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Effects of an orally active non-peptide bradykinin B_2 receptor antagonist, FR173657, on plasma exudation in rat carrageenin-induced pleurisy

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1 Effects of an orally active non-peptide (BK) B_2 receptor antagonist, FR173657 ((E)-3-(6-acetamido-3-pyridyl)-N-[N-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-N-methylaminocarbonylmethyl] acrylamide) on the plasma exudation in rat carrageenin-induced pleurisy were investigated.

2 Plasma exudation induced by intrapleural injection of bradykinin (BK, 3 nmol per rat) into male SD strain rats (SPF, 8 weeks old) were significantly inhibited by oral administration of novel B_2 receptor antagonist FR173657 (3–30 mg kg^{-1}, 1 h before BK injection) in a dose-dependent manner, whereas that induced by histamine was not.

3 The inhibitory effect of 30 mg kg^{-1} FR173657 persisted for more than 4 h.

4 Intrapleural injection of λ-carrageenin (2% (w/v), 0.1 ml per rat) caused marked plasma exudation and accumulation of exudates from 1 h after carrageenin injection. The maximum plasma exudation response was observed 5 h after carrageenin. The oral administration of FR173657 to rats (30 mg kg^{-1}, 1 h before carrageenin) significantly (by 50–77%) blunted the plasma exudation 1, 3, 5, and 7 h after carrageenin, causing a significant parallel reduction (by 42–57%) in the volume of exudates.

5 The anti-inflammatory effect of FR173657 on rat carrageenin-induced pleurisy was almost equipotent with that of the peptide B_2 antagonist Hoe140 (1 mg kg^{-1}, i.v.), a plasma kallikrein inhibitor, soy bean trypsin inhibitor (0.3 mg per rat, intrapleural injection) and bromelain (10 mg kg^{-1}, i.v.).

6 In pleurisy induced by intrapleural injection of a histamine releaser, compound 48/80, the plasma exudation was observed only within 20 min after the injection. This plasma exudation was not affected by FR173657, although it was completely inhibited by a mixture of pyrilamine (5 mg kg^{-1}, i.v.) and methysergide (3 mg kg^{-1}, i.v.).

7 These results indicate that FR173657 is an orally active, promising anti-inflammatory agent for kinin-dependent inflammation.

Keywords: Bradykinin B_2 receptor; orally active; non-peptide; antagonist; carrageenin-induced pleurisy; plasma exudation

Introduction

Bradykinin (BK) is a potent, biologically active peptide that induces dilatation of the resistance blood vessels, increases in venular permeability, sensations of pain, prostaglandin release and increases in the glomerular filtration rate and in sodium and water excretion from the kidneys (Bhoola *et al*., 1992; Burch *et al*., 1990; Majima & Katori, 1995; Katori & Majima, 1996). Potent and metabolically stable antagonists of kinin receptors are required to demonstrate the pathophysiological roles of kinins *in vivo*. At least two subtypes of BK receptors (B_1 and B_2 receptors) have been identified by pharmacological analysis and molecular cloning (Regoli & Barabe, 1980; Hess *et al*., 1992; Menke *et al*., 1994). B_2 receptors mediate most of the biological actions of the kinins (Bhoola *et al*., 1992). More than ten years ago, the first BK B_2 receptor antagonist, [D-Phe^7]-BK, a peptide analogue of BK, was obtained (Vavrek & Stewart, 1985). Further replacements of amino acid residues at position 5 and 8 of [D-Phe^7]-BK with β-(2-thienyl)-alanine potentiated antagonistic activity (Stewart, 1995). Recently developed potent B_2 antagonists such as Hoe140 (DArg-[Hyp^3,Thi^5,DTic^7,Oic^8]-BK), S16118 (p-guanidobenzoyl-[Hyp^3,Thi^5,DTic^7,Oic^8]-BK), and CP-0127 (bis-succinimidohexane dimer of DArg^0-[Hyp^3,Thi^5,DTic^7,Oic^8]-BK), are all peptide analogues of BK that contain unnatural or conformationally restrained amino acid residues or dimeric structures (Hock *et al*., 1991; Wirth *et al*., 1991; Cheronis *et al*., 1992; Feletou *et al*., 1995). These can be used *in vivo*, but, because of their poor bioavailabilities in oral administration, there are limitations to their therapeutic use. Several non-peptide BK antagonists, such as WIN64338 ([[4-[[2-[[bis(cyclohexylamino)methylene]amino]-3-(2-naphthalenyl)-1-oxopropyl]amino]phenyl]methyl]-tributylphosphoniumchloride monohydrochloride) have been synthesized (Salvino *et al*., 1993; Sawutz *et al*., 1994), but they have not been administered orally (Hall *et al*., 1995).

FR173657 ((E)-3-(6-acetamido-3-pyridyl)-N-[N-[2,4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-N-methylaminocarbonylmethyl]acrylamide) is another non-peptide B_2 antagonist that has recently been developed (Asano *et al*., 1997). This compound has a high oral bioavailability and is the first orally active compound (Asano *et al*., 1997). Oral administration of FR173657 inhibits BK-induced bronchoconstriction in guinea-pigs and carrageenin-induced paw oedema in rats (Asano *et al*., 1997). In the present study, we examined the effect of this antagonist on plasma exudation in rat carrageenin-induced pleurisy, in which the plasma kallikrein-kinin system was activated to generate kinin in the pleural cavity. The contribution of generated kinin to induce the inflammatory response has been fully established by us and other groups (Katori *et al*., 1979; Uchida *et al*., 1983; Tissot *et al*., 1985; Majima *et al*., 1996a).

Methods

Animals

Male Sprague-Dawley (SD) rats (8 weeks old, specific pathogen free) were purchased from SLC (Hamamatsu, Japan). All

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animals were kept in constant temperature and humidity, and were deprived of food overnight before experiments.

All the animal experiments were performed in accordance with the Kitasato University School of Medicine guidelines for animal experiments.

Hypotensive responses induced by bradykinin and acetylcholine

Changes in mean arterial blood pressure (MABP) were determined in conscious, unrestrained SD rats as described in our earlier study (Majima *et al*., 1994). Briefly, a polyethylene cannula (PE-10, Clay-Adams, Parsippany, N.J., U.S.A.) was inserted into the abdominal aorta through the femoral artery under light ether anaesthesia and was connected to a PE-50 cannula (Clay-Adams) and exteriorized in the interscapular region. A blood pressure transducer (TP-200T, Nihon Koden, Tokyo) was attached to the other end of the intra-arterial cannula, and MABP was monitored on a polygraph (WS-641-G, Nihon Koden, Tokyo). Another polyethylene cannula (PE-10 plus PE-50) was also inserted into the vena cava and was exteriorized.

On the next day, solutions of bradykinin (BK, 5 nmol kg^{-1}, at a concentration of 5 nmol ml^{-1} in physiological saline containing 0.1% (w/v) gelatine) and acetylcholine (ACh, 50 nmol kg^{-1}, at a concentration of 50 nmol ml^{-1} in physiological saline) were injected intravenously, followed by an injection of physiological saline (1 ml kg^{-1}) to wash out the route of administration.

