0026-895X/97/020171-06$3.00/0

MOLECULAR PHARMACOLOGY, **51:**171–176 (1997).

ACCELERATED COMMUNICATION

Novel Subtype-Selective Nonpeptide Bradykinin Receptor Antagonists FR167344 and FR173657

ICHIRO ARAMORI, JUNKO ZENKOH, NORIYUKI MORIKAWA, NUALA O'DONNELL, MASAYUKI ASANO, KOZO NAKAMURA, MORITA IWAMI, HITOSHI KOJO, and YOSHITADA NOTSU

Molecular Biological Research Laboratory (I.A., J.Z., N.M., N.O., K.N., M.I., H.K., Y.N.) and Exploratory Research Laboratories (M.A.), Fujisawa Pharmaceutical Co., Ltd., Tsukuba 300–26, Japan

Received October 2, 1996; Accepted October 22, 1996

SUMMARY

We describe the receptor binding and antagonistic properties of two novel nonpeptide antagonists, FR167344 (3-bromo-8-[2,6-dichloro-3-[*N*-[(*E*)-4-(*N*,*N*-dimethylcarbamoyl)cinnamidoacetyl]-*N*-methylamino]benzyloxy]-2-methylimidazo[1,2-a]pyridine hydrochloride) and FR173657 (8-[3-[*N*-[(*E*)-3-(6-acetamidopyridin-3-yl)acryloylglycyl]-*N*-methylamino]-2,6-dichlorobenzyloxy]-2-methylquinoline), for the human bradykinin receptor subtypes (B_1 and B_2). In competitive experiments using membranes prepared from Chinese hamster ovary cells expressing the bradykinin receptor subtypes, FR167344 and FR173657 showed a high affinity binding to the B_2 receptor with IC_{50} values of 65 and 8.9 nM, respectively, and no binding affinity for the B_1 receptor. FR167344 and FR173657 inhibited the B_2 receptor-mediated phosphatidylinositol (PI) hydrolysis and produced a concentration-dependent rightward shift in the dose-response curve to bradykinin. This shift was accompanied by a progressive reduction of maximal response. Estimated pA_2 values for the antagonism of bradykinin-induced PI hydrolysis by FR167344 and FR173657 were 8.0 and 9.0, respectively. FR167344 and FR173657 showed no stimulatory effects on PI hydrolysis. Therefore, FR167344 and FR173657 are potent, highly selective, and insurmountable antagonists for the human bradykinin B_2 receptor.

Kinins are members of a family of peptides that exhibit a variety of biological activities, including vasodilation, increased vascular permeability, contraction of smooth muscle cells, and activation of sensory neurons (1–3). Bradykinin and kallidin (Lys-bradykinin) are released from high- and low-molecular-weight kininogens by the proteolytic action of kallikreins. Removal of the carboxyl terminus of these peptides by a carboxypeptidase generates des-Arg^9-bradykinin and des-Arg^{10}-kallidin, respectively (1, 2). The biological effects elicited by kinins are mediated through the activation of two bradykinin receptor subtypes, B_1 and B_2 (1, 3, 4). The cDNA sequences encoding these receptors have been reported (5–7). The two bradykinin receptors have seven hydrophobic segments and share a significant sequence similarity with other G protein-coupled receptors. B_1 receptor exhibits a rank order of binding affinities as follows: des-Arg^{10}-kallidin > kallidin > des-Arg^9-bradykinin ≫ bradykinin (4, 7); a rank order of potency for the B_2 receptor is bradykinin = kallidin ≫ des-Arg^9-bradykinin (4–6).

The involvement of kinins in the pathology of human diseases, including pain, inflammation, trauma, burns, shock, allergy, and some cardiovascular diseases, has been suggested by studies of animal models and humans (1–3). Bradykinin receptor antagonists thus have therapeutic potential as novel analgesics and anti-inflammatory agents. To assess the disparate roles of the multiple receptor subtypes in each disease and to develop a clinically useful bradykinin antagonist, it is important to study the precise pharmacological properties of the two individual bradykinin receptors. Previously, several peptide and nonpeptide bradykinin receptor antagonists have been reported (4, 8, 9). However, the accurate determination of the potencies of antagonists for individual receptors has been hampered by the existence of multiple receptor subtypes in tissues or cell preparations

ABBREVIATIONS: FR167344, 3-bromo-8-[2,6-dichloro-3-[*N*-[(*E*)-4-(*N*,*N*-dimethylcarbamoyl)cinnamidoacetyl]-*N*-methylamino]benzyloxy]-2-methylimidazo[1,2-*a*]pyridine hydrochloride; FR173657, 8-[3-[*N*-[(*E*)-3-(6-acetamidopyridin-3-yl)acryloylglycyl]-*N*-methylamino]-2,6-dichlorobenzyloxy]-2-methylquinoline; Hoe140, D-Arg-[hydroxyproline^3,β-thienylalanine^4,D-Tic^7,Oic^8]bradykinin; PCR, polymerase chain reaction; PI, phosphatidylinositol; IP_1, inositol monophosphate; IP_2, inositol bisphosphate; IP_3, inositol trisphosphate; CHO, Chinese hamster ovary; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; PBS, phosphate-buffered saline; DR, dose-ratio.

