ligand binding to a o_{2}- and b_{2}-adrenergic receptors (34); subtype specificity and major pharmacological differences between human and rodent 5-hydroxytryptamine receptors as-

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**Table V**

| Ligands | B_{1}R | Construct 6 | B_{1}[L294T]R | B_{1}[F302Y]R |
|---------|-------|------------|---------------|---------------|
| Agonists |       |            |               |               |
| BK      | 6,280 ± 1,070 | 9,750 ± 192 | >10,000       | 7,060 ± 502   |
| desArg^{10}-BK | 2,600 ± 603 | 5,810 ± 1,680 | >10,000       | 2,950 ± 454   |
| Antagonists |       |            |               |               |
| NPC 567 | 145 ± 33 | 849 ± 281 | 4.47 ± 0.77^a | 84.3 ± 22.3   |
| desArg^{10}-Hoe140 | 21.9 ± 2.7 | 10.6 ± 2.0^a | 1.74 ± 0.70^a | 0.63 ± 0.21^a |
| Hoe140 | 1,810 ± 930 | 138 ± 50^a | 26.2 ± 6.8^a | 277 ± 58^a    |
| FR 173657 | >10,000 | >10,000 | >10,000       | >10,000       |

^a Significantly different than wild-type B_{1} receptor, with \( p < 0.05 \).
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scribed to a single amino acid of TM VII (35); changes in 5HT1b-adrenergic specificity (36).

Interestingly, the two mutations conferring high affinity binding for kinin pseudopeptide antagonists did not improve BK binding, suggesting a different binding site for kinin agonists and antagonists. Our results are consistent with previous data indicating that the binding pocket for Hoe140 might be deeper in the transmembrane domain and closer to TM VII than the BK binding site (11). In this study, a number of alanine amino acid replacements in the rat B2 receptor were found to affect Hoe140 binding but not BK binding, including substitution of Tyr297, which corresponds to Tyr295 of the human B2 receptor. Another approach using antibodies raised against peptides derived from the putative extracellular domains of the B2 receptor also suggested that the binding sites for peptidic agonists and antagonists onto the B2 receptor did not overlap (9).

By combining chimeric constructions and a mutagenesis approach, we have demonstrated that the TMVII of the human B1 receptor is involved in the efficacy of G-protein coupling and also possesses important determinants for the binding of B1-selective ligands such as desArg10-KD and desArg10-[Leu9]-KD. In the B2 receptor, extensive analysis of the transmembrane and the extracellular domains by alanine-scanning mutagenesis or epitope-specific antibodies binding experiments has identified a few residues important for agonist binding (7, 9, 11). In the B1 receptor, recent studies proposed that TMIII and TMVI bear important residues for the binding of the B1 agonist desArg10-KD (12, 13). However, no information has been published on determinants important for pseudopeptide antagonists binding such Hoe140 in the kinin receptors. We propose that the residues Leu294 and Phe302 in the upper part of the B1 TMVII and their homologous residues Thr287 and Tyr295 in the B2 receptor contribute to the induction of conformations that selectively recognize the various peptidic or pseudopeptidic kinin antagonists.

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