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European Journal of Pharmacology 333 (1997) 79–86

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Pharmacological characterization of a novel, orally active, nonpeptide bradykinin B_2 receptor antagonist, FR167344

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Received 14 November 1996; revised 13 June 1997; accepted 17 June 1997

Abstract

To investigate the pathophysiological role of bradykinin and to develop a drug for inflammatory diseases, we discovered an orally active, nonpeptide bradykinin B_2 receptor antagonist, FR167344, *N*-[*N*-[3-[(3-bromo-2-methylimidazo[1,2-a]pyridin-8-yl)oxymethyl]-2,4-dichlorophenyl]-*N*-methylaminocarbonylmethyl]-4-(dimethylaminocarbonyl) cinnamylamide hydrochloride. This compound competitively displaced [^{3}H]bradykinin binding to bradykinin B_2 receptors present in guinea-pig ileum membrane with an IC_{50} value of 6.6×10^{-10} M. In isolated guinea-pig ileum preparations, it also antagonized bradykinin-induced contraction with a pA_2 value of 9.3. In human lung fibroblast IMR-90 cells, FR167344 displaced [^{3}H]bradykinin binding to human bradykinin B_2 receptors with an IC_{50} value of 1.3×10^{-8} M, but not [^{3}H]des-Arg^{10}–kallidin binding to human bradykinin B_1 receptors. In vivo, oral administration of FR167344 inhibited bradykinin-induced bronchoconstriction in guinea pigs and the bradykinin-induced hypotensive response for 6 h in rats. These results show that FR167344 is a potent, selective, orally active and long acting bradykinin B_2 receptor antagonist. © 1997 Elsevier Science B.V.

Keywords: Bradykinin; Bradykinin receptor antagonist; FR167344; FR172357; Bradykinin B_2 receptor; Orally active

1. Introduction

Bradykinin, a nonapeptide (Arg–Pro–Pro–Gly–Phe–Ser–Pro–Phe–Arg), is released from kininogens by the action of kallikreins. Bradykinin elicits a variety of biological responses including pain, inflammation, and hypotension (Bhoola et al., 1992; Burch et al., 1990). To investigate the physiological and pathological role of bradykinin and to make an anti-inflammatory drug, many bradykinin receptor antagonists have been synthesized (Burch et al., 1990; Stewart, 1995). [D-Phe^7]bradykinin was the first bradykinin receptor antagonist (Vavrek and Stewart, 1985). Incorporation of β-(2-thienyl)-alanine residues at position 5 and 8 of [D-Phe^7]bradykinin converted a weak antagonist to a much more potent antagonist (Stewart, 1995). Although these first-generation bradykinin receptor antagonists were useful for studying the involvement of bradykinin in many pathophysiological processes, they do not have a good therapeutic potential in vivo yet. Recently, second-generation bradykinin B_2 receptor antagonists have been reported (Hock et al., 1991; Cheronis et al., 1992). One of the most successful antagonists, Hoe 140 (D-Arg–[Hyp^3, Thi^5, D-Tic^7, Oic^8]bradykinin), is highly potent and long-acting against bradykinin-induced responses and can be used to inhibit inflammation in vivo (Wirth et al., 1991). However, these antagonists are all peptide analogs and their therapeutic use is limited because of their poor oral bioavailability. Especially when the antagonists are used as treatment for a chronic disease, oral activity is a prerequisite. Recently, some nonpeptide bradykinin B_2 receptor antagonists such as WIN 64338 ([[4-[[2-[[bis(cyclohexylamino)methylene] amino]-3-(2-naphthyl)-1-oxopropyl]amino]phenyl]methyl]tributylphosphonium chloride monohydrochloride) have been reported (Sawutz et al., 1994), but it has not been reported that these antagonists are orally active.

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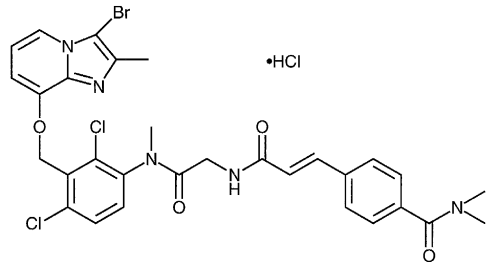

Fig. 1. Structure of FR167344: *N*-[*N*-[3-[(3-bromo-2-methylimidazo[1,2-a]pyridin-8-yl)oxymethyl]-2,4-dichlorophenyl]-*N*-methylaminocarbonylmethyl]-4-(dimethylaminocarbonyl) cinnamylamide hydrochloride.