studied. Furthermore, it has become apparent that the ligand-receptor interaction should not be extrapolated across species without independent validation, because equivalent receptors between species can exhibit distinct pharmacological properties (4, 10). The functional expression of a human cDNA clone for each bradykinin receptor subtype in the same cell type can provide a useful system to study the pharmacological profiles of antagonists for a single receptor subtype without any ambiguity resulting from the multiple receptor subtypes and from species differences in these receptors. FR167344 and FR173657 are novel nonpeptide bradykinin receptor antagonists that are effective on bradykinin-induced bronchoconstriction in guinea pigs and hypotensive response in rats (11).[1] In this investigation, we examined the potencies, selectivities and antagonistic properties of FR167344 and FR173657 for the two human bradykinin receptor subtypes in transfected CHO cells.

Experimental Procedures

Materials. Materials were obtained from the following sources: human kidney and aorta cDNA from Clontech (Palo Alto, CA); α-minimal essential medium lacking ribonucleosides and deoxyribonucleosides from Flow Laboratories (Irving, UK); Dulbecco's modified Eagle medium from Nissui (Tokyo, Japan); dialyzed fetal bovine serum from Sigma Chemical (St. Louis, MO); kallidin, [des-Arg^{10}], [3,4-Prolyl-3,4-^{3}H]-([^{3}H]des-Arg^{10}-kallidin) and bradykinin, [2,3-Prolyl-3,4-^{3}H]-([^{3}H]bradykinin)from Dupont-New England Nuclear (Boston, MA); *myo*-[2-^{3}H]inositol (18.8 Ci/mmol) from Amersham (Arlington Heights, IL); bradykinin, kallidin, des-Arg^9-bradykinin and des-Arg^9-[Leu^8]-bradykinin from Peptide Institute (Osaka, Japan); des-Arg^{10}-kallidin from Peninsula Laboratories (Belmont, CA). FR167344, FR173657, and Hoe140 (8) were prepared by Exploratory Research Laboratories of Fujisawa Pharmaceutical (Osaka, Japan). The chemical structures of FR167344 and FR173657 are shown in Fig. 1.

Receptor cDNA. The cDNA clones coding for the human B_1 and B_2 receptors were obtained by PCR performed on human aorta cDNA and kidney cDNA, respectively. To amplify the full-length B_1 and B_2 receptor-coding region, sets of primers were designed according to the published nucleotide sequences (6, 7) (B_1: set 1, 5′-GGACTGGTCTGTGCATGGCATCATCCTGGC-3′; 5′-GCGTCGACGGTTCAATGCTGTTTTAATTCCGCC-3′; B_2: set 1, 5′-CGGAATTCATCAATGTTTCTGTCTGTTCGTGA-3′; 5′-AAGGGCAGCCAGCAGATGATG-3′; set 2, 5′-TTTCCTGATGCTGGTGAGCAT-3′; 5′-CGGGATCCTTACACAAATTCACAGCAGCCCT-3′). PCR amplification was performed by using the GeneAMP DNA amplification reagent kit according to the following schedule: B_1, 30 sec at 94° and 5 min at 65° for 30 cycles; B_2, 1 min at 94°, 2 min at 55°, and 3 min at 72° for 25 cycles followed by one cycle of 10 min at 72°. The resultant PCR products, the 1.1 kb pair B_1 receptor cDNA fragment and the 0.55 and 0.65 kb pair B_2 receptor cDNA fragments, were subcloned individually into pBluescript SK(−) after digestion with a mixture of *Spe*I and *Sal*I (pBS-hB_1) and of *Eco*RI, *Sst*I, and *Bam*HI (pBS-hB_2), respectively. The identities of the obtained clones were confirmed by nucleotide sequence analyses (6, 7).

Transfection and stable expression of the cloned bradykinin receptors. The CHO cell lines expressing the human bradykinin receptors were established according to the procedures described previously (12). B_1 and B_2 receptor cDNA were subcloned individually into a eukaryotic expression vector containing the simian virus 40 early promoter and the mouse dihydrofolate reductase cDNA as a selective marker. The resultant plasmids were transfected into CHO (dhfr$^-$) cells by the calcium phosphate method (13). Cell lines expressing a bradykinin receptor together with dihydrofolate reductase were selected in the α-minimal essential medium lacking ribonucleosides and deoxyribonucleosides, supplemented with 10% dialyzed fetal bovine serum. From selected cell populations, clonal cell lines were isolated by single cell cloning.