In the preliminary experiments in another group of animals, BK (1–300 nmol kg^{-1}, i.v.) was injected, and maximum hypotensive responses from the baseline values were estimated. The ED_{50} value of hypotensive response was determined to be 5.1 nmol kg^{-1}. Thus, 5 nmol kg^{-1} BK was used to test the effect of FR173657. ACh (50 nmol kg^{-1}), which induced the same magnitude of hypotension as 5 nmol kg^{-1} BK, was also selected in the present experiment. After the hypotensive response had been recorded without FR173657, this antagonist was administered orally (30 mg kg^{-1}, suspended at 30 mg ml^{-1} with 5% gum arabic). BK (5 nmol kg^{-1}) and ACh (50 nmol kg^{-1}) were administered intravenously 1, 2, 3, 5, and 7 h after the administration of FR173657.

For vehicle control rats, 1 ml kg^{-1} of 5% gum arabic was administered.

Intrapleural injections of bradykinin and histamine

SD rats were lightly anaesthetized with ether and 1.0 ml of a solution of BK (0.3, 1, 3 and 10 nmol ml^{-1}, in sterile physiological saline containing 0.1% (w/v) gelatine) was injected into the right pleural cavity. The rate of exudation of plasma from the circulation into the pleural cavity was determined by the leakage of dye (pontamine sky blue, 50 mg kg^{-1} ml^{-1} saline, Tokyo Kasei, Tokyo, Japan) for 20 min. This dye had been injected intravenously under light ether anaesthesia just before BK injection. Blood was collected during exsanguination for measurements of the concentration of the dye in the serum. The concentrations of dye in the exudate and in the serum were determined spectrophotometrically from the absorbance at 630 nm of known concentrations of the dye. The rate of exudation of plasma was calculated from the amount of dye exuded during the course of 20 min, corrected by reference to the concentration of dye in serum, since the concentration of dye in the serum differed from rat to rat. FR173657 was administered orally 1 h before BK injection at doses of 3, 10, and 30 mg kg^{-1} (suspended at 3, 10 and 30 mg ml^{-1} with 5% gum arabic).

To estimate duration of effectiveness, FR173657 (30 mg kg^{-1}) was administered orally from one to seven hours before BK injection.

To test the selectivity of the antagonist, 1.0 ml of histamine solution (1 μmol ml^{-1} in physiological saline) was injected intrapleurally. FR173657 was administered orally 1 h before histamine injection at a dose of 30 mg kg^{-1}.

Induction of carrageenin-induced pleurisy in rats

SD rats were lightly anaesthetized with ether and 0.1 ml of a solution of λ-carrageenin (2% (w/v) in sterile physiological saline, Picnin-A) was injected into the right pleural cavity as previously described (Katori *et al*., 1979). For the determination of the rate of exudation, pontamine sky blue was used as described above.

FR173657 was administered orally (30 mg kg^{-1}, suspended at 30 mg ml^{-1} with 5% gum arabic) 1 h before carrageenin injection. To estimate the effect of FR173657 in rats more than 5 h after carrageenin injection, the same dose of FR173657 was administered orally again 3.5 h after the carrageenin injection. For vehicle rats, 5% gum arabic solution was given, and then the carrageenin was injected.

To compare the effect of FR173657 with that of a peptide antagonist of the B_2 receptor, Hoe 140 (1 mg kg^{-1}, dissolved in the physiological saline at 1 mg ml^{-1}, Hoechst) was intravenously administered 10 min before carrageenin injection. For vehicle treated rats, only saline was administered (1 ml kg^{-1}, i.v.).

To inhibit the generation of BK from high-molecular-weight (HMW)-kininogen by plasma kallikrein (Oh-ishi & Katori, 1979), a plasma kallikrein inhibitor, soy bean trypsin inhibitor (SBTI), was applied topically. A solution of SBTI (0.3 ml of a 1 mg ml^{-1} physiological saline) was injected into the pleural cavity of the inflamed rats 40 min before the collection of pleural exudate. In vehicle-control rats, only physiological saline (0.3 ml) was injected into the pleural cavity.

Further, brolemain (10 mg kg^{-1} ml^{-1} in physiological saline), a thiolprotease from pineapple stem, was intravenously administered to rats 30 min before the injection of carrageenin to breakdown the prekallikrein and HMW kininogen in plasma (Oh-ishi *et al*., 1979). For vehicle control rats, only physiological saline was administered (1 ml kg^{-1}, i.v.).

Determination of kininogen levels in plasma and pleural exudates

Blood (1.8 ml per rat) was collected from the carotid artery into plastic tubes containing 0.2 ml of 3.8% sodium citrate, and the plasma was separated by centrifugation (1500 *g*, for 15 min at 20°C). The plasma levels of HMW and low-molecular-weight (LMW) kininogens were determined by the method described previously by Uchida *et al*. (1986), in which kininogens were converted to bradykinin, and the amounts of kinin generated were measured by a bradykinin ELISA kit (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan) (Majima *et al*., 1996a). Kininogen levels were expressed as ng bradykinin equivalent mg^{-1} plasma protein.

After collection of blood and exsanguination 3 h after carrageenin injection, the pleural fluid was collected, and 1/9 volume of 3.8% sodium citrate was added and centrifuged (1500 *g*, for 15 min at 20°C). The supernatant was used for the kininogen determination, as described previously (Uchida *et al*., 1983).

The total protein in citrated plasma or exudate was measured by the method of Lowry *et al*. (1951).

Determination of a stable bradykinin metabolite, BK-(1–5), in the pleural exudates

After exsanguination of rats 3 h after carrageenin injection, 5 ml of ice-cold absolute ethanol was injected into the pleural cavity to prevent further degradation of the metabolites of BK. The fluid in the pleural cavity was collected in a plastic tube and centrifuged for 15 min at $1500 \times g$ at 4°C after being kept at 70°C for 10 min. The ethanol extracts (supernatants) were evaporated to dryness and washed with diethylether to remove the lipids. The washed samples were dissolved in 4 ml of distilled water that had been acidified with 0.2 ml of 0.01 N HCl and were applied to a Sep-Pak C_{18} cartridge column. After the samples had been washed with 12 ml of distilled water and

4 ml of 0.1 M acetic acid, BK and products of its degradation were eluted with 6 ml of 80% (v/v) acetonitrile containing 0.1 M acetic acid (Kauker *et al*., 1984). The kinin fraction was evaporated under reduced pressure and the residue was dissolved in 800 μl of the assay buffer, and the levels of BK-(1–5) was determined with a newly developed ELISA kit (Dainippon Pharmaceutical Co. Ltd., Osaka, Japan; Majima *et al*., 1996b).

Induction of compound 48/80-induced pleurisy in rats

SD rats were lightly anaesthetized with ether and 0.5 ml of a solution of a histamine releaser, compound 48/80 (5 μg ml^{-1} (v/v) in sterile physiological saline) was injected into the right pleural cavity as previously described (Tanaka *et al*., 1986). The rate of plasma exudation was also determined as mentioned above.

FR173657 was administered orally (30 mg kg^{-1}, suspended at 30 mg ml^{-1} with 5% gum arabic) 1 h before compound 48/80 injection.