In order to obtain orally active bradykinin receptor antagonists, we tried to make nonpeptide bradykinin receptor antagonists. First of all, we discovered a seed compound containing the imidazo[1,2-a]pyridine structure by random screening of several thousand chemicals, and converted it to more potent compounds by chemical modification. Finally, we obtained FR167344, *N*-[*N*-[3-[(3-bromo-2-methylimidazo [1,2-a]pyridin-8-yl)oxymethyl]-2,4-dichlorophenyl]-*N*-methylaminocarbonylmethyl]-4-(dimethylaminocarbonyl) cinnamylamide hydrochloride (Fig. 1). In the present study, we have characterized the pharmacological activity of FR167344 in vitro, and demonstrated that it antagonizes the in vivo functional response of bradykinin and is active by oral administration.

2. Materials and methods

2.1. Receptor binding

2.1.1. Guinea-pig ileum

The specific binding of [^{3}H]bradykinin (a high-affinity bradykinin B_2 receptor ligand) was assayed according to the method previously described (Manning et al., 1986) with minor modifications. Male Hartley guinea pigs (from Charles River Japan) were killed by exsanguination under anaesthesia. The ilea were removed and homogenized in ice-cold buffer (50 mM *N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) and 1 mM 1,10-phenanthroline, pH 6.8) with a Polytron. The homogenate was centrifuged to remove cellular debris ($1000 \times g$, 20 min, 4°C) and the supernatant was centrifuged ($100\,000 \times g$, 60 min, 4°C). Then, the pellet was resuspended in ice-cold assay buffer (50 mM TES, 1 mM 1,10-phenanthroline, 140 μg/ml bacitracin, 1 mM dithiothreitol, 1 μM captopril and 0.1% bovine serum albumin, pH 6.8) and was stored at −80°C until use.

In the binding assay, membranes were incubated with [^{3}H]bradykinin (final concentration 0.06 nM) and various concentrations of test compounds or unlabelled bradykinin at room temperature for 60 min. Receptor-bound [^{3}H]bradykinin was harvested by filtration through Whatman GF/B glass fibre filters under reduced pressure and the filter was washed 5 times with 300 μl of ice-cold buffer (50 mM Tris–HCl). The radioactivity retained on the washed filter was measured with a liquid scintillation counter. Specific binding was calculated by subtracting the non-specific binding (determined in the presence of 1 μM unlabelled bradykinin) from the total binding. The experiments were performed three or four times in duplicate. Mean IC_{50} values are shown.

2.1.2. Rat uterus

Uteri from female Sprague–Dawley rats (from Japan SLC) were removed immediately after killing of the animals by exsanguination under anaesthesia. The uteri were homogenized and centrifuged with the same method as described above for guinea-pig ileum. Membrane preparations of rat uteri were used for the binding assay using the same methods as described above for the guinea-pig ileum. The experiments were performed three or four times in duplicate. Mean IC_{50} values are shown.

2.1.3. Human fibroblast

IMR-90, human fetal lung fibroblasts (obtained from the American Type Culture Collection), were grown in Dulbecco's modified Eagle's minimum essential medium (DMEM) containing penicillin (100 μg/ml), streptomycin (100 μg/ml), and 10% fetal bovine serum. The cells were cultured in the 24-well tissue culture plates at a concentration of 10^5 cells/well prior to assay. In the bradykinin B_1 receptor binding assay, IMR-90 cells were treated with interleukin-1β (1 ng/ml) for 6 h before assay to enhance bradykinin B_1 receptor expression. The cells were washed twice with phosphate-buffered saline containing 0.1% bovine serum albumin, then incubated with [^{3}H]bradykinin (final concentration 1 nM) for the bradykinin B_2 receptor binding assay or [^{3}H]des-Arg^{10}–kallidin (a high-affinity bradykinin B_1 receptor ligand, final concentration 1 nM) for the bradykinin B_1 receptor binding assay and test compounds for 90 min at room temperature in 0.5 ml of assay buffer (20 mM HEPES, 125 mM *N*-methyl-D-glucamine, 5 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM 1,10-phenanthroline, 1 mM dithiothreiol, 1 μM captopril and 0.1% bovine serum albumin, pH 7.4). Non-specific binding was determined in the presence of 1 μM unlabelled bradykinin or des-Arg^{10}–kallidin. At the end of the incubation, the buffer was aspirated and the cells were washed three times with phosphate-buffered saline containing 0.1% bovine serum albumin. Bound radioactivity was determined by solubilizing the cells with 1% sodium dodecyl sulfate containing 0.05 M NaOH and measuring the radioactivity in a liquid scintillation counter. The experiments were performed three or four times in triplicate. Mean IC_{50} values are shown. In Scatchard analysis, concentrations of [^{3}H]bradykinin varied from 0.03 to 1 nM.