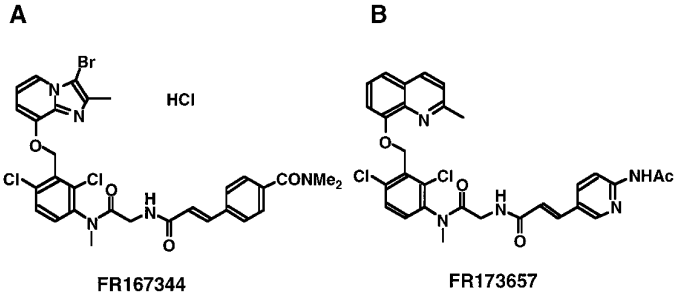

Fig. 1. Structure of FR167344 (A) and FR173657 (B).

Ligand binding of bradykinin receptors. For the determination of the receptor densities and ligand binding selectivities of the bradykinin receptors expressed in clonal cells, the isolation of crude membranes and subsequent ligand binding assays were performed as described previously (14–16). Binding assays for the B_1 and B_2 receptor were carried out by using [^{3}H]des-Arg^{10}-kallidin and [^{3}H]bradykinin, respectively. Cell membranes (12.5–75 μg/ml) were incubated with various concentrations (saturation experiments) or 500 pM (displacement experiments) of [^{3}H]des-Arg^{10}-kallidin or [^{3}H]bradykinin for 90 min in 0.25 ml of the binding solution containing 20 mM HEPES, pH 7.4, 125 mM *N*-methyl-D-glucamine, 5 mM KCl, 0.1% BSA, 1 mM 1,10-phenanthrolin monohydrate, 1 mM dithiothreitol, 1 μM captopril and 140 μg/ml bacitracin (for the B_1 receptor) or 25 mM trimethylaminoethanesulfonic acid, pH 6.8, 0.1% BSA, 1 mM 1,10-phenanthrolin monohydrate, 1 mM dithiothreitol, 1 μM captopril, and 140 μg/ml bacitracin (for the B_2 receptor). All experiments were carried out at least three times in duplicate. The specific binding was calculated by subtracting the nonspecific binding, determined in the presence of 1 μM unlabeled des-Arg^{10}-kallidin (for the B_1 receptor) or bradykinin (for the B_2 receptor), from the total binding. The specific binding activity amounted to 90–92% of the total binding activity.

Measurements of PI hydrolysis. PI hydrolysis was measured essentially as described previously (12). CHO (dhfr$^-$) cells expressing individual bradykinin receptors were seeded in 12-well plates at a density of 1×10^5 cells per well and cultured for 1 day. The cells were labeled with [^{3}H]inositol (1 μCi/ml) for 24 hr. The cells were washed twice with PBS containing 0.2% BSA and incubated with the same solution for 30 min and then with PBS containing 0.2% BSA and 10 mM LiCl for 30 min at 37°. Agonist stimulation was started by replacing the medium with fresh PBS (1 × = 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.4) containing 0.2% BSA, 10 mM LiCl, and test reagents. The reaction was terminated by 5% (w/v) trichloroacetic acid after incubation for 30 min at 37°. Separation of [^{3}H]inositol phosphates was carried out by BioRad (Richmond, CA) AG1×8 chromatography essentially as described (17). IP_1, IP_2, and IP_3 were eluted serially with 5 mM disodium tetraborate and 180 mM sodium formate, 0.1 M formic acid and 0.4 M ammonium formate and 0.1 M formic acid and 1.0 M ammonium formate, respectively. The radioactivity in the eluates was determined by a liquid scintillation spectrometer.

Data analysis. In the radioligand binding experiments, displacement data were fitted to the equation $\%B = 100/\{1 + (x/IC_{50})^{n_H}\}$, where $\%B$ is percentage of a bound radioligand to the total specific binding, x is the concentration of a competing ligand, IC_{50} values represent the concentrations of ligands to inhibit the specific radioligand binding of receptors by 50%, and n_H is the pseudo-Hill coef-

[1] N. Inamura, M. Asano, C. Hatori, H. Sawai, T. Fujiwara, A. Katayama, H. Kayakiri, S. Satoh, Y. Abe, T. Inoue, Y. Sawada, K. Nakahara, T. Oku, and M. Okuhara, unpublished observations.

