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LF 16.0335, a novel potent and selective nonpeptide antagonist of the human bradykinin B_2 receptor

[1,2]Didier Pruneau, [1]Jean-Michel Luccarini, [1]Chantal Fouchet, [1]Evelyne Defrêne, [1]Rose-Marie Franck, [1]Bruno Loillier, [1]Hervé Duclos, [1]Claude Robert, [1]Béatrice Cremers, [1]Pierre Bélichard & [1]Jean-Luc Paquet

[1]Groupe de Pharmacochimie des Récepteurs, Centre de Recherche, Laboratoires Fournier, 50 Rue de Dijon, 21121-Daix, France

1 In the present paper, we describe the *in vitro* pharmacological properties of LF 16.0335 (1-[[3-[(2,4-dimethylquinolin-8-yl)oxymethyl]-2,4-dichloro-phenyl]sulphonyl] -2(S) - [[4 -[4-(aminoiminomethyl)phenyl-carbonyl]piperazin-1-yl]carbonyl]pyrrolidine), a novel and potent nonpeptide antagonist of the human bradykinin (BK) B_2 receptor.

2 LF 16.0335 displaced [^{3}H]-BK binding to membrane preparations from CHO cells expressing the cloned human B_2 receptor, INT 407 cells and human umbilical vein with K_i values of 0.84 ± 0.39 nM, 1.26 ± 0.68 nM and 2.34 ± 0.36 nM, respectively.

3 In saturation binding studies performed in INT 407 cell membranes in the presence or absence of LF 16.0335, B_{max} values of [^{3}H]-BK were not significantly changed suggesting that LF 16.0335 behaves as a competitive antagonist.

4 LF 16.0335 had no affinity for the cloned human kinin B_1 receptor stably expressed in 293 cells. In addition, this compound at 1 μM did not significantly bind to a range of 40 different membrane receptors and eight ion channels except muscarinic M_2 and M_1 receptors for which an IC_{50} value of 0.9 and 1 μM was obtained.

5 BK stimulates in a concentration-dependent manner phosphoinositosides (IPs) production in cultured INT 407 cells. Concentration-response-curves to BK were shifted to the right in the presence of LF 16.0335 (0.1 μM) without reduction of the maximum. LF 16.0335 inhibited the concentration-contraction curve to BK in the human umbilical vein giving a pA_2 value of 8.30 ± 0.30 with a Schild plot slope that was not different from unity.

6 These results demonstrate that LF 16.0335 is a potent, selective and competitive antagonist of the human bradykinin B_2 receptor.

Keywords: bradykinin; B_2 receptor antagonist; nonpeptide; *in vitro*; binding; human umbilical vein; phosphoinositosides

Introduction

Kallidin and bradykinin are endogenous peptides which are thought to play important roles in pathological situations associated to inflammation and pain (Regoli & Barabé, 1980; Bhoola *et al*., 1992; Hall, 1992). Two subtypes of kinin receptor belonging to the family of G-protein coupled receptors with seven transmembrane-spanning domains have been identified (Regoli *et al*., 1977; Regoli & Barabé, 1980; Hess *et al*., 1992; Menke *et al*., 1994). The B_2 receptor is activated by bradykinin and kallidin and is constitutively expressed whilst the B_1 receptor, which recognises C-terminal des-arginated derivatives of kinins, is induced under stressful and inflammatory conditions (Bouthillier *et al*., 1987; Pruneau *et al*., 1994; Marceau, 1995). Bradykinin-related peptide B_2 receptor antagonists have been particularly useful in demonstrating detrimental roles of bradykinin in the development and the maintenance of diseases such as asthma (Akbary *et al*., 1996), allergic rhinitis (Austin *et al*., 1994; Dear *et al*., 1996) post-traumatic and post-ischemic cerebral oedema (Narotam *et al*., 1995; Relton *et al*., 1997), cystitis (Giuliani *et al*., 1993; Ahluwalia *et al*., 1994), pancreatitis (Griesbacher *et al*., 1993; Yotsumoto *et al*., 1993) and rheumatoid arthritis (Sharma & Wirth, 1996). The first nonpeptide B_2 receptor antagonist, WIN 64338, was a weak antagonist and possessed a number of non-specific properties (Sawutz *et al*., 1994). More recently, FR173657, a new nonpeptide B_2 receptor antagonist with potent *in vitro* and *in vivo* activities, was described (Aramori *et al*., 1997; Asano *et al*., 1997). For example in competition binding assay at the cloned human B_2 receptor expressed in CHO cells, this compound displayed a IC_{50} value of 8.9 nM and had no affinity for the kinin B_1 receptor (Aramori *et al*., 1997).

Although human, rat, rabbit and mouse B_2 receptors share a high degree of homology in term of their amino-acid composition (Maceachern *et al*., 1991; Hess *et al*., 1992; 1994; Bachvarov *et al*., 1995), they differ markedly in their pharmacological profiles. Thus, WIN 64338 is more potent at the guinea-pig than the human B_2 receptor (Salvino *et al*., 1993; Marceau *et al*., 1994; Pruneau *et al*., 1995; Sawutz *et al*., 1994). Similarly, FR173657 was found to be at least ten times more potent at bradykinin B_2 receptors in the pig coronary artery and guinea-pig ileum than at bradykinin B_2 receptors in the human umbilical vein (Rizzi *et al*., 1997). Therefore, it is important to take into account inter-species pharmacological differences when searching for new compounds with the aim of treating human diseases.