2.2. Smooth muscle contraction

2.2.1. Guinea-pig ileum

Guinea-pig ileum contraction produced by bradykinin was measured by the method of Hock et al. (1991). Segments (1.5 cm) of ileum were isolated from male Hartley guinea pigs and suspended in 25 ml organ baths containing Tyrode solution (g/l: NaCl 8.0, KCl 0.2, $MgCl_2 \cdot 6H_2O$ 0.1, $CaCl_2 \cdot 2H_2O$ 0.2, $NaHCO_3$ 1.0, $NaHPO_4 \cdot 2H_2O$ 0.05, Glucose 1.0), maintained at 37°C and bubbled with 95% O_2, 5% CO_2. Tension was measured isometrically with force transducers and responses were recorded on a multi-channel polygraph recorder. Initial tension was set at 1.0 g. After an equilibration period of about 30 min, a stable baseline tone was reached and two or three contractions were obtained in response to bradykinin (6×10^{-8} M). After the contraction, the isolated tissue was washed three times. After a rest of 10 min the segments relaxed to baseline levels. The segments were incubated with the bradykinin receptor antagonists for 10 min before bradykinin was added. The percent inhibition produced by bradykinin receptor antagonists was calculated from the following formula: % inhibition = ((the last bradykinin-induced change in tension in the absence of the antagonists) − (bradykinin-induced change in tension in the presence of the antagonists)) ÷ (the last bradykinin-induced change in tension in the absence of the antagonists) × 100. IC_{50} was calculated from the % inhibition. In the pA_2 study, the segments were contracted with bradykinin in cumulative concentrations of 10^{-9} to 10^{-5} M. Different concentrations of FR167344 were applied 10 min before the bradykinin-induced contraction was measured in its presence. The pA_2 value was calculated from Schild plots (Schild, 1947). A dose ratio (DR) was calculated from the ED_{50} of the concentration–response curve in the presence of the antagonist divided by the ED_{50} for the individual concentration–response curve of bradykinin alone.

Acetylcholine or histamine-induced contraction was measured by the same method as the bradykinin-induced contraction with minor modifications. Initial tension was set at 0.5 g. The temperature of the organ baths was maintained at 27°C. Acetylcholine or histamine was added at the concentration of 1×10^{-6} or 5×10^{-7} M, respectively. The percent inhibition and IC_{50} were calculated with the same methods as described above for bradykinin.

2.2.2. Reversibility

After the concentration–response curves for bradykinin were obtained in the presence of 6 nM FR167344, the segments of ileum were washed 5 times with Tyrode solution. After incubation, the washing step was repeated. Then, following a rest of 15 min, the segments were contracted with bradykinin in cumulative concentrations of 10^{-9} to 10^{-5} M again.

2.2.3. Rabbit aorta

Experiments were performed according to Regoli et al. (1977) with some modifications. The thoracic aorta was isolated from male New Zealand White rabbits, cut in a spiral and divided into small strips. The aortic strips were suspended in 25 ml organ baths containing Krebs solution at 37°C. Contractions of the strips were recorded isometrically with a basal tension of 2 g on the tissue. After an equilibration period of 5 h, FR167344 was tested for its ability to inhibit bradykinin B_1 receptor-mediated contractions elicited by des-Arg^9–bradykinin (5×10^{-7} M).