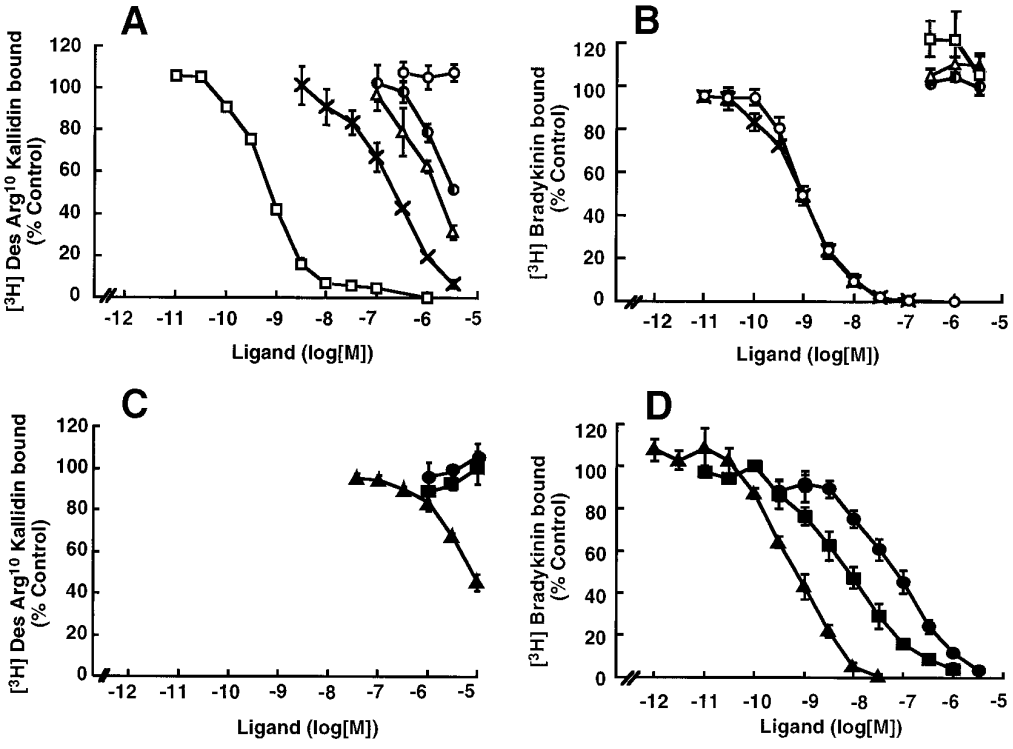

Fig. 2. Displacements of specific radioligand binding to membranes of clonal cells expressing the human bradykinin receptor subtypes by kinin peptides (A, B) and the antagonists (C, D). Experimental details are described in Experimental Procedures. The unlabeled ligands added to the binding assays of the B_1 receptor (A, C) and the B_2 receptor (B, D) are as follows: bradykinin (○), kallidin (×), des-Arg^9-bradykinin (◐) and des-Arg^9-[Leu^8]-bradykinin (△), des-Arg^{10}-kallidin (□), FR167344 (●), FR173657 (■), and Hoe140 (▲).

ficient. In the functional assay, the dose-response curves for bradykinin obtained in the absence and presence of antagonists were fitted to the equation $R = [(R_{max} - 1)/\{1 + (EC_{50}/x)^{n_H}\}] + 1$, where R is the inositol phosphates (fold increase), R_{max} is the maximal response (fold increase), EC_{50} values are the effective concentrations of half-maximal response, x is the concentration of bradykinin, and n_H is the Hill coefficient. pA_2 values were estimated from DRs calculated from the horizontal distances between ascending regions of the dose-response curves of bradykinin obtained in the absence and presence of antagonists (18). DRs were determined from the effective concentrations of bradykinin in the absence and presence of antagonists that cause 10% of the maximal response in the absence of antagonists. The slopes were determined by linear regression by the method of least squares.

Results and Discussion

Stable expression of the bradykinin receptors. The cDNA clones coding for the human B_1 and B_2 bradykinin receptors were obtained by PCR, and their identities were confirmed by nucleotide sequence analyses. There were some nucleotide differences between our B_1 bradykinin receptor cDNA clone and those of GenBank U12512, resulting in amino acid substitution. The amino acid sequence deduced from our B_1 receptor cDNA sequence showed substitutions of R (CGG) at residue 146 and RCGGR (AGGTGCGGGGGCCGC) at residues 239–243, in place of the sequence predicted from GenBank data, G (GGA) and RVRGP (AGAGTGCGGGGGCCG), respectively. The integrity of our nucleotide sequence was confirmed by the comparison with the sequence of B_1 receptor gene cloned from the human genomic DNA.[2] CHO (dhfr$^-$) cells were transfected with a vector-directing expression of the cDNA for each of the human bradykinin receptor subtypes. A mouse dihydrofolate reductase cDNA was used as a selective marker that allowed receptor-expressing cell populations to grow in the medium lacking ribonucleosides and deoxyribonucleosides. More than 20 clonal cell lines that stably expressed bradykinin receptors at various levels were identified for each of the B_1 and B_2 receptor by ligand binding of [^{3}H]des-Arg^{10}-kallidin and [^{3}H]bradykinin, respectively. No binding of these radioligands was observed in untransfected CHO cells (data not shown). Clonal cells expressing maximal levels of the B_1 and B_2 receptors were identified by saturation binding analyses, and the receptor densities of the B_1 receptor-expressing and B_2 receptor-expressing cell lines were estimated to be 0.2 and 1.2 pmol/mg of membrane proteins with a dissociation constant (K_d) of 110 pM and 66 pM, respectively (data not shown). The K_d values obtained for the B_1 and B_2 receptors agreed with those reported previously (6, 7). These two cell lines permanently expressing the B_1 and B_2 receptors were used for subsequent competition binding experiments and analyses of PI hydrolysis.