LF16.0335,1-[[3-[(2,4-dimethylquinolin- 8 - yl)oxymethyl] -2, 4-dichloro - phenyl]sulphonyl]-2(S)-[[4-[4-(aminoiminomethyl)-phenylcarbonyl]piperazin-1-yl]carbonyl]pyrrolidine (Figure 1), is a new potent nonpeptide B_2 receptor antagonist. In the present study, we report its competition binding properties to the cloned and native human B_2 receptor from INT 407 cells

[2] Author for correspondence.

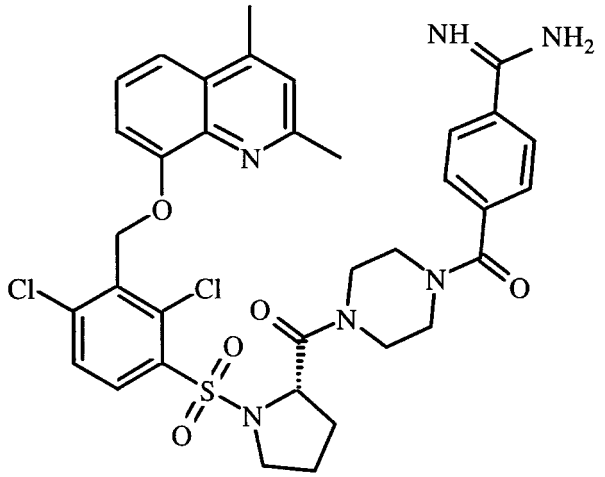

Figure 1 Structure of LF 16.0335 (1-[[3-[(2,4-dimethylquinolin-8-yl)oxymethyl]-2,4-dichloro-phenyl]sulphonyl]-2(S)-[[4-[4-(aminoiminomethyl)phenylcarbonyl]piperazin-1-yl]carbonyl]pyrrolidine).

and human umbilical vein membrane preparations. In addition, we show that LF 16.0335 inhibits the phosphoinositosides production in response to B_2 receptor stimulation and is a potent competitive antagonist of bradykinin-induced contractions of the isolated human umbilical vein.

Methods

Cloning and expression of the human B_2 and B_1 receptor

Cloning and stable expression of the human B_2 receptor was performed as follows. Briefly, the coding region of the human receptor was amplified by Polymerase Chain Reaction (PCR) using the human genomic DNA from HepG2 cells as a template and adapted 36-mers oligonucleotides. The PCR product was subcloned into the *ECo*RI and *Xba*I sites of the vector pBlueScript SK^- (Stratagene, Ozyme, Montigny le Bretoneux, France). The DNA sequence analysis of the subcloned PCR product confirmed that it was identical to the one published by Hess *et al*., (1992). The recombinant plasmid was digested with *Eco*RI and *Xba*I and the insert was subcloned into the eukaryotic expression vector pcDNA3 (Invitrogen, Leek, Netherlands). CHO cells were maintained in HAM F12 containing 10% foetal calf serum, 4.5 g/l glucose, 100 mg/l streptomycin and 10^5 units/l penicillin. Cells were transfected with the cDNA containing vector (10 μg/plate of 150 mm in diameter) using the calcium phosphate precipitation method (Chen & Okayama, 1987). Transfected cells were allowed to recover 3 days and were then subjected to selection pressure with 500 μg/ml geneticin (Gibco, Cergy-Pontoise, France). Resistant cells were propagated and individual cell clones were isolated by limiting dilution plating. Cell clones were screened for receptor expression and then propagated.

Cloning and stable expression of the human kinin B_1 receptor was performed in 293 cells as previously described in details (Bastian *et al*., 1997).

Membrane preparation

Cells were scrapped in binding buffer solution of the following composition: 25 mM TES (pH 6.8), 1 mM 1,10-phenantroline, 140 μg/ml bacitracine and 0.1% bovine serum albumin. As recently described (Gessi *et al*., 1997), human umbilical veins that were collected post-delivery and kept frozen at -20°C were thinly chopped off in 15 ml binding buffer solution (composition as above) and homogenised for 30 s using a Polytron (setting 10). Homogenates were centrifugated at 100 g for 10 min at 4°C in order to discard fat and connective tissues and debris. Supernatants or cells were then homogenized for 10 s using a Polytron (setting 6). Membranes were then pelleted at $40{,}000 \times g$ for 20 min at 4°C and resuspended in ice cold binding buffer. Protein concentration was determined according to the method of Bradford (1976) using a Bio-Rad protein assay kit.

Binding assays

Saturation isotherms were obtained with [^{3}H]-BK (0.1 to 5 nM) in a total volume of 0.5 ml for 90 min at room temperature. Nonspecific binding was evaluated by adding BK at 10 μM. Reactions were terminated by filtration using a Brandel Tissue Harvester onto GF/B filters that had been previously soaked for 2 h in 0.1% (w/v) polyethyleneimine. Filters were washed with ice-cold 50 mM Tris (pH 7.4) and counted in a Beckman liquid scintillation counter. Competition experiments were carried out by incubating membranes with 11 concentrations of LF 16.0335 and 200 – 800 pM of [^{3}H]-BK in a final volume of 0.5 ml for 90 min at room temperature.