2.3. Bradykinin-induced bronchoconstriction

Male Hartley guinea pigs were fasted overnight and anaesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg), and the trachea, jugular vein, and oesophagus were cannulated. The animals were ventilated at a tidal volume of 10 ml/kg and at a frequency of 60 breaths/min through the tracheal cannula. To suppress spontaneous respiration, alcuronium chloride (0.5 mg/kg) was administered intravenously through the jugular vein cannula. Then, propranolol (10 mg/kg) was also administered subcutaneously. After a 10 min period, bradykinin (5 μg/kg, dissolved in saline with 0.1% bovine serum albumin) was administered intravenously through the jugular vein. Bronchoconstriction was measured by the modified Konzett and Rössler (1940) method as the peak increase in pulmonary insufflation pressure (Asano et al., 1992). FR167344 suspended in 0.5% methylcellulose solution or vehicle was administered through the oesophageal cannula. After a 30 min period, bradykinin was administered and the bronchoconstriction was measured in the same manner. 0% response was determined as the pulmonary insufflation pressure before the administration of bradykinin. 100% response was determined as the first bradykinin-induced bronchoconstriction before drug administration. The percent response was calculated from the following formula: % response = (increase in pulmonary insufflation pressure after drug administration) ÷ (increase in pulmonary insufflation pressure before drug administration) × 100.

2.4. Bradykinin-induced hypotensive response

Male Wistar rats were fasted overnight and anaesthetized with sodium pentobarbital (50 mg/kg i.p.). Left femoral arteries and left femoral veins of the animals were cannulated. Systemic blood pressure was measured directly in the left femoral artery by use of a pressure transducer, connected to a multi-channel recorder. Bradykinin was dissolved in saline with 0.1% bovine serum albumin and given as a bolus injection (10 μg/kg) into the left femoral vein through the cannula. One hour after the first bradykinin injection, FR167344 dissolved in 0.05 M HCl or vehicle was administered orally. At 1, 3 and 6 h after the oral administration, bradykinin was given in the same manner. Additional administration of sodium pentobarbital was performed at 15 min before the following bradykinin injections.

2.5. Materials

FR167344, FR 167377, FR172357, Hoe 140, and des-Arg^{10}–[leu^{9}]kallidin were chemically synthesized in Fujisawa Pharmaceutical. All other compounds were purchased from commercial sources.

2.6. Statistical analysis

The results are expressed as the means ± S.E.M., and the statistical significance of difference between groups was analyzed by means of analysis of variance (ANOVA) followed by Dunnett's test. IC_{50}, or ED_{50} values were obtained by using non-linear curve-fitting methods with a specific computer program. The K_i value was calculated by the method of Cheng and Prusoff (1973).

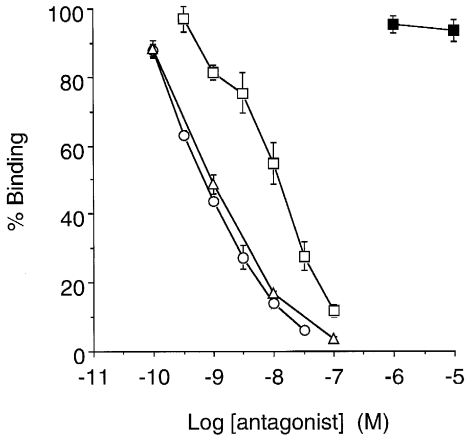

Fig. 2. Effect of FR167344 on radioligand binding to bradykinin B_1 or B_2 receptors in guinea-pig, rat and human tissues. Guinea-pig ileum membrane (○), rat uterus membrane (△), and IMR-90 cells (□) were incubated with [^{3}H]bradykinin and increasing concentrations of FR167344. Interleukin-1β-treated IMR-90 cells (■) were incubated with [^{3}H]des-Arg^{10}–kallidin and 10^{-6} or 10^{-5} M FR167344. Data are expressed as means ± S.E.M. ($n = 3$–4).

3. Results

3.1. Receptor binding

3.1.1. Guinea-pig ileum

FR167344 (HCl salt) and Hoe 140 (a potent bradykinin B_2 receptor antagonist) displaced [^{3}H]bradykinin binding to bradykinin B_2 receptors in guinea-pig ileum membrane preparations, but des-Arg^{10}–[Leu^{9}]kallidin (a potent bradykinin B_1 receptor antagonist) did not (Fig. 2, Table 1). The IC_{50} value and the K_i value of FR167344 were 6.6×10^{-10} and 1.2×10^{-10} M, respectively. FR167377 (salt free) and FR172357 (CH_3SO_3H salt) had the same activity as FR167344 in displacing [^{3}H]bradykinin binding to the receptors (IC_{50} values are 6.7×10^{-10} and 8.8×10^{-10} M, respectively).