The histamine antagonist, pyrilamine maleate (5 mg ml^{-1}, in physiological saline), and the 5-hydroxytryptamine antagonist, methysergide hydrogen maleinate (3 mg ml^{-1}, in physiological saline), were administered intravenously (1 ml kg^{-1}) 10 min before compound 48/80 injection. For vehicle control rats, physiological saline was injected (1 ml kg^{-1}, i.v.).

Drugs

BK was purchased from Peptide Institute (Minoh, Osaka, Japan). ACh was obtained from Wako Pure Chemicals (Osaka, Japan). FR173657 was supplied from Fujisawa Pharmaceutical Co. Ltd. (Tokyo, Japan). Hoe140 was a gift from Hoechst AG (Frankfurt, Germany). Soy bean trypsin inhibitor (SBTI) was purchased from Worthington Biochemial Co. (Cleveland, OH, U.S.A.). Bromelain was supplied from Jintandorf Corp. Ltd. (Tokyo, Japan). Pyrilamine maleate was a gift from May and Baker (Dagenham, U.K.) and methysergide hydrogen maleinate was from Sandoz AG (Basel, Switzerland). Compound 48/80 was obtained from Sigma, Chemical Corp., (St. Louis, MO, U.S.A.). λ-Carrageenin (Picnin-A) was purchased from Zushi Chemical Institute (Zushi, Kanagawa, Japan) and pontamine sky blue was obtained from Tokyo Kasei (Tokyo, Japan). Other reagents were all of analytical grade and were obtained from commercial sources.

Statistical analysis

Values are expressed as mean ± s.e.mean. For comparison of data from two groups, Student's *t* test was used to evaluate the significance of differences. When variances were heterogeneous, statistical analyses were performed by the Aspin-Welch method or by Wilcoxon's rank sum test.

For comparison of data from multiple groups, one-way ANOVA followed by *post-hoc* Scheffe's test was used.

Results

Effect of FR173657 on BK-induced hypotension

The mean arterial blood pressure (MABP) of non-treated rats was 108 ± 3 mmHg (n = 6). Intravenous injection of BK (5 nmol kg^{-1}, i.v., bolus) caused a rapid but transient reduction in MABP, the maximum reduction was −31.4 ± 3.1 mmHg. ACh (50 nmol kg^{-1}, i.v., bolus) also reduced the MABP, and the magnitude of the reduction was almost the same as that induced by BK (5 nmol kg^{-1}).

FR173657 (30 mg kg^{-1}, p.o.) did not influence the basal MABP throughout the experimental period, but, as shown in Figure 1, it significantly blocked BK-induced hypotensive responses by 95%, 91%, 90% and 79%, 1, 2, 3 and 5 h after administration of FR173657, respectively. By contrast, there

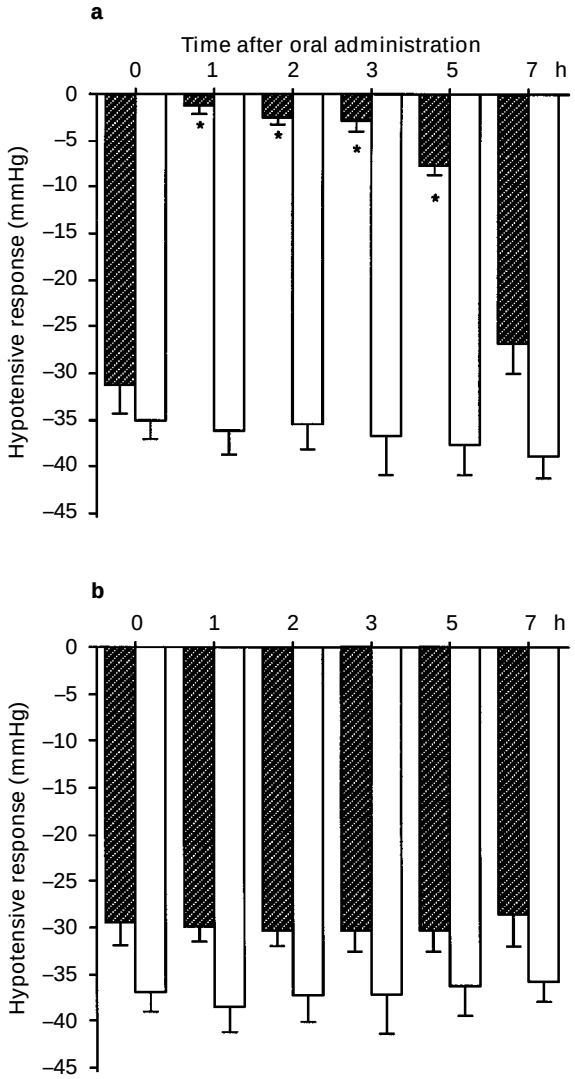

Figure 1 Effect of FR173657 on the hypotension induced by bradykinin and acetylcholine in conscious and unrestrained rats. FR173657 (30 mg kg^{-1}) (a) or vehicle solution (b) was orally administered. Hypotensive responses in mean arterial blood pressure were estimated by the maximum hypotension produced by the bolus intravenous injection of bradykinin (BK, 5 nmol kg^{-1}) or acetylcholine (ACh, 50 nmol kg^{-1}). Each value represents the mean ± s.e.mean from six animals. The hatched columns shows hypotension induced by BK, and the open columns that induced by ACh. Comparisons were made with each basal value (time 0 hour), $*P<0.05$.

were no marked changes in the hypotensive responses induced by intravenous administration of ACh over a 7 h experimental period.

The rats receiving only vehicle solution of FR173657 had the same hypotensive responses to BK or ACh throughout the experimental periods (Figure 1b).

Effect of FR173657 on BK-induced plasma exudation into the pleural cavity

As Figure 2a shows, intrapleural injections of BK (0.3–10 nmol per rat) caused plasma exudation in a dose-dependent manner. This exudation was inhibited by 85–97% by FR173657 (30 mg kg^{-1}, p.o.). Also, Figure 2b shows that the plasma exudation induced by BK 3 nmol per rat was markedly inhibited by oral administration of FR173657 (3–30 mg kg^{-1}, p.o.), the maximum effect being obtained with 30 mg kg^{-1}. The suppressed levels seen with 30 mg kg^{-1} FR173657 were almost the same as those observed in rats treated with only