Receptor-binding studies of the bradykinin antagonists. The transfection and functional expression of cDNA clones for single receptor subtypes in the same cell type are useful for the accurate characterization of ligand-receptor interaction because uncertainties arising from the presence of multiple receptor subtypes and the species differences of the equivalent receptors can be eliminated successfully. We determined the potencies and selectivities of the nonpeptide antagonists, FR167344 and FR173657, in inhibiting specific radioligand binding to membranes prepared from CHO cells expressing each bradykinin receptor subtype. Competition curves of radioligand binding by the kinin peptides FR167344, FR173657, and Hoe140, a peptide antagonist, are presented in Fig. 2. IC_{50} values to inhibit specific radioligand binding to both receptor subtypes are summarized in Table 1. When the receptor binding properties of kinins were exam-

[2] J. Zenkoh, I. Aramori, N. Morikawa, and Y. Notsu, unpublished observations.

TABLE 1

Selectivities and affinities of kinin peptides and antagonists for binding to the human bradykinin receptor subtypes.

IC_{50} values of kinin peptides and antagonists are given as the means ± standard error of the data obtained from three separate experiments.

Ligand	IC_{50} ± standard error (n = 3)	
	B_1	B_2
	nM	
Bradykinin	>3,200	1.1 ± 0.17
Kallidin	210 ± 27	0.89 ± 0.12
Des-Arg^{10}-Kallidin	0.81 ± 0.042	>3,200
Des-Arg^{9}-Bradykinin	3,200 ± 86	>3,200
Des-Arg^{9}-[Leu^{8}]-Bradykinin	1,500 ± 30	>3,200
FR167344	>10,000	65 ± 20
FR173657	>10,000	8.9 ± 3.9
Hoe140	6,700 ± 350	0.74 ± 0.14

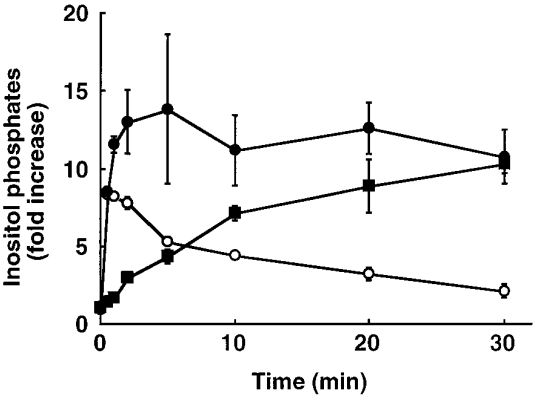

Fig. 3. Time courses of bradykinin-stimulated accumulation of inositol phosphates in clonal cells expressing the human B_2 receptor. Experimental details are described in Experimental Procedures. A cell line expressing the human B_2 receptor was incubated with bradykinin (100 nM) for the times indicated and then determined for IP_1 (■), IP_2 (●), and IP_3 (○) formation. The inositol phosphate formation is expressed as the fold increase in inositol phosphate levels, compared with cells not treated with bradykinin at the corresponding times. The values are means ± standard deviation of triplicate determinations.

ined, the two human bradykinin receptor subtypes expressed in clonal cells showed distinguishable rank orders of binding affinities to these peptides. The rank orders and the IC_{50} values of the peptides were consistent with the reported data (4–7). Bradykinin receptor antagonists were then tested for their potencies in displacing specific bindings of the receptors. FR167344 and FR173657 were potent inhibitors of [^{3}H]bradykinin binding to the human B_2 receptor. This was in marked contrast to their inability to inhibit [^{3}H]des-Arg^{10}-kallidin binding to the human B_1 receptor. The IC_{50} values of FR167344 and FR173657 for the human B_2 receptor were 65 ± 20 nM and 8.9 ± 3.9 nM, respectively. In separate binding experiments using membranes of animal tissues and cultured cells, FR167344 and FR173657 did not inhibit specific radioligand binding to the other G protein-coupled receptors, including adenosine receptors, α_1-adrenergic receptors, muscarinic receptors, endothelin ET_A receptor, and tachykinin NK_1 receptor at the concentration of 1 μM (11).[1] Therefore, FR167344 and FR173657 are selective ligands for the human bradykinin B_2 receptor.

The present study reasonably predicts the pharmacological action of FR167344 and FR173657 in humans. We also have shown that FR167344 and FR173657 competed potently with the specific binding of [^{3}H]bradykinin to guinea pig ileum membranes with IC_{50} values of 0.75 and 0.56 nM, respectively (11),[1] suggesting that the guinea pig receptor assay would be a suitable animal model.