For kinin B_1 receptor competition binding experiments, 0.5 – 1 nM [^{3}H]-desArg^{10}-[Leu^9]-kallidin was used as ligand in the presence and absence of various concentrations of LF 16.0335.

Selectivity studies

Competition binding assays of LF 16.0335 at 1 μM were performed in human and non human cell membrane preparations for adenosine A_1 and A_2, α_1- and α_2-adrenoceptors, β_1-, β_2- and β_3-adrenoceptors, angiotensin AT_1 and AT_2, cholecystokinin CCK_1 and CCK_2, dopamine D_1 D_2 and D_3, endothelin ET_A and ET_B, $GABA_A$ and $GABA_B$, glutamate AMPA, histamine H_1, H_2 and H_3, leukotriene BLT and *CysLT*$_1$, acetylcholine muscarinic M_1 and M_2 and nicotinic ([^{3}H]nicotine as a non selective radioligand), tachykinin NK_1, NK_2 and NK_3, neuropeptide Y ([^{3}H]neuropeptide Y as a non selective radioligand), neurotensin NST_1, opioid (μ, δ and κ; [^{3}H]naloxone as a non selective radioligand), 5-hydroxytryptamine 5-HT_{1A} 5-HT_{1D} 5-HT_{2A} 5-HT_3 and 5-HT_4, vasopressin V_1 and V_2 receptors and for L-type Ca^{2+} channels, N-type Ca^{2+} channels, ATP-sensitive K channels, voltage-dependent K channels, Ca^{2+}-dependent K channels, Na channels sites one and two and Cl channels using appropriate radiolabelled ligands and standard methods. Since LF 16.0335 was found to inhibit at 1 μM the binding of specific radioligand to acetylcholine muscarinic human cloned M_1 and M_2 receptors, its affinity for these receptors was determined from competition binding assays using [^{3}H]pirenzepine and [^{3}H]AF-DX 384, respectively.

LF 16.0335 was also tested at 1 μM against angiotensin-converting enzyme and cyclooxygenase 2.

Measurement of inositol phosphates (IPs)

CHO cells grown in 12 well plates were labelled for 18 h with 1 μCi/ml [^{3}H] *myo*-inositol in serum free medium 199 (Gibco, Cergy-Pontoise, France). Cells were washed with phosphate buffer solution and then incubated in 500 μl of IPs assay buffer of the following composition (in mM): NaCl 116, KCl 4.7,

$MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 5, glucose 11, HEPES 20, captopril 0.01, LiCl 10 and 140 μg/ml bacitracine for 15 min at 37°C. Cells were incubated with the antagonist 20 min before addition of the agonist and the incubation was continued for an additional 15 min period. The reaction medium was then removed and the reactions were stopped by adding 500 μl of an ice-cold solution of 5% perchloric acid containing 50 μg/ml phytic acid. After 15 min on ice, the mixture was neutralized with a 2 M K_2CO_3 solution. Different IPs components were then separated by anion exchange chromatography according to the method as described by Berridge *et al*., (1982).

Isolated organs experiments

With the approval of the Ethical Committee of Clinique de Chenôve (Chenôve, France), human umbilical cords were collected post delivery and immediately placed in a Krebs solution of the following composition (in mM): NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $NaHCO_3$ 25, $CaCl_2$ 2.5, ethylenediaminetetracetic acid (EDTA) 0.026, glucose 5.5, bubbled with 95% O_2 plus 5% CO_2 and maintained at 4°C. Thirty minutes later, the umbilical vein was dissected out and cleared of surrounding connective and fat tissues whilst maintained in Krebs solution. Rings (3–4 mm in length) were prepared and the endothelium was rubbed off by gently moving a catheter (0.7 mm in outside diameter, Biotrol-Merck, Paris, France) back and forth several times. Vein rings were set up in 8 ml jacketed organ baths containing Krebs solution and maintained at 37°C. Rings were left unstretched for 2 h and were then stretched in a stepwise fashion by 250 mg tension increments up to 1 g. After a 1 h resting period, Krebs solution of the organ bath was replaced by a high potassium containing Krebs solution (KPSS) in which NaCl was replaced by KCl in order to assess the contractile capacity of the tissue. After washing twice with normal Krebs and return to the baseline, the following compounds were added into the organ bath: mepyramine (1 μM), atropine (1 μM), indomethacin (3 μM), N^G-nitro-L-arginine (L-NOARG, 30 μM), captopril (10 μM), thiorphan (1 μM), DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid (MERGETPA, 5 μM) and nifedipine (0.1 μM). Mepyramine and atropine were used to block histaminergic and cholinergic receptors. Indomethacin and L-NOARG inhibited prostanoids formation and NO-synthase pathways, respectively. MERGETPA, captopril and thiorphan were used to prevent the degradation of BK by carboxypeptidases, angiotensin converting enzyme and neutral endopeptidase (EC 3.4.24.11), respectively. Nifedipine blocked the occurrence of spontaneous contractions without affecting the tonic response to BK. Thirty minutes later responses to cumulative BK were obtained in the presence or the absence of LF 16.0335 added 15 min before BK. At the end of the experiments, after washing and return to the baseline level the maximal contraction of each vein segment was obtained by adding the thromboxane A_2 mimetic, U46619 (1 μM).