3.1.2. Rat uterus

In rat uterus, FR167344 also antagonized [^{3}H]bradykinin binding to bradykinin B_2 receptors (Fig. 2, Table 1). Hoe 140 was also effective in this assay.

3.1.3. Human fibroblast

FR167344 inhibited [^{3}H]bradykinin binding to human bradykinin B_2 receptors with an IC_{50} value of 1.3×10^{-8} M and a K_i value of 1.6×10^{-9} M, but not [^{3}H]des-Arg^{10}–kallidin binding to human bradykinin B_1 receptors even at 10^{-5} M (Fig. 2, Table 1). Hoe 140 was potent in inhibiting bradykinin B_2 receptor binding, but not bradykinin B_1 receptor binding. Des-Arg^{10}–[Leu^{9}]kallidin inhibited bradykinin B_1 receptor binding with an IC_{50} value of 2.6×10^{-9} M but not bradykinin B_2 receptor binding (Table 1). Scatchard analysis was performed in the absence and presence of this compound. FR167344 reduced the absolute value of the slope, but did not change the *x*-axis intercept (Fig. 3). Therefore, FR167344 increased the K_d value without changing the B_{max} value. In our assay system, untreated IMR-90, human fibroblast cells expressed about 70 000 bradykinin B_2 receptors per cell, and IMR-90 cells treated by interleukin-1β expressed about 40 000 bradykinin B_1 receptors per cell (data not shown). These data are consistent with the data previously described (Menke et al., 1994).

3.2. Smooth muscle contraction

3.2.1. Guinea-pig ileum

In isolated guinea-pig ileum, FR167344 had no agonistic effect, but antagonized bradykinin-induced contractions dose-dependently (Table 2). FR167344 did not inhibit acetylcholine or histamine-induced guinea-pig ileum con-

Table 1
Effect of FR167344 on radioligand binding to bradykinin B_1 or B_2 receptors in guinea-pig, rat and human tissues

Receptor binding	IC_{50} (M)		
	FR167344	Hoe 140	des-Arg^{10}–[Leu^{9}]kallidin
Guinea-pig ileum B_2	6.6×10^{-10}	8.9×10^{-11}	$> 10^{-5}$
Rat uterus B_2	1.2×10^{-9}	1.6×10^{-10}	N.T.
Human B_2	1.3×10^{-8}	2.7×10^{-9}	$> 10^{-5}$
Human B_1	$> 10^{-5}$	$> 10^{-5}$	2.6×10^{-9}

Data are expressed as means ($n = 3$–4). N.T.: not tested.

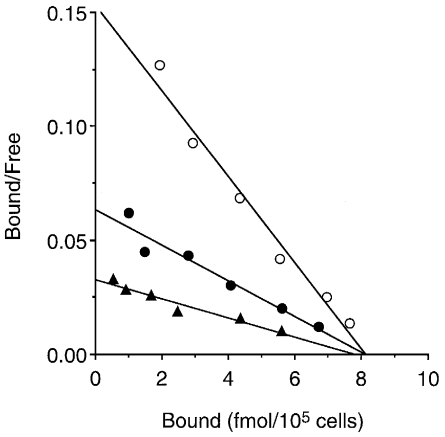

Fig. 3. Effect of FR167344 on Scatchard analysis of specific [^{3}H]bradykinin binding to bradykinin B_2 receptors in human fibroblasts, IMR-90 cells. IMR-90 cells were incubated with various concentrations of [^{3}H]bradykinin in the absence (○) or presence of 1 (●) or 2 nM (▲) FR167344. Data represent a typical experiment that was repeated twice with similar results.

tractions even at 10^{-6} M (Table 2). FR167344 caused parallel rightward shifts of the concentration–response curves for bradykinin at 10^{-9}, 3×10^{-9}, 6×10^{-9} M of the antagonist. At 10^{-8} M, FR167344 also caused a parallel rightward shift of the concentration–response curve, but a maximal response to bradykinin (10^{-9} to 10^{-5} M) was not obtained (Fig. 4A). Analysis of the data yielded a pA_2 value of 9.3 and a slope of 1.5 (Fig. 4B).