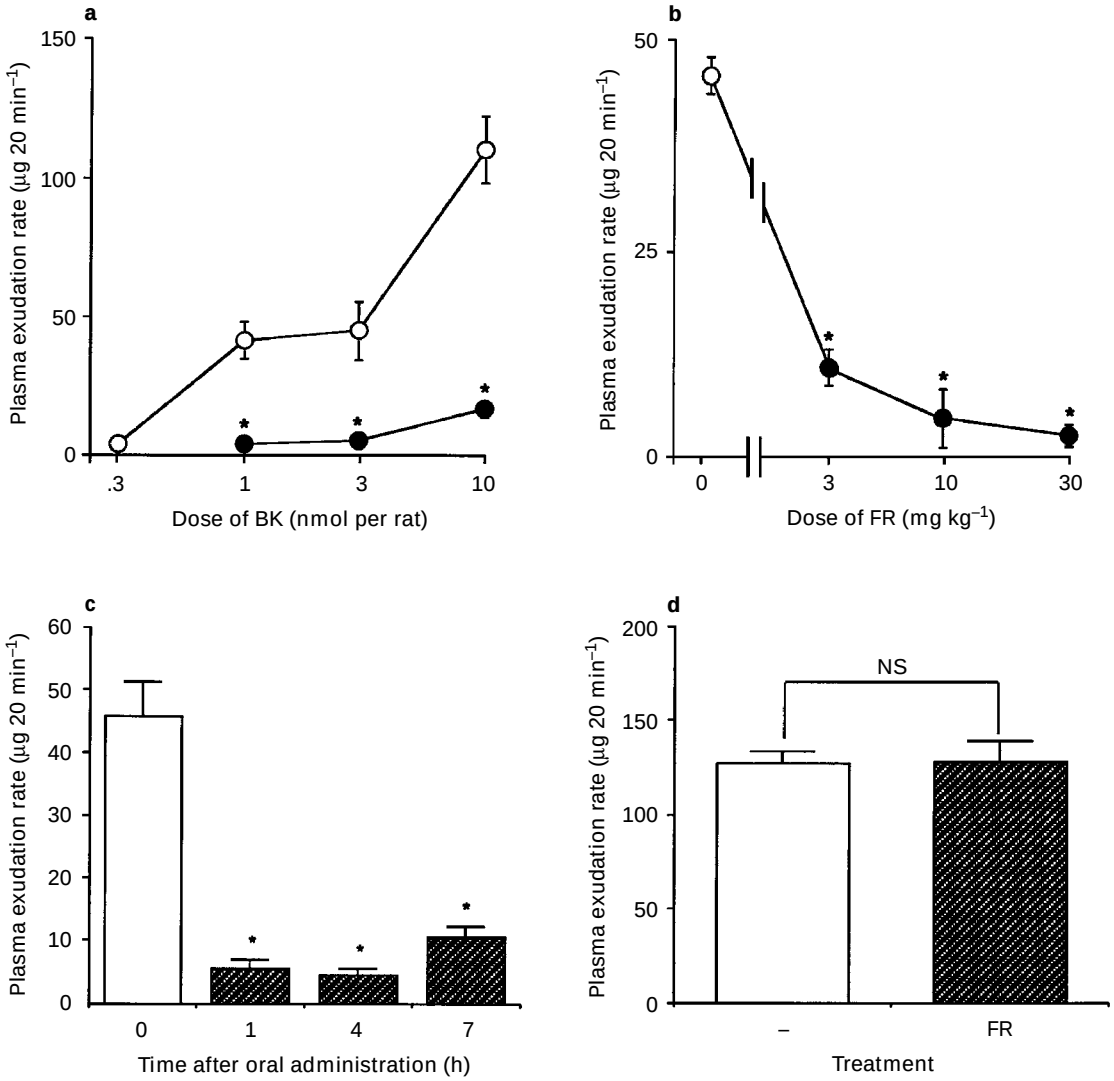

Figure 2 Effect of FR173657 on the plasma exudation into the pleural cavity induced by bradykinin or histamine. In (a), intrapleural injections of bradykinin (BK, 1–10 nmol per rat) were made 1 h after oral administration (30 mg kg^{-1}) of FR173657 (FR). In (b), intrapleural injections of bradykinin (3 nmol per rat) were made 1 h after the oral administration of three different doses (3–30 mg kg^{-1}) of FR. In (c), intrapleural injections of BK (3 nmol per rat) were made at the indicated times after the oral administration of FR (30 mg kg^{-1}). In (d), intrapleural injections of histamine (1 μmol per rat) were made 1 h after oral administration of FR (30 mg kg^{-1}). Each value represents the mean and vertical lines show s.e.mean from four to six animals. The hatched columns and (●) show the results from rats administered FR. Comparisons were made with control values from rats receiving only vehicle solution of FR (○) and open columns. $*P<0.05$.

vehicle (1 ml of saline containing 0.1% gelatine) injections into the pleural cavity (3.2 ± 0.5 μg 20 min^{-1}, $n=4$).

The inhibitory effect of FR173657 (30 mg kg^{-1}, p.o.) persisted for at least 4 h (Figure 2c). Thereafter (7 h after BK), the inhibitory effect on plasma exudation became incomplete.

The plasma exudation induced by intrapleural injection of histamine (1 μmol per rat) was not affected by FR173657 (30 mg kg^{-1}, p.o.) (Figure 2d).

Effect of FR173657 on exudation of plasma and accumulation of pleural fluid in rat carrageenin-induced pleurisy

Figure 3a shows the time course of changes in the volume of exudate in rats with carrageenin-induced pleurisy. The accumulation of pleural exudate in vehicle-treated rats continued to increase for 7 h. FR173657 (30 mg kg^{-1}, p.o.) significantly reduced the volume of exudate 3, 5, and 7 h after carrageenin by 41%, 47%, and 57%, respectively. When different doses of FR173657 (3 and 10 mg kg^{-1}, p.o.) were administered, the volume of exudate 3 h after carrageenin was 0.70 ± 0.08 ml ($n=4$) and 0.55 ± 0.07 ml ($n=4$), respectively; the effect of the higher dose (10 mg kg^{-1}) was stastically significant.

As Figure 3b shows, the rates of exudation of plasma, determined by leakage of dye from the circulation over 20 min periods, peaked at 5 h and were significantly reduced by FR173657 (30 mg kg^{-1}, p.o.) 1, 3, 5, and 7 h after carrageenin (by 52%, 53%, 76% and 67%, respectively). After treatment with FR173657 (3 and 10 mg kg^{-1}, p.o.), the plasma exudation rates 3 h after carrageenin were 96 ± 9 μg 20 min^{-1} ($n=4$) and 73 ± 5 μg 20 min^{-1} ($n=4$), respectively, and the effect of the higher dose (10 mg kg^{-1}) was statistically significant.

Figure 4 depicts the inhibitory effects of agents that modify the kallikrein-kinin system. The plasma exudation 1 h after carrageenin was significantly inhibited by SBTI and bromelain (by 56% and 54%, respectively; Figure 4a). The plasma exudation 3 h after carrageenin injection was also significantly inhibited (by 52%) by a bradykinin B_2 receptor antagonist, Hoe140 (Figure 4b). This was also true in rats treated with SBTI and bromelain, in which the inhibition rates were 62% and 57%, respectively (Figure 4b). Seven hours after carrageenin, the plasma exudation was also significantly inhibited