Effects of FR167344 and FR173657 on bradykinin-induced PI hydrolysis in clonal cells expressing the human B_2 receptor. The bradykinin receptors have been shown to mediate the stimulation of PI hydrolysis via a G protein (19). We examined the effects of bradykinin application on IP_1, IP_2, and IP_3 formation in cells expressing the human B_2 receptor. The results of time course of PI hydrolysis are presented in Fig. 3. Exposure to 100 nM bradykinin resulted in a substantial stimulation of inositol formation in clonal cells expressing the human B_2 receptor. IP_2 and IP_3 levels increased rapidly, whereas IP_1 was elevated slowly probably as a result of dephosphorylation of IP_2 and IP_3. No bradykinin-induced response of PI hydrolysis was observed in untransfected CHO cells (data not shown). Next, we determined the dose-response curve of bradykinin for the stimulation of inositol phosphate formation in B_2 receptor-expressing cells (Fig. 4). In this experiment, PI hydrolysis was measured by incubating cells with bradykinin for 30 min and monitoring the maximal formation of a mixture of IP_1, IP_2, and IP_3. The EC_{50} value of bradykinin was 1.1 nM, consistent with the binding affinity of bradykinin for the B_2 receptor (IC_{50} = 1.1 nM). This functional assay of B_2 receptor activation allowed the quantitative measurement of the potencies of antagonists for a single receptor subtype. Then, FR167344, FR173657, and Hoe140 were tested by measuring the ability to inhibit bradykinin-stimulated inositol phosphate formation in clonal cells stably expressing the human B_2 receptor. In this experiment, receptor-expressing cells were preincubated with different concentrations of the antagonist for 30 min. The amount of total inositol phosphates (IP_1 + IP_2 + IP_3) was determined after the activation of B_2 receptor with various concentrations of bradykinin for 30 min in the presence of the antagonist. In the presence of FR167344, FR173657, and Hoe140, the dose-response curves of bradykinin were shifted to the right in a concentration-dependent manner, and the maximal effect of bradykinin was significantly decreased (Fig. 4). To estimate pA_2 values, the DRs were calculated from the horizontal distances between ascending parallel portions of the dose-response curves for bradykinin in the absence and presence of the antagonists. Schild analyses (18) of the antagonism of bradykinin-induced PI hydrolysis by FR167344 and FR173657 yielded estimated pA_2 values of 8.0 and 9.0, respectively. The values were comparable to that of Hoe140 (estimated pA_2 = 8.6). The slopes of the regression lines for the antagonism by FR167344, FR173657, and Hoe140 were 1.2, 1.0, and 1.0, respectively. These results indicate that FR167344 and FR173657 selectively interact with the B_2 receptor and produce insurmountable inhibitory effects on bradykinin-induced PI hydrolysis. FR167344, FR173657, and Hoe140 itself showed no significant stimulatory effects on PI hydrolysis in B_2 receptor-expressing cells at the concentration of 10 μM (data not shown). Therefore, the results demonstrate that FR167344 and FR173657 are potent, highly selective, and insurmountable nonpeptide antagonists of the human bradykinin B_2 receptor.

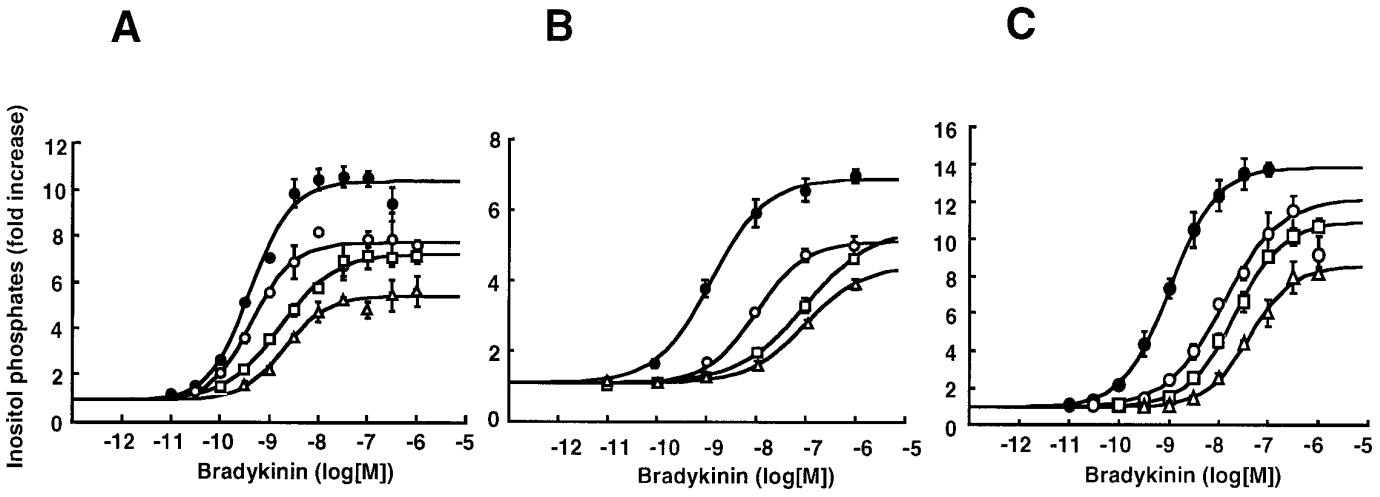

Fig. 4. Effect of the bradykinin antagonists on bradykinin-induced inositol phosphate formation in clonal cells expressing the bradykinin receptor subtypes. Experimental details are described in Experimental Procedures. A cell line expressing the human B_2 receptor was preincubated in the absence (●) and presence of FR167344 (A), FR173657 (B), and Hoe140 (C) at 10 nM (○), 32 nM (□), and 100 nM (△) for 30 min. The cells were incubated with indicated concentrations of bradykinin for 30 min in the absence and presence of the antagonists tested, and then total inositol phosphate formation was measured. For other explanations, see Fig. 3.