3.2.2. Reversibility in guinea-pig ileum

After the removal of FR167344 (6 nM) by washing, the contractile response to bradykinin was restored, and the parallel leftward shifts of the concentration–response curves were observed. ED_{50} changed from 1200 ± 610 to 210 ± 95 nM (Table 3). Washout did not completely restore the bradykinin response, because the concentration–response curves did not return to the original position.

Table 2
Effect of FR167344 on smooth muscle contractions in isolated guinea-pig ileum and rabbit aorta

Smooth muscle contraction	IC_{50} (M)	
	FR167344	Hoe 140
Guinea-pig ileum		
bradykinin-induced	3.0×10^{-9}	6.7×10^{-9}
acetylcholine-induced	$>10^{-6}$	N.T.
histamine-induced	$>10^{-6}$	N.T.
Rabbit aorta		
des-Arg^9–bradykinin-induced	$>10^{-6}$	N.T.

Data are expressed as means ($n=3$). N.T.: not tested. The percent inhibition by bradykinin receptor antagonists was calculated from the following formula: % inhibition = ((the stimulant-induced change in tension in the absence of the antagonists) − (the stimulant-induced change in tension in the presence of the antagonists)) ÷ (the stimulant-induced change in tension in the absence of the antagonists) × 100.

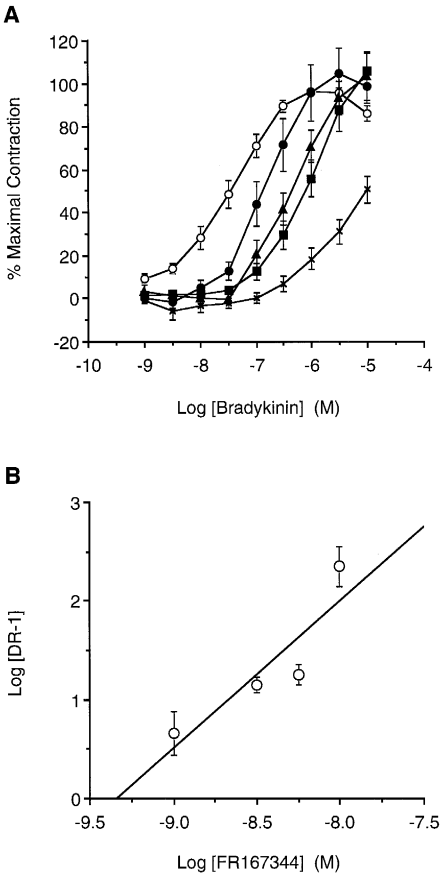

Fig. 4. Effect of FR167344 on bradykinin-induced contractions in isolated guinea-pig ileum preparations. (A) Dose–response curves. Data are expressed as means ± S.E.M. ($n=5$). Open circles (○) show values in the absence of FR167344. Closed circles (●), triangles (▲), squares (■) and crosses (×) show values in the presence of 10^{-9}, 3×10^{-9}, 6×10^{-9} and 10^{-8} M FR167344, respectively. (B) Schild analysis. Data are expressed as means ± S.E.M. ($n=5$). The data yield a pA_2 value of 9.3 and a slope of 1.5.

3.2.3. Rabbit aorta

FR167344 did not inhibit des-Arg^9–bradykinin-induced rabbit aorta contractions even at 10^{-6} M (Table 2).

3.3. Bradykinin-induced bronchoconstriction

Exogenously administered bradykinin (5 μg/kg i.v.) induced an increase in pulmonary insufflation pressure, indicating bronchoconstriction, in guinea pigs (29.2 ± 2.2

Table 3
The reversibility of the effect of FR167344 in guinea-pig ileum

Bradykinin-induced contraction	ED_{50} of bradykinin (nM)
In the absence of FR167344	48 ± 12
In the presence of 6 nM FR167344	1200 ± 610
After washing	210 ± 95

ED_{50} was obtained from concentration–response curves for bradykinin ($n=5$).