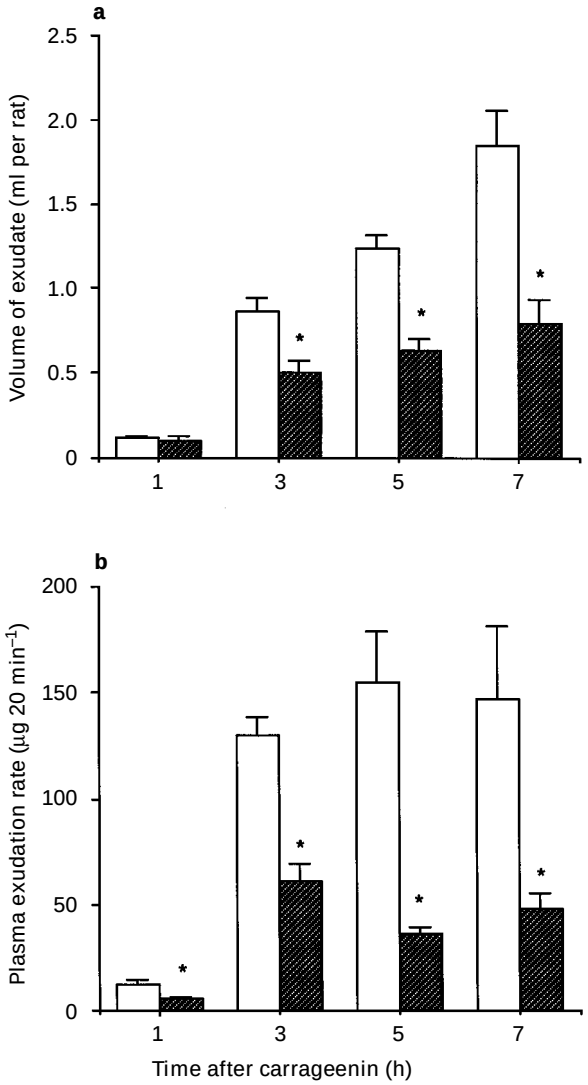

Figure 3 Effects of FR173657 on the volume of the pleural exudates (a) and the plasma exudation rates (b) in rat carrageenin-induced pleurisy. FR173657 was administered orally (30 mg kg^{-1}, hatched columns) 1 h before carrageenin injection and 3.5 h after carrageenin injection (in rats for 5 and 7 h). Each value represents the mean ± s.e.mean from six to nine animals. Comparisons were made with vehicle-treated animals (open columns), $^*P<0.05$.

by SBTI and bromelain (by 75% and 74%, respectively; Figure 4c). These inhibitory effects were essentially the same as those of FR173657 (30 mg kg^{-1}) (Figure 3b).

Changes in the levels of kininogens and a BK degradation product in rat carrageenin-induced pleurisy

Figure 5a depicts the plasma kininogen levels in rats 3 h after carrageenin injection. FR173657 (30 mg kg^{-1}) did not affect plasma HMW or LMW kininogens in rats with carrageenin-induced pleurisy.

In the inflammatory exudates in vehicle control rats with carrageenin-induced pleurisy, the HMW kininogen levels were much lower than those in plasma, although the LMW kininogen levels in the exudates were not different from those in plasma (Figure 5b). FR173657 administration (30 mg kg^{-1}) did not affect the intrapleural kininogen levels in rats with inflammation, and the HMW kininogen levels were also very low in FR173657-treated rats (Figure 5b).

Figure 6 shows the intrapleural levels of the bradykinin degradation product BK-(1 – 5) 3 h after carrageenin injection. The level of BK-(1 – 5) in vehicle-control rats was 8.1 ± 0.5 ng per rat ($n=6$). The administration of FR173657 (30 mg kg^{-1}) significantly (by 59%) reduced the intrapleural levels of BK-(1 – 5).

Effect of FR173657 on exudation of plasma and accumulation of pleural fluid in rat compound 48/80-induced pleurisy

Figure 7a and b shows the time course of changes in the volume of exudate and plasma exudation in rat compound 48/80-induced pleurisy. Compound 48/80 caused obvious plasma exudation at 20 min, although the volume of pleural exudate did not change further after 1, 2 or 3 h in this model. A mixture of pyrilamine and methysergide reduced the plasma exudation by 95%. FR173657 (30 mg kg^{-1}, p.o.) did not reduce the plasma exudation at all, as observed at the initial phase of this model.

Discussion

Abundant knowledge on the components of the kallikrein-kinin system, such as kallikreins, kininogens and kininases has been accumulated since kallikrein was discovered in 1925 by the surgeon Emil-Karl Frey. However, progress in the kinin field has been hampered for the following two reasons; bradykinin receptor antagonists, which were developed previously, are of limited utility *in vivo* because of metabolic lability or poor oral bioavailability, and it is difficult to detect kinin even in the sites where it is generated because of its rapid degradation (Ferreira & Vane, 1967). A very different approach to increasing the potency of bradykinin receptor antagonists was taken by many researchers. To overcome the latter difficulty associated with the detection of kinin, assays for the residual levels of precursor proteins of the kinin-forming system, e.g., plasma prekallikrein (Oh-ishi & Katori, 1979), high- and low-molecular-weight (HMW and LMW) kininogens (Uchida *et al*., 1986), and assays for stable products of kinin degradation (Majima *et al*., 1989; 1993) in biological fluids were developed.

FR173657 is a non-peptide B_2 antagonist which has been developed recently (Asano *et al*., 1997). In the present study, we used rats as experimental animals. FR173657 is considered to be active in rat tissues as it displaced [^{3}H]-BK binding to B_2 receptors in the rat uterus (IC_{50} 1.5×10^{-9} M), as well as in guinea-pig ileum membranes (IC_{50} 5.6×10^{-10} M) and in human lung fibroblast IMR-90 cells (IC_{50} 2.9×10^{-9} M) (Asano *et al*., 1997). In contrast to another non-peptide bradykinin B_2 antagonist, WIN64338 (Salvino *et al*., 1993), FR173657 did not have different potencies dependent upon the species tested. In fact, FR173657 inhibited carrageenin-induced paw oedema in rats (Asano *et al*., 1997). FR173657 is not active on B_1 receptors, as it did not reduce [^{3}H]-des-Arg^{10}-kallidin binding in human lung fibroblast IMR-90 cells (Asano *et al*., 1997). Other non-peptide B_2 receptor antagonists have been synthesized (Salvino *et al*., 1993; Sawutz *et al*., 1994), but FR173657 has been shown to be the most potent B_2 receptor antagonist, with a pA_2 value in guinea-pig isolated ileum of 9.2 (Asano *et al*., 1997). FR173657 also has a high oral bioavailability (Asano *et al*., 1997).

In the present experiments, the oral administration of FR173657 inhibited BK-induced plasma exudation into the pleural cavity significantly (Figure 2b), but did not inhibit histamine-induced plasma exudation (Figure 2d). The plasma exudation into the pleural cavity due to histamine activated neither the plasma kallikrein-HMW kininogen system nor the glandular kallikrein-LMW kininogen system to generate kinin, as judged by the lack of increase in the kinin degradation products (Katori *et al*., 1989a). Thus, it is unlikely that FR173657 would inhibit the plasma exudation elicited by histamine (Figure 2d). These findings indicate that FR173657 has good selectivity for kinin receptors.