In a separate series of experiments, we assessed the reversibility of BK-induced contractions following application of LF 16.0335 and washings. After, concentration-response curves to BK were obtained in the absence or presence of LF 16.0335 (0.1 μM) incubated for 15 min, HUV preparations were then washed five times with fresh Krebs solution. After 30 min, the washing step was repeated and 75 min later, the response-curve to BK was obtained. The same washing procedure was repeated so that the third response-curve to BK was performed 3 h after the introduction of LF 16.0335 or its vehicle in the organ bath.

Analysis of data

Binding competition data and concentration-response curves for IPs hydrolysis and BK-induced contractions were analysed using GraphPADInPlot (GraphPAD Software, San Diego, CA, U.S.A.). The maximal binding of [^{3}H]-BK at equilibrium (B_{max}) and the equilibrium dissociation constant (K_D) were derived from saturation curves fitted with one site ligand binding model. Data from competition experiments were analysed according to the equation:

$$B_c/B = [C]^{nH}/([C]^{nH} + [IC_{50}]^{nH})$$

where B and B_c are the binding observed in the absence and presence of the competing ligand C, IC_{50} is the concentration of C reducing specific binding by 50% and n_H is the pseudo Hill coefficient. Values of pK_i ($-\log K_i$) were obtained from the Cheng-Prusoff equation (Cheng & Prusoff, 1973):

$$K_i = [IC_{50}]/1 + [L]/K_D$$

where K_i is the apparent dissociation constant, L and K_D are the concentration and equilibrium dissociation constant of the radioligand, respectively and IC_{50} is as previously defined.

In functional assays, EC_{50} calculation was performed using a linear regression within the two half-log concentrations surrounding the 50% value. pK_B value ($-\log K_B$) was obtained according to the equation:

$$K_B = [A]/(\text{concentration/ratio} - 1)$$

where [A] is the concentration of the antagonist and concentration ratio is the EC_{50} in the presence of the antagonist divided by the EC_{50} in the absence of antagonist. Schild analysis was used to calculate pA_2 values when Schild plot slopes did not differ from unity and when maximum responses to BK were not significantly affected whatever the concentration of antagonist.

Statistical analysis were performed using Statview (Abacus Concept, Palo Alto, CA, U.S.A.). A one-way analysis of variance followed by a Student's *t*-test was used to establish significant differences between K_i values. A P value less than 0.05 was considered as statistically significant.

Drugs

[^{3}H] Bradykinin (90 to 120 Ci mmol^{-1}) and [^{3}H]-desArg^{10}-[Leu^9]-kallidin (74 to 77 Ci mmol^{-1}) were from New England Nuclear (Les Ullis, France). MERGETPA (DL-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid) was obtained from Calbiochem (La Jolla, CA, U.S.A.). All molecular biology and cell culture reagents were purchased from Life Technologies (Cergy-Pontoise, France). Other chemicals were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). LF 16.0335 was synthesised at Laboratoires Fournier (Daix, France). This compound was used as a dichlorhydrate salt and dissolved in water.

Results

Binding experiments

Saturation experiments showed that [^{3}H]-bradykinin bound to membrane preparations of CHO cells expressing the human B_2 receptor, INT407 cells and human umbilical vein. In each case, the specific binding of [^{3}H]-bradykinin was saturable and Scatchard analyses gave linear plots consistent with the presence of a single class of high-affinity binding sites (Figure

2). Values of K_D and B_{max} were: 0.45 ± 0.12 nM and 4135 ± 654 fmol/mg protein for the cloned receptor ($n=6$), 0.24 ± 0.04 nM and 435 ± 65 fmol/mg for INT407 cells ($n=8$) and 0.46 ± 0.13 nM and 94 ± 29 fmol/mg for human umbilical vein preparations ($n=6$), respectively.

In competition binding experiments, LF 16.0335 inhibited in a concentration-dependent manner the binding of [^{3}H]-BK to the human cloned B_2 receptor giving a K_i value of 0.84 ± 0.39 nM (Figure 3). LF 16.0335 also bound to the native B_2 receptor in membrane preparations from INT407 cells and human umbilical veins giving K_i values of 1.26 ± 0.68 nM and 2.34 ± 0.36 nM, respectively (Figure 3). In each case the Hill coefficient (n_H) of LF 16.0335 was not different from unity, which suggested single-site competitive inhibition. Saturation binding experiments for [^{3}H]-BK in the presence and absence of LF 16.0335 (0.1, 0.3, 1 nM) were performed in INT407 membrane preparations to confirm the competitive nature of the binding inhibition. Figure 4 shows that increasing concentrations of LF 16.0335 produced an increase in equilibrium dissociation constant (K_D) without significant change in receptor density (B_{max}), which is consistent with competitive antagonism. In the presence of 0.1, 0.3 and 1 nM LF 16.0335, the mean K_D value was 0.73 ± 0.11 nM, 1.46 ± 0.41 nM, 1.55 ± 0.56 nM, respectively, and in the absence of LF 16.0335, it was 0.46 ± 0.10 nM.