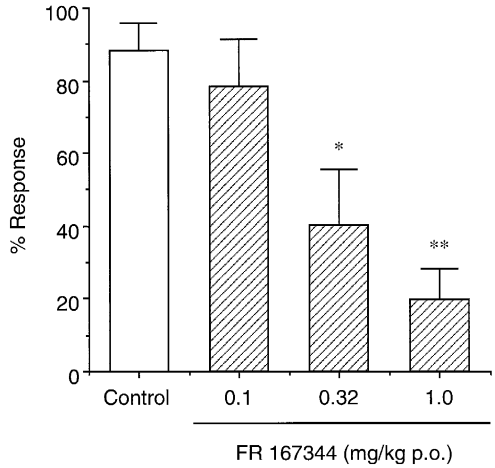

Fig. 5. Inhibition of bradykinin-induced bronchoconstriction by oral administration of FR167344 in guinea pigs. Data are expressed as means ± S.E.M. ($n = 5$–14). Open column shows a value in control animals. Hatched columns show values in FR167344-administered animals. * $P < 0.05$, ** $P < 0.01$ vs. control (Dunnett's test).

cm H_2O). Then, oral administration of FR167344 dose-dependently inhibited the bradykinin-induced increase in pulmonary insufflation pressure with an ED_{50} value of 0.28 mg/kg (Fig. 5).

3.4. Bradykinin-induced hypotensive response

Intravenous injection of bradykinin (10 μg/kg) provoked reproducible transient hypotension. The oral administration of FR167344 did not induce any significant changes in blood pressure but significantly inhibited the bradykinin-induced hypotensive response dose-dependently (Fig. 6). The bradykinin antagonism elicited by the 1 or 10 mg/kg oral administration of FR167344 lasted for more than 6 h (Fig. 6).

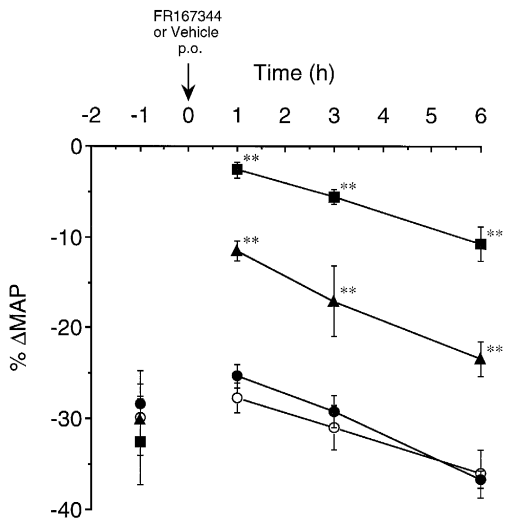

Fig. 6. Inhibition of bradykinin-induced hypotension by oral administration of FR167344 in rats. The ordinate shows % change in mean arterial pressure (% ΔMAP). Data are expressed as means ± S.E.M. ($n = 5$ or 6). Open circles (○) show values in control animals. Closed circles (●), triangles (▲) and squares (■) show values in FR167344-administered animals at a dose of 0.1, 1, and 10 mg/kg, respectively. ** $P < 0.01$ vs. control (Dunnett's test).

4. Discussion

To investigate the pathophysiological role of bradykinin and to develop a drug for inflammatory diseases, we tried to make nonpeptide bradykinin receptor antagonists. We have obtained an orally active nonpeptide bradykinin receptor antagonist, FR167344, by optimization of a seed compound discovered by random screening. The present study demonstrates that FR167344 is a potent, specific, orally active, and long acting bradykinin B_2 receptor antagonist.

The effects of bradykinin are mediated through specific G-protein-coupled cell surface receptors (Burch and Axelrod, 1987). At least two subtypes of bradykinin receptor, designated as B_1 and B_2, have been identified by molecular cloning and pharmacological means (Regoli and Barabé, 1980; Hess et al., 1992; Menke et al., 1994). Most biological actions of bradykinin are thought to be mediated by the bradykinin B_2 receptors. FR167344 antagonized bradykinin binding to bradykinin B_2 receptors in guinea-pig, rat and human tissues (Fig. 2, Table 1). In the human bradykinin B_1 receptor binding assay, FR167344 had no effect on the binding of bradykinin B_1 radioactive ligand to the receptors even at 10^{-5} M. FR167344 inhibited the bradykinin-induced contraction in guinea-pig ileum, but did not inhibit the acetylcholine or histamine-induced contraction in guinea-pig ileum and the des-Arg^9–bradykinin-induced contraction in rabbit aorta. These results confirm that FR167344 is a selective antagonist against bradykinin B_2 receptors.