Next, we tested the effect of FR173657 on the plasma exudation in rat carrageenin-induced pleurisy, in which the

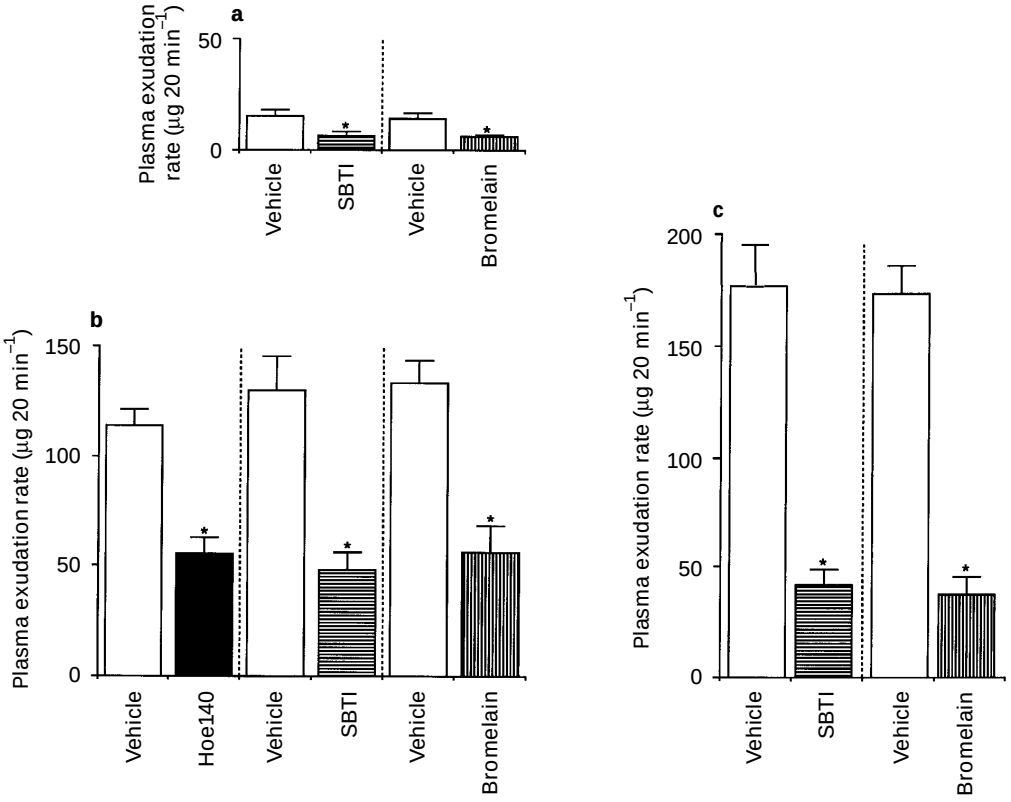

Figure 4 Effects of Hoe140, soy bean trypsin inhibitor (SBTI) and bromelain (hatched or solid columns) on the plasma exudation rates in rat carrageenin-induced pleurisy. (a), (b) and (c) represent the results 1, 3 and 5 h after carrageenin, respectively. Each value represents mean ± s.e.mean from five to eight animals. Comparisons were made with vehicle-treated animals (open columns), $*P<0.05$.

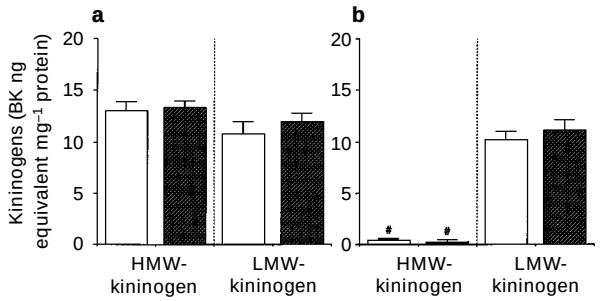

Figure 5 The kininogen levels in the plasma (a) and the pleural exudate (b) in rat carrageenin-induced pleurisy. The open columns depict results from vehicle-treated rats, and the hatched columns those from FR173657-treated rats (30 mg kg^{-1}, p.o.), 3 h after carrageenin injection. Each value represents mean ± s.e.mean from five to six animals. Comparison was made between the kininogen levels in the plasma and the pleural exudate, $\#P<0.05$.

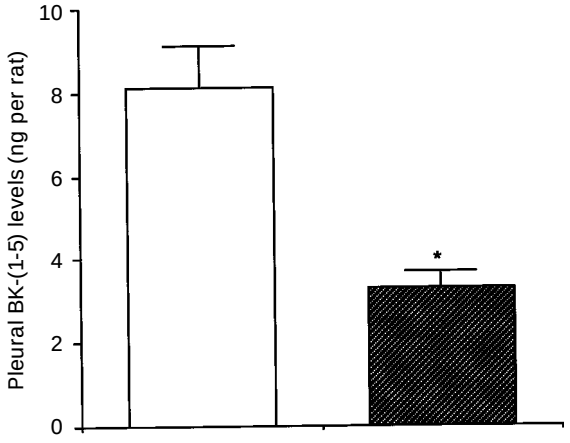

Figure 6 Bradykinin-(1 – 5) levels in the pleural exudate in rat carrageenin-induced pleurisy. Open column depicts results from vehicle-treated rats, and hatched column those from FR173657-treated rats (30 mg kg^{-1}, p.o.), 3 h after carrageenin injection. Each value represents mean ± s.e.mean from five rats. Comparison was made with vehicle-treated rats, $*P<0.05$.

plasma kallikrein-HMW kininogen system is selectively activated to generate kinin in the pleural cavity (Uchida *et al*., 1983). In this model, the BK degradation product BK-(1 – 5) is continuously generated (Majima *et al*., 1996b), and the levels in plasma prekallikrein and HMW kininogen are markedly reduced throughout the experimental period (Uchida *et al*., 1983) as a result of activation of this system. Activated plasma kallikrein acts solely on the HMW kininogen, and not on the LMW kininogen, which is a good substrate for tissue or glandular kallikrein (Uchida & Katori, 1986; Katori *et al*., 1989b). Both the plasma exudation rates and the volumes of accumulated fluid were significantly reduced by orally administered FR173657 (Figure 3). The inhibitory potency of FR173657 at a sufficient dose (such as 30 mg kg^{-1}) was essentially the same as those observed during the treatment with the B_2 receptor blocker Hoe140 or with treatments that inhibit BK generation in the pleural cavity, either by inhibiting activated plasma kallikrein (Oh-ishi & Katori, 1979) or by depletion of precursor proteins with bromelain (Oh-ishi *et al*., 1979). The duration of the effect of FR173657 was more than 4 h, as estimated by the blockade of hypotension and plasma exudation induced by intravenous and intrapleural injections of BK, respectively (Figure 1 and Figure 2c). These findings supported our surmise that FR173657, when administered orally acts on the B_2 receptor to antagonize the kinin generated in the pleural cavity. As Figure 5 shows, even if rats were pretreated with