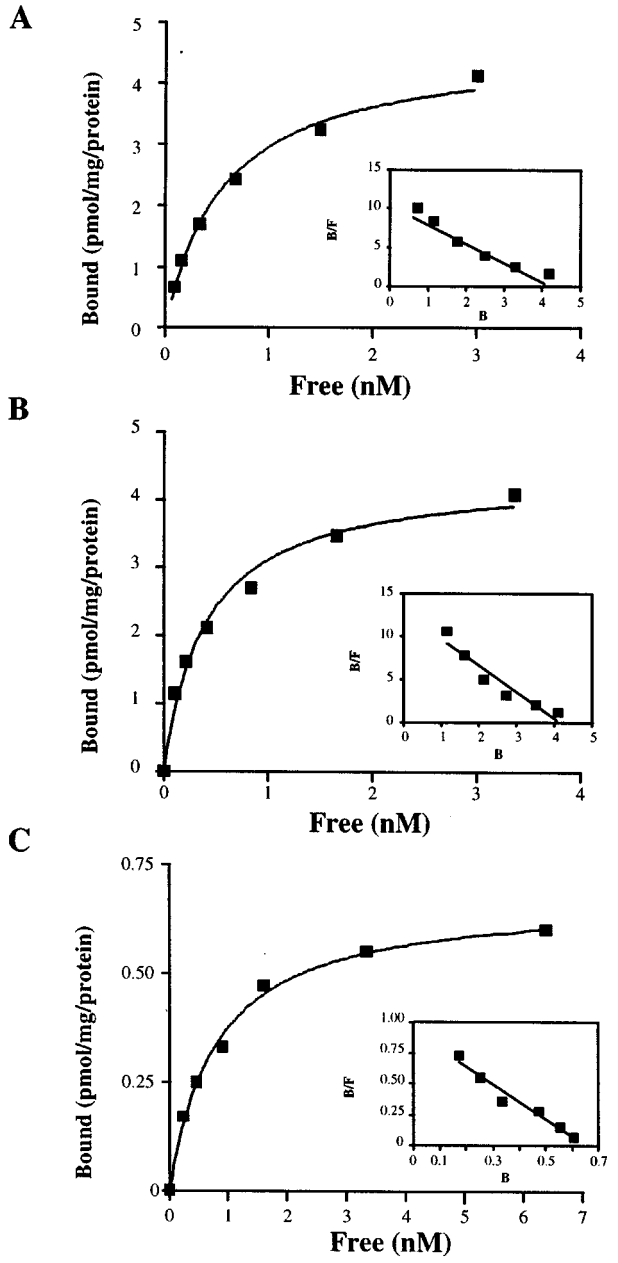

Figure 2 Binding of [^{3}H]-BK to membrane preparations from CHO cells expressing the human bradykinin B_2 receptor (A), INT 407 cells (B) and human umbilical vein (C). Scatchard plots of corresponding saturation binding data are inserted. Values represent one typical experiment performed in duplicate.