In Scatchard analysis, FR167344 increased the K_d value without changing the B_{max} value (Fig. 3), suggesting that FR167344 may competitively inhibit [^{3}H]bradykinin binding to bradykinin B_2 receptors in IMR-90 cells. In isolated guinea-pig ileum, FR167344 caused parallel rightward shifts of the concentration–response curves for bradykinin at 10^{-9}, 3×10^{-9} and 6×10^{-9} M of the antagonist (Fig. 4), suggesting that this compound may also competitively inhibit bradykinin-induced contractions. Although the maximal response to bradykinin (10^{-9} to 10^{-5} M) was not obtained in the presence of 10^{-8} M FR167344, on the basis of the concentration–response curve higher concentrations of bradykinin ($> 10^{-5}$ M) would have produced the same maximal response as control. These data suggest that FR167344 may be a competitive antagonist in guinea-pig ileum. However, the slope of the Schild plot is larger than 1, suggesting that FR167344 is not a simple competitive antagonist. It may have other properties such as the slow-equilibrium kinetics at high concentrations.

Our data indicate that the effect of FR167344 is partially reversible (Table 3). Therefore, it is speculated that this compound may tightly bind to B_2 receptors by non-covalent bonds. Recently we identified a non-peptide B_2 full agonist whose chemical structure is closely related to that of FR167344 (Aramori et al., 1997). It is a highly potent and selective agonist, suggesting that these types of nonpeptide B_2 agonists and antagonists may bind to the bradykinin binding site in B_2 receptors. But further investigation is required for elucidating the exact antagonistic mechanism of FR167344.

At some concentrations (10^{-9} to 3×10^{-8} M) bradykinin caused relaxation in the presence of 10^{-8} M FR167344 (Fig. 4A). This phenomenon suggests that there may be another subtype of bradykinin receptor in guinea-pig ileum. Bradykinin may have caused a slight relaxation of the ileum through this subtype, but FR167344 may not have antagonized the response.

Although FR167344 was less potent than Hoe 140 in inhibiting bradykinin binding to bradykinin B_2 receptors in guinea-pig ileum (Table 1), it was more potent than Hoe 140 in inhibiting bradykinin-induced guinea-pig ileum contractions (Table 2). The pA_2 value of FR167344 was 9.3, that is, larger than that of Hoe 140 (8.9) previously reported (Rhaleb et al., 1992). The reason for this discrepancy may be that FR167344 is more resistant to peptidases in tissue and penetrates tissue better than Hoe 140, because FR167344 is a nonpeptide and a smaller compound. FR167344 is also more potent than WIN 64338, a nonpeptide B_2 receptor antagonist that has a pA_2 value of 8.2 in guinea-pig ileum and a K_i value of 6.4×10^{-8} M in human B_2 receptor binding assay (Sawutz et al., 1994).

To evaluate the in vivo effectiveness of FR167344 as a bradykinin receptor antagonist, we examined the effects of the oral administration of FR167344 on bradykinin-induced in vivo responses. In guinea pigs, FR167344 showed potent inhibitory activity against bradykinin-induced bronchoconstriction by the oral administration (Fig. 5). This result demonstrates that FR167344 is orally active. In rats, the oral administration of FR167344 inhibited the bradykinin-induced hypotensive response for 6 h at a dose of 1 mg/kg. These data confirm the oral activity of FR167344 and show that FR167344 is a long acting antagonist in vivo.

Bradykinin provokes a variety of biological responses such as pain, inflammation, bronchoconstriction and hypotension. Therefore it seems that bradykinin has an important role in inflammatory diseases. Especially, it has been proposed that bradykinin is a pivotal mediator in asthma (Proud and Kaplan, 1988; Farmer, 1991). Bradykinin induces the release of tachykinins (Saria et al., 1988) and histamine (Ishizaka et al., 1985), which cause bronchoconstriction and microvascular leakage (Barnes et al., 1988). In asthmatic patients, bradykinin inhalation causes bronchoconstriction at a lower dose than that of histamine (Fuller et al., 1987). Kininogenase activity and immunoreactive kinins are increased in bronchoalveolar lavage fluid of asthmatic patients (Christiansen et al., 1987). These findings suggest that bradykinin receptor antagonists have therapeutic potential against asthma. In the case of a chronic disease such as asthma, the oral activity of FR167344 is beneficial for compliance with therapy.

In conclusion, this study described a nonpeptide bradykinin B_2 receptor antagonist, FR167344, that is potent, selective, orally active, and long acting. This compound will not only be a good tool for studying the pathophysiological role of bradykinin but also a useful treatment for asthma and other inflammatory diseases.

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