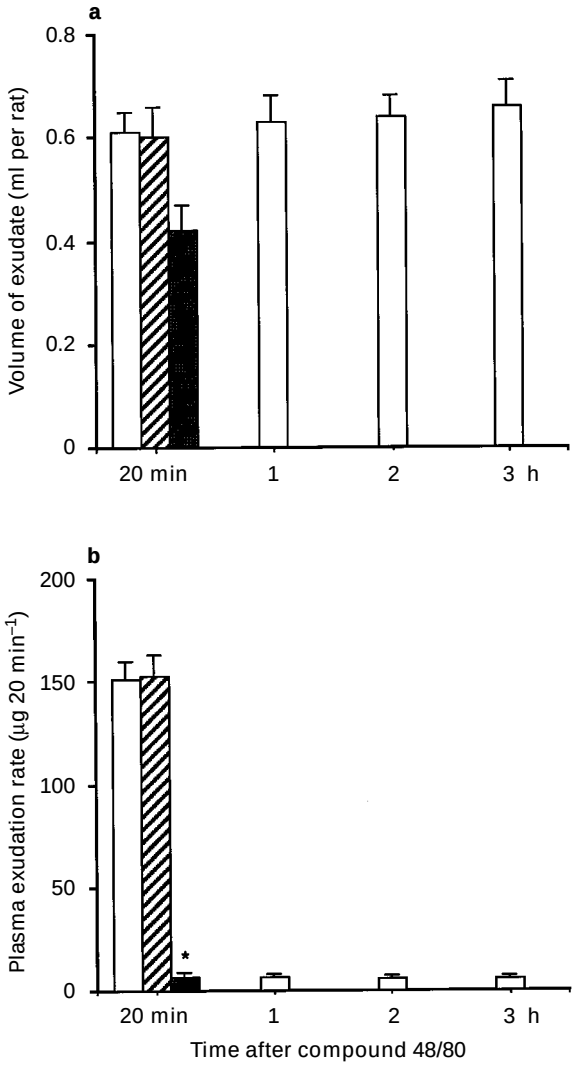

Figure 7 Effects of FR173657 and a cocktail of pyrilamine and methysergide on the plasma exudation rates in rat compound 48/80-induced pleurisy. Open columns depict results from vehicle-treated rats (saline, i.v.), and hatched columns those from FR173657-treated rats (30 mg kg^{-1}, p.o.). Stippled column represents results from pyrilamine plus methysergide-treated rats. Each value represents mean ± s.e.mean from five rats. Comparisons were made with vehicle-treated rats, $*P<0.05$.

FR173657, the HMW kininogen was consumed completely, suggesting the full conversion of HMW kininogen to BK. Since all the plasma newly supplied from the circulation into the pleural cavity becomes the source of the kinin generated in the pleural cavity, FR173657 treatment reduced the formation of kinin by reducing the supply of HMW kininogen in the plasma, which is a substrate for plasma kallikrein. The reduced kinin formation with FR173657 treatment was confirmed by the reduced levels of BK-(1 – 5) in the pleural cavity (Figure 6). FR173657 may block the positive kinin generation feedback mechanism mediated by the increased plasma exudation due to kinin.

In compound 48/80-induced pleurisy, active plasma exudation occurred during the first 20 min after injection, but did not increase further over the 3 h experimental period (Figure 7). In this model, the kallikrein-kinin system was not activated, similar to our observations with histamine-induced pleural exudation. Indeed, the kallikrein-kinin system may be inhibited by mast cell-derived chymase causing degradation of Factor XII, an activator of kinin generation, as found previously (Majima *et al*., 1987; Majima & Katori, 1989). Compound 48/80 releases both histamine and 5-hydroxytryptamine from rodent mast cells (Schwartz & Austen, 1984). The observation that a combination of pyrilamine and methysergide, but not the kinin receptor-selective antagonist, FR173657, inhibited plasma exudation by compound 48/80 supports a role for these amines and not for BK in compound 48/80-induced exudation.

In conclusion, the first orally active, non-peptide B_2 antagonist, FR173657, was shown to be effective in inhibiting plasma exudation in rat carrageenin-induced pleurisy. The effect lasted for more than four hours and was selective for BK. This compound may open a new door to the therapeutic use of B_2 antagonists for kinin-related diseases.

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References

ASANO, M., INAMURA, N., HATORI, C., SAWAI, H., FUJIWARA, T., KATAYAMA, A., KAYAKIRI, H., SATOH, S., ABE, Y., INOUE, T., SAWADA, Y., NAKAHARA, K., OKU, T. & OKUHARA, M. (1997). An orally active, nonpeptide bradykinin B_2 receptor antagonist, FR173657. *Br. J. Pharmacol.*, **120,** 617 – 624.

BHOOLA, K.D., FIGUEROA, C.D. & WORTHY, K. (1992). Bioregulation of kinins: Kallikreins, kininogens, and kininases. *Pharmacol. Rev.*, **44,** 1 – 80.

BURCH, R.M., FARMER, S.G. & STEANKA, L.R. (1990). Bradykinin receptor antagonists. *Med. Res. Rev.*, **10,** 237 – 269.

CHERONIS, J.C., WHALLEY, E.T., NGUYEN, K.T., EUBANKS, S.R., ALLEN, L.G., DUGGAN, M.J., LOY, S.D., BONHAM, K.A. & BLODGETT, J.K. (1992). A new class of bradykinin antagonists: synthesis and in vitro activity of bissuccinimidoalkaline peptide dimers. *J. Med. Chem.*, **35,** 1563 – 1572.

FELETOU, M., LONACHAMPT, M., ROBINEAU, P., JAMONNEAU, I., THURIEAU, C., FAUCHERE, J.L., VILLA, P., GHEZZI, P., PROST, J.F. & CANET, E. (1995). Effects of the bradykinin B_2 receptor antagonist S16118 (p-guanidobenzyl-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin) in different in vivo animal models of inflammation. *J. Pharmacol. Exp. Ther.*, **273,** 1078 – 1084.

FERREIRA, S.H. & VANE, J.R. (1967). The disappearance of bradykinin and eledoisin in the circulation and vascular beds of the cat. *Br. J. Pharmacol.*, **30,** 417 – 424.

HALL, J.M., FIGINI, M., BUTT, S.K. & GEPPETTI, P. (1995). Inhibition of bradykinin-evoked trigeminal nerve stimulation by the non-peptide bradykinin B_2 receptor antagonist WIN64338 *in vivo* and *in vitro*. *Br. J. Pharmacol.*, **116,** 3164 – 3168.

HESS, J.F., BORKOWSKI, J.A., YOUNG, G.S., STRADER, C.D. & RANSOM, R.W. (1992). Cloning and pharmacological characterization of a human bradykinin (BK-2) receptor. *Biochem. Biophys. Res. Commun.*, **184,** 260 – 268.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARD, H.J., WIEMER, G., HENKE, S., BREIPOHL, G., KONIG, W., KNOLLE, J. & SCHOLKENS, B.A. (1991). Hoe 140 a new potent and long acting bradykinin-antagonist: *in vitro* studies. *Br. J. Pharmacol.*, **102,** 769 – 773.

KATORI, M., HARADA, Y., UENO, A., MAJIMA, M., ODA, T. & KAWAMURA, M. (1989a). Modulation of plasma exudation by PGE_2 and that of leukocyte migration by LTB_4 in inflammatory models. *Dermatologia*, **179,** 60 – 63.