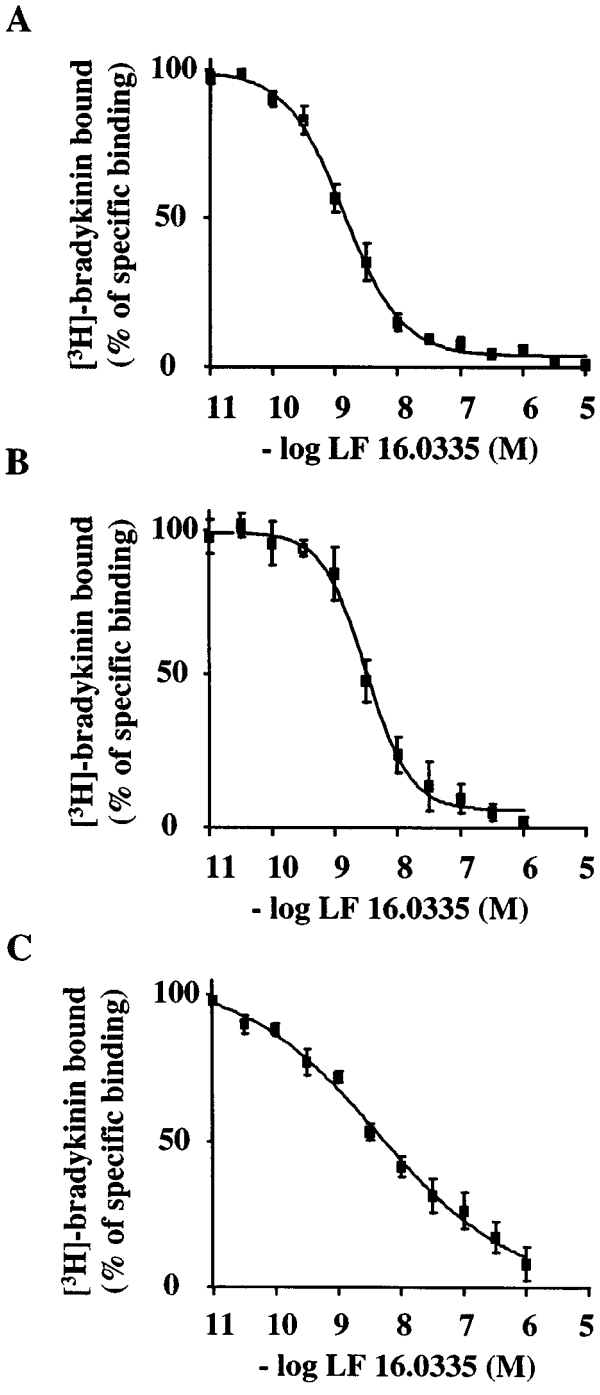

Figure 3 Competition binding of [^{3}H]-BK to membrane preparations from CHO cells expressing the human bradykinin B_2 receptor (A), INT 407 cells (B) and human umbilical vein (C) by LF 16.0335. Nonspecific binding was determined in the presence of 10 μM unlabelled BK. Values represent mean $\pm$ s.e.mean from three to five experiments performed in duplicate.

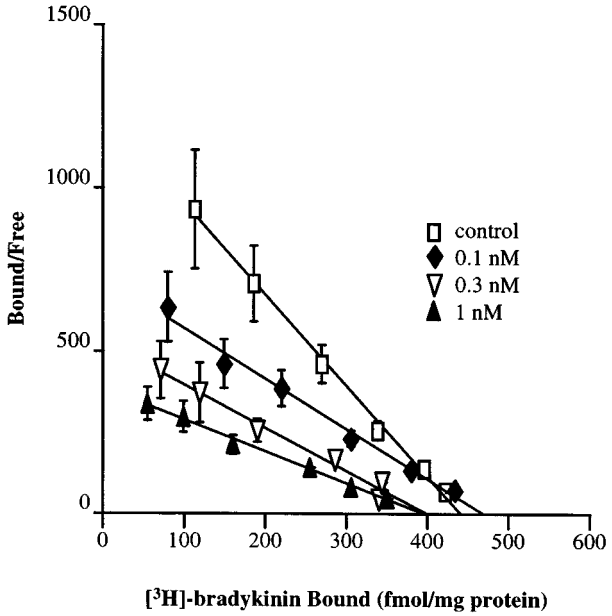

Figure 4 Scatchard plots of [^{3}H]-BK binding to INT 407 cell plasma membranes in the absence and presence of LF 16.0335. Binding conditions are described in the Methods section. Specific binding was analysed by the Scatchard method and values represent mean± s.e.mean from three to four experiments performed in duplicate.

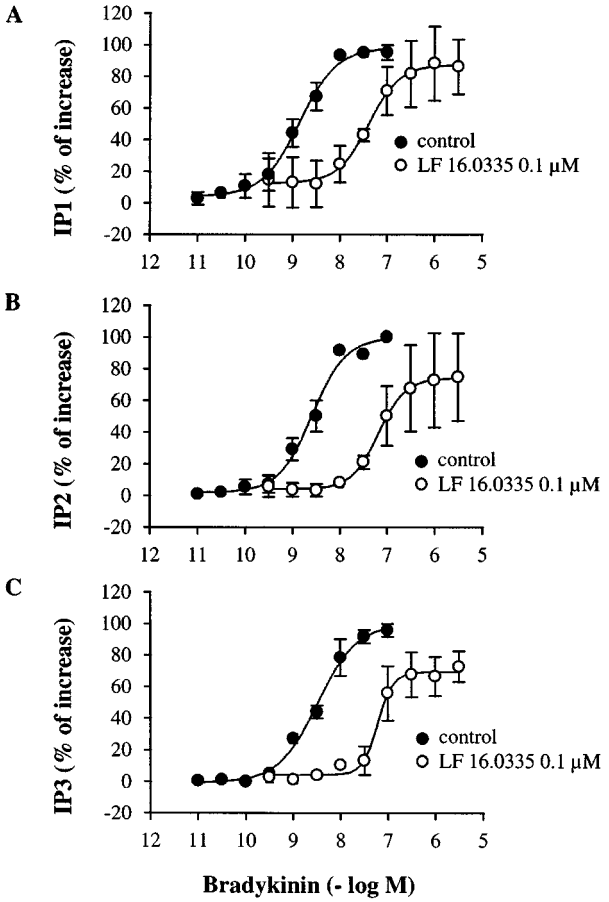

Figure 5 BK-induced production of IP1 (A), IP2 (B) and IP3 (C) in CHO cells expressing the human bradykinin B_2 receptor in the absence and presence of LF 16.0335. Values represent mean± s.e.mean from three experiments.