The molecular mechanism underlying the interaction of these insurmountable antagonists with the B_2 receptor remains to be elucidated. However, the B_2 receptor antagonists described here differ markedly from the competitive antagonists we have described previously for the endothelin ET_A receptor (20) and for the tachykinin NK_1 receptor (21), which induce a parallel shift in the dose-response curve of agonist-induced PI hydrolysis with no depression of the maximal response in CHO cells expressing ET_A receptor and NK_1 receptor, respectively. In previous studies, Hoe140 has been shown to elicit a rightward shift in the dose-response curve of the bradykinin-induced contraction and a progressive decrease of the maximal response in guinea pig ileum (22) and trachea (23), rabbit jugular vein (24, 25), and rat uterus (26). The noncompetitive behavior of Hoe140 has been suggested to be caused by a long-lasting interaction or, alternatively, an allosteric interaction with the receptor (22, 24). We also have observed that FR167344 exhibits long-lasting inhibition of bradykinin-induced hypotension in rats.[1] The type of antagonism exhibited by FR167344 and FR173657 may be responsible, at least in part, for the tight binding to the human B_2 receptor.

FR167344 and FR173657 are highly selective antagonists for the B_2 receptor and lack agonist activity and, as such, will facilitate the elucidation of the physiological properties of the B_2 receptor. We have shown that FR167344 and FR173657 inhibit the bradykinin-induced contraction of guinea pig ileum with pA_2 values of 9.4 and 9.2, respectively (11).[1] These pA_2 values are comparable with those determined in this paper for the antagonism of PI hydrolysis. We also demonstrated that FR167344 and FR173657 inhibit bradykinin-induced bronchoconstriction in guinea pigs and bradykinin-induced hypotension and carrageenan-induced paw edema in rats (11),[1] the actions of which are all believed to be mediated via B_2 receptor. Therefore, highly potent bradykinin receptor antagonists, such as FR167344 and FR173657, may have a therapeutic potential as novel anti-inflammatory agents. The use of cloned human receptors expressed in mammalian cells described in this study should facilitate the development of new bradykinin antagonists that will be valuable as human therapeutic agents.

Acknowledgments

We thank Professor Marc G. Caron and Dr. Stephen S.G. Ferguson for their critical reading of the manuscript.

References

1. Regoli, D., and J. Barabé. Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.* **32:**1–46 (1980).
2. Proud, D., and A. P. Kaplan. Kinin formation: mechanisms and role in inflammatory disorders. *Annu. Rev. Immunol.* **6:**49–83 (1988).
3. Dray, A., and M. Parkins. Bradykinin and inflammatory pain. *Trends Neurosci.* **16:**99–104 (1993).
4. Regoli, D., N.-E. Rhaleb, S. Dion, and G. Drapeau. New selective bradykinin receptor antagonists and bradykinin B_2 receptor characterization. *Trends Pharmacol. Sci.* **11:**156–161 (1991).
5. McEachern, A. E., E. R. Shelton, S. Bhakta, R. Obernolte, C. Bach, P. Zuppan, J. Fujisaki, R. W. Aldrich, and K. Jarnagin. Expression cloning of a rat B_2 bradykinin receptor. *Proc. Natl. Acad. Sci. USA* **88:**7724–7728 (1991).
6. Hess, J. F., J. A. Borkowski, G. S. Young, C. D. Strader, and R. W. Ransom. Cloning and pharmacological characterization of a human bradykinin (BK-2) receptor. *Biochem. Biophys. Res. Commun.* **184:**260–268 (1992).
7. Menke, J. G., J. A. Borkowski, K. K. Bierilo, T. MacNeil, A. W. Derrick, K. A. Schneck, R. W. Ransom, C. D. Strader, D. L. Linemeyer, and J. F. Hess. Expression cloning of a human B_1 bradykinin receptor. *J. Biol. Chem.* **269:**21583–21586 (1994).
8. Hock, F. J., K. Wirth, U. Albus, W. Linz, H. J. Gerhards, G. Wiemer, S. Henke, G. Breipohl, W. König, J. Knolle, and B. A. Schölkens. Hoe140 a new potent and long acting bradykinin-antagonist: *in vitro* studies. *Br. J. Pharmacol.* **102:**769–773 (1991).
9. Sawutz. D. G., J. M. Salvino, R. E. Dolle, F. Casiano, S. J. Ward, W. T. Houck, D. M. Faunce, B. D. Douty, E. Baizman, M. M. A. Awad, F. Marceau, and P. R. Seoane. The nonpeptide WIN 64338 is a bradykinin B_2 receptor antagonist. *Proc. Natl. Acad. Sci. USA* **91:**4693–4697 (1994).
10. Hall, J. M., M. P. Caulfield, S. P. Watson, and S. Guard. Receptor subtypes or species homologues; relevance to drug discovery. *Trends Pharmacol. Sci.* **14:**376–383 (1993).
11. Asano, M., N. Inamura, C. Hatori, H. Sawai, T. Fujiwara, A. Katayama, H. Kayakiri, S. Satoh, Y. Abe, T. Inoue, Y. Sawada, K. Nakahara, T. Oku, and M. Okuhara. An orally active, nonpeptide bradykinin B_2 receptor antagonist, FR173657. *Br. J. Pharmacol.,* in press.
12. Aramori, I., and S. Nakanishi. Coupling of two endothelin receptor subtypes to differing signal transduction in transfected Chinese hamster ovary cells. *J. Biol. Chem.* **267:**12468–12474 (1992).
13. Graham, F. L., and A. J. van der Eb. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52:**456–467 (1973).
14. Shigemoto, R., Y. Yokota, K. Tsuchida, and S. Nakanishi. Cloning and