KATORI, M., MAJIMA, M., ODI-ADOME, R., SUNSHARA, N. & UCHIDA, Y. (1989b). Evidence for the involvement of a plasma kallikrein-kinin system in the immediate hypotension produced by endotoxin in anaesthetized rats. *Br. J. Pharmacol.*, **78,** 1383–1391.

KATORI, M. & MAJIMA, M. (1996). Pivotal role of renal kallikrein-kinin system in the development of hypertension and approaches to new drugs based on this relationship. *Jpn. J. Pharmacol.*, **70,** 95–128.

KATORI, M., UCHIDA, Y., OH-ISHI, S., HARADA, Y., UENO, A. & TANAKA, K. (1979). Involvement of plasma kallikrein-kinin system in rat carrageenin-induced pleurisy. *Eur. J. Rheumatol. Inflammation*, **2,** 217–219.

KAUKER, M.L., CROFTON, J.T., SHARE, L. & NASJELETTI, A. (1984). Role of vasopressin in regulation of renal kinin excretion in Long-Evans and diabetes insipidus rats. *J. Clin. Invest.*, **73,** 824–831.

LOWRY, O.H., ROSEBROUGH, N.T., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193,** 265–275.

MAJIMA, M. & KATORI, M. (1989). Degradation of Hageman factor by chymotrypsin-like protease released from rat mast cells during degranulation. *Adv. Exp. Med. Biol.*, **247A,** 485–487.

MAJIMA, M. & KATORI, M. (1995). Approaches to the development of novel antihypertensive drugs: crucial role of the renal kallikrein-kinin system. *Trends Pharmacol. Sci.*, **16,** 239–246.

MAJIMA, M., KATORI, M., OGINO, M., SAITO, M., SUGIMOTO, K., ADACHI, K., OHNO, T., SUNAHARA, N., KATOH, K., TATEMICHI, N. & TAKEI, Y. (1996a). Failure of endogenous blood kinin levels elevated by captopril to induce hypotension in normotensive and hypertensive rats: A study using a newly developed ELISA for kinin. *Biomed. Res.*, **17,** 15–25.

MAJIMA, M., MIZOGAMI, S., KURIBAYASHI, Y., KATORI, M. & OH-ISHI, S. (1994). Hypertension induced by a nonpressor dose of angiotensin II in kininogen-deficient rats. *Hypertension*, **24,** 111–119.

MAJIMA, M., NISHIYAMA, K., IGUCHI, Y., YAO, K., OGINO, M., OHNO, T., SUNAHARA, N., KATOH, K., TATEMICHI, N., TAKEI, Y. & KATORI, M. (1996b). Determination of bradykinin-(1–5) in inflammatory exudate by a new ELISA as a reliable indicator of bradykinin generation. *Inflamm. Res.*, **45,** 416–423.

MAJIMA, M., SUNAHARA, N., HARADA, Y. & KATORI, M. (1993). Detection of the degradation products of bradykinin by enzyme immunoassays as markers for the release of kinin in vivo. *Biochem. Pharmacol.*, **45,** 559–567.

MAJIMA, M., TANI, Y. & KATORI, M. (1987). Loss of the activity of human coagulation factor XII by a chymotrypsin-like protease activated in rat mast cells during degradation with compound 48/80. *Thromb. Res.*, **46,** 855–867.

MAJIMA, M., UENO, A., SUNAHARA, N. & KATORI, M. (1989). Measurement of des-Phe^8-Arg^9-bradykinin by enzyme immunoassay – A useful parameter of plasma kinin release. *Adv. Exp. Med. Biol.*, **247B,** 535–540.

MENKE, J.G., BORKOWSKI, J.A., BIERILO, K.K., MACNEIL, T., DERRICK, A.W., SCHNECK, K.A., RANSOM, R.W., STRADER, C.D., LINEMEYER, D.L. & HESS, J.F. (1994). Expression cloning of a human B_1 bradykinin receptor. *J. Biol. Chem.*, **269,** 21583–21586.

OH-ISHI, S. & KATORI, M. (1979). Fluorometric assay method for plasma prekallikrein using peptidylmethyl-coumarylamide as a substrate. *Thromb. Res.*, **14,** 551–559.

OH-ISHI, S., UCHIDA, Y., UENO, A. & KATORI, M. (1979). Bromelain, a thiolprotease from pineapple stem, depletes high-molecular-weight kininogen by activation of Hageman factor (factor XII). *Throm. Res.*, **14,** 665–672.

REGOLI, D. & BARABE, J. (1980). Pharmacology of bradykinin and related peptides. *Pharmacol. Rev.*, **32,** 1–46.

SALVINO, J.M., SEOANE, P.R., DOUTY, B.D., AWAD, M.M., DOLLE, R.E., HOUCK, W.T., FAUNCE, D.M. & SAWUTZ, D.G. (1993). Design of potent non-peptide competitive antagonists of human bradykinin B_2 receptor. *J. Med. Chem.*, **36,** 2583–2584.

SAWUTZ, D.G., SALVINO, J.M., DOLLE, R.E., CASIANO, F., WARD, S.J., HOUCK, W.T., FAUNCE, D.M., DOUTY, B.D., BAIZMAN, E., AWAD, M.M., MARCEAU, F. & SEOANE, P.R. (1994). The nonpeptide WIN 64338 is a bradykinin B_2 receptor antagonist. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 4693–4697.

SCHWARTZ, L.B. & AUSTEN, K.F. (1984). Structure and function of the chemical mediators of mast cells. *Prog. Allergy*, **34,** 271–321.

STEWART, J.M. (1995). Bradykinin antagonist: development and applications. *Biopolymers*, **37,** 143–155.

TANAKA, K., UENO, A. & KATORI, M. (1986). Roles of leukotrienes in two rat allergic inflammatory models: IgE-mediated and IgG-antigen complex-induced pleurisies. *Prostaglandins*, **31,** 1099–1116.

TISSOT, M., REGOLI, D. & GIROUD, J.P. (1985). Bradykinin levels in inflammatory exudates. *Inflammation*, **9,** 419–424.

UCHIDA, Y. & KATORI, M. (1986). Independent consumption of high and low molecular weight kininogens *in vivo*. *Adv. Exp. Med. Biol.*, **198,** 113–117.

UCHIDA, Y., MAJIMA, M. & KATORI, M. (1986). A method of determination of human plasma HMW and LMW kininogen levels by bradykinin enzyme immunoassay. *Pharmacol. Res. Commun.*, **18,** 831–846.

UCHIDA, Y., TANAKA, K., HARADA, Y., UENO, A. & KATORI, M. (1983). Activation of plasma kallikrein-kinin system and its significant role in rat carrageenin-induced pleurisy. *Inflammation*, **7,** 121–131.

VAVREK, R. & STEWART, J.M. (1985). Competitive antagonists of bradykinin. *Peptides*, **6,** 161–164.

WIRTH, K., HOCK, F.J., ALBUS, U., LINZ, W., ALPERMANN, H.G., ANAGNOSTOPOULOS, H., HENKE, S., BREIPOHL, G., KONIG, W., KNOLLE, J. & SCHOLKENS, B.A. (1991). Hoe 140 a new potent and long acting bradykinin-antagonist: in vivo studies. *Br. J. Pharmacol.*, **102,** 774–777.

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