Selectivity profile

LF 16.0335, to concentrations up to 10 μM, did not alter the binding of [^{3}H]-des-Arg^{10}-[Leu^9]bradykinin in membranes from 293 cells stably expressing the human kinin B_1 receptor. Selectivity of LF 16.0335 was further assessed by testing it in 40 receptor binding, two enzyme and eight ion channel assays. LF 16.0335 at 1 μM had no significant effect in this battery of assays, except for the human muscarinic M_2 and M_1 receptor binding assay in which it had an IC_{50} of 0.9 μM and 1 μM. These values are >500 fold higher than its value for the bradykinin B_2 receptor.

Functional responses

Bradykinin caused a concentration-dependent increase in phosphoinositosides production in CHO cells expressing the human B_2 bradykinin receptor (Figure 5). The EC_{50} was 0.087±0.015 nM, 0.052±0.030 nM and 0.21±0.20 nM for IP1, IP2 and IP3, respectively (n=3). LF 16.0335 (10 nM) shifted to the right the concentration-response curves to bradykinin without depressing the maxima. The calculated pK_B value of LF 16.0335 was 8.27±0.06, 8.28±0.28 and 8.21±0.18 for IP1, IP2 and IP3 response-curves, respectively.

The human isolated umbilical vein contracts in response to bradykinin (Gobeil *et al*., 1996; Figure 6). In this preparation, LF 16.0335 caused a rightward shift of the concentration-contraction curve to bradykinin without reducing the maximum (Figure 6). Schild analysis gave a slope of 0.86±0.07 which was not significantly different from unity, indicating competitive antagonism (n=5–7). The calculated pA_2 of LF 16.0335 was 8.30±0.30.

Repetitive washing of HUV preparations after LF 16.0335 (0.1 μM) application was used to evaluate the reversibility of BK-induced contractions. The EC_{50} of BK concentration-response curves in the absence and presence of LF 16.0335 for 15 min were 14.4±6.5 nM and 472.4±61.9 nM, respectively (n=3). The corresponding *EC50* ratio was 23.8. When BK response-curves were obtained in the same preparations 2 and 3 h following washings, the *EC50* ratio was reduced to 4.2 and 2.1.

Discussion

This study has demonstrated that the nonpeptide compound, LF 16.0335, binds with high affinity to the human bradykinin B_2 receptor and that it is a potent competitive antagonist of this receptor. Although LF 16.0335 showed some weak affinity for the human muscarinic M_2 and M_1 receptors it did not bind to a range of other G-protein coupled receptors including the human kinin B_1 receptor.

Although the kinin-kallikrein system does not appear to play any important roles under normal physiological conditions, it is strongly activated in inflammatory situations (Bhoola *et al*., 1992; Dray & Bevan, 1993). Thus, increased local production of bradykinin occurs in patients suffering from ischemic stroke (Makevnina *et al*., 1994), sepsis (Karlsrud *et al*., 1996) allergic rhinitis (Proud *et al*., 1983) and asthma (Christiansen *et al*., 1987). Any detrimental effects of acutely elevated bradykinin are mediated by the activation of B_2 receptors which are constitutively expressed in a number of tissues. In this regard, stimulation of bradykinin B_2 receptors