expression of a rat neuromedin K receptor cDNA. *J. Biol. Chem.* **265:**623–628 (1990).
15. Schneck, K. A., J. F. Hess, G. Y. Stonesifer, and R. W. Ransom. Bradykinin B_1 receptors in rabbit aorta smooth muscle cells in culture. *Eur. J. Pharmacol.* **266:**277–282 (1994).
16. Manning, D. C., R. Vavrek, J. M. Stewart, and S. H. Snyder. Two bradykinin binding sites with picomolar affinities. *J. Pharmacol. Exp. Ther.* **237:**504–512 (1986).
17. Berridge, M. J., R. M. C. Dawson, C. P. Downes, J. P. Heslop, and R. F. Irvine. Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. *Biochem. J.* **212:** 473–482 (1983).
18. Arunlakshana, O., and H. O. Schild. Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.* **14:**48–58 (1959).
19. Yano, K., H. Higashida, R. Inoue, and Y. Nozawa. Bradykinin-induced rapid breakdown of phosphatydylinositol 4,5-bisphosphate in neuroblastoma × glioma hybrid NG108–15 cells. *J. Biol. Chem.* **259:**10201–10207 (1984).
20. Aramori, I., H. Nirei, M. Shoubo, K. Sogabe, K. Nakamura, H. Kojo, Y. Notsu, T. Ono, and S. Nakanishi. Subtype selectivity of a novel endothelin antagonist, FR139317, for the two endothelin receptors in transfected Chinese hamster ovary cells. *Mol. Pharmacol.* **43:**127–131 (1993).
21. Aramori, I., M. Morikawa, J. Zenkoh, N. O'Donnell, M. Iwami, H. Kojo, Y. Notsu, M. Okuhara, S. Ono, and S. Nakanishi. Subtype- and species-selectivity of a tachykinin antagonist, FK888, for cloned rat and human tachykinin receptors. *Eur. J. Pharmacol.* **269:**277–281 (1994).
22. Griesbacher, T., and F. Lembeck. Analysis of the antagonistic actions of Hoe140 and other novel bradykinin analogues on guinea-pig ileum. *Eur. J. Pharmacol.* **211:**393–398 (1992).
23. Trifilieff, A., A. D. Silva, Y. Landry, and J.-P. Gies. Effect of Hoe 140, a new B_2 noncompetitive antagonist, on guinea pig tracheal bradykinin receptors. *J. Pharmacol. Exp. Ther.* **263:**1377–1382 (1992).
24. Rhaleb, N.-E., N. Rouissi, D. Jukic, D. Regoli, S. Henke, G. Breipohl, and J. Knolle. Pharmacological characterization of a new highly potent B_2 receptor antagonist (Hoe140: D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin). *Eur. J. Pharmacol.* **210:**115–120 (1992).
25. Félétou, M., M. Germain, C. Thurieau, J.-L. Fauchère, and E. Canet. Agonistic and antagonistic properties of the bradykinin B_2 receptor antagonist, Hoe140, in isolated blood vessels from different species. *Br. J. Pharmacol.* **112:**683–689 (1994).
26. Liebmann, C., S. Nawrath, B. Ludwig, and I. Paegelow. Pharmacological and molecular actions of the bradykinin B_2 receptor antagonist, Hoe140, in the rat uterus. *Eur. J. Pharmacol.* **235:**183–188 (1993).

Send reprint requests to: Ichiro Aramori, Ph.D., Molecular Biological Research Lab, Fujisawa Pharmaceutical Co., Ltd., 5–2-3 Tokodai, Tsukuba 300–26, Japan. E-mail: ichiro__aramori@rnd.fujisawa.co.jp