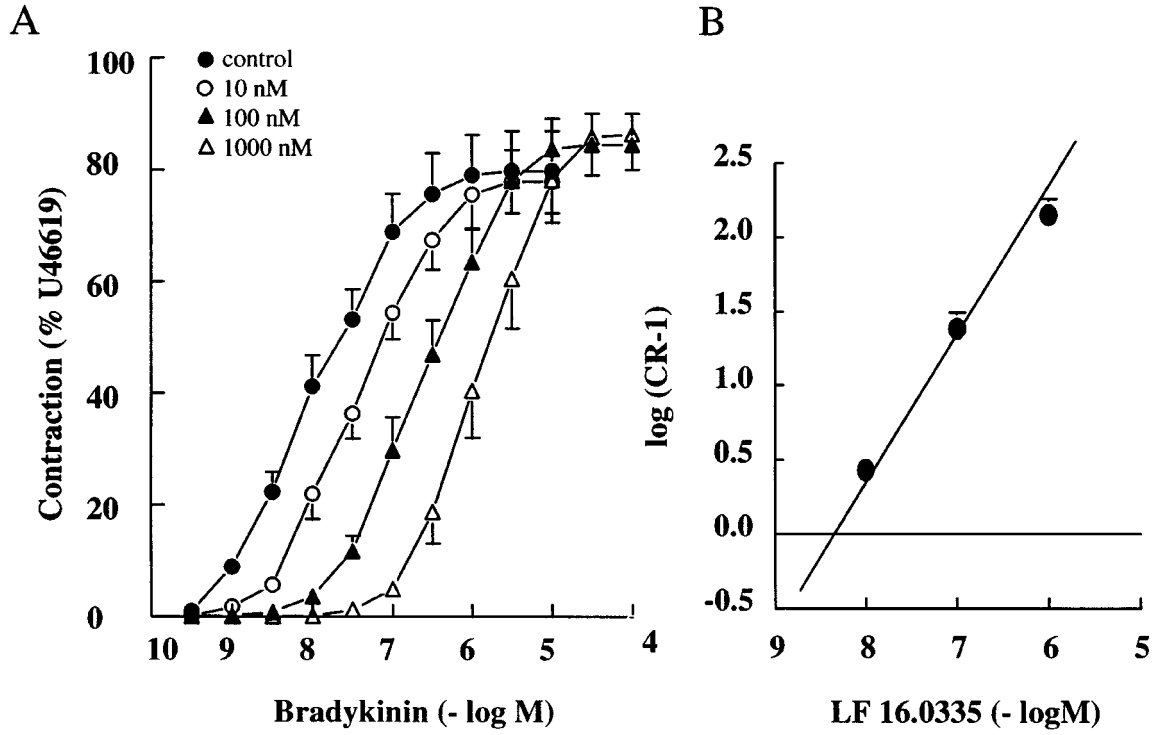

Figure 6 (A) Concentration-response curves of BK in the human umbilical vein in the absence and presence of LF 16.0335. (B) Schild plot analysis is inserted. Values represent mean ± s.e.mean from five to seven experiments.

increases vascular permeability and produces vasodilatation, both effects leading ultimately to tissue oedema (Kamiya *et al*., 1993; Breil *et al*., 1995; Schilling & Wahl, 1997). Bradykinin is also a potent algogenic agent which produces pain by stimulating nociceptive nerve terminals and sensitising them to other stimuli, including mechanical stimulation and heat (Rang *et al*., 1991; Walker *et al*., 1995; Cesare & McNaughton, 1996). Therefore, compounds which selectively inhibit the kinin-kallikrein system may have substantial clinical relevance. Hoe 140 represented the first potent and stable peptide bradykinin B_2 receptor antagonist (Hock *et al*., 1991) and WIN 64338 the first non peptide B_2 receptor antagonist (Sawutz *et al*., 1994). However, the former compound turned out to be a weak and non selective functional antagonist of the human B_2 receptor (Marceau *et al*., 1994; Sawutz *et al*., 1994; Gobeil *et al*., 1996). More recently, the nonpeptide compounds, FR 173657 and FR 167344, were reported to be potent antagonists of the human bradykinin B_2 receptor (Aramori *et al*., 1997; Asano *et al*., 1997). LF 16.0335, which bears a quinoline moiety in its chemical structure as FR 173657, was also a potent and competitive antagonist of the human B_2 receptor. In this respect, LF 16.0335 did not significantly change the B_{max} of bradykinin in competition binding studies, suggesting that it is a competitive inhibitor of [^{3}H]-BK binding. In addition, the affinities of LF 16.0335 for bradykinin B_2 receptors in membrane preparations from human umbilical vein, INT407 cells and CHO cells expressing the B_2 receptor were all in the nanomolar range. This latter finding indicates that LF 16.0335 binds equally well to cloned and native receptors.

In addition to high potency, a receptor antagonist must also be highly selective if it is to be useful clinically. In this respect, LF 16.0335 has no affinity for the human kinin B_1 receptor. This is not surprising since human kinin B_2 and B_1 receptors share only 36% homology in amino-acid composition (Menke *et al*., 1994) and differ markedly in their pharmacological profiles (Bastian *et al*., 1997). LF 16.0335, however, displayed some affinity for acetylcholine muscarinic receptors but the binding affinities were >500 fold lower than that for the bradykinin B_2 receptor. Taken together these results suggest that LF 16.0335 is a selective bradykinin B_2 receptor antagonist.

Bradykinin B_2 receptors are coupled *via* G_q proteins to phospholipase C in a number of cell types (i.e. Liao & Homcy, 1993) and their activation produces a concentration-dependent increase in intracellular inositol phosphates (Yano *et al*., 1984). In CHO cells expressing the human bradykinin B_2 receptor, bradykinin-induced IP1, IP2 and IP3 response-curves were shifted to the right in the presence of LF 16.0335 without a depression of the maximum, suggesting a competitive antagonism. The isolated human umbilical vein contracts in response to bradykinin through activation of B_2 receptors (Gobeil *et al*., 1996). LF 16.0335 produced a rightward shift of the concentration-response curve to bradykinin without affecting the maximum. Schild plot analysis gave a calculated pA_2 of 8.30 with a slope not different from unity indicating that LF 16.0335 behaves as a competitive antagonist. We also investigated the reversibility of LF 16.0335 effect in HUV rings contracted with BK. It appeared that the effect of LF 16.0335 was slowly reversible. It might be related to peculiar physicochemical properties of LF 16.0335 or, more likely, to the presence of a large number of spare B_2 receptors in this tissue. Interestingly, although FR173657 was an apparent competitive antagonist in the isolated human umbilical vein (Rizzi *et al*., 1997), it behaved as a non competitive antagonist against bradykinin-induced inositol phosphate formation in CHO cells expressing the human B_2 receptor (Aramori *et al*., 1997). It also applies to Hoe 140 which was an apparent competitive antagonist in HUV and guinea-pig trachea whilst it behaved as a non-competitive antagonist in the human

bronchi and guinea-pig ileum (Félétou *et al*., 1995; Pruneau *et al*., 1995). Therefore, further experiments are needed using different tissues and animal species in order to establish the competitive antagonist behaviour of LF 16.0335.

In conclusion, LF 16.0335 represents a useful tool to further understand the physiological and pathophysiological roles of B_2 receptor activation and to dissect molecular interactions of bradykinin with its receptor.

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