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Effects of the orally active non-peptide bradykinin B_2 receptor antagonist, FR173657, on plasma extravasation in guinea pig airways

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Abstract

We investigated the effect of the orally active non-peptide bradykinin B_2 receptor antagonist, FR173657 (*E*)-3-(6-acetamido-3-pyridyl)-*N*-[*N*-[2,4-di-chloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-*N*-methy-laminocarbonylmethyl] acrylamide), on plasma extravasation mediated by activation of sensory nerves in guinea pig airways. Plasma extravasation was assessed by the photometric measurement of the extravasated Evans blue after formamide extraction. We found that the increase in Evans blue dye extravasation evoked by an aerosol of bradykinin (0.1 mM, 2 min) in the presence of phosphoramidon (2.5 mg/kg, i.v.) was abolished completely by FR173657 (20 mg/kg, p.o.) in the trachea and main bronchi. In sensitized guinea pigs pretreated with phosphoramidon, FR173657 (20 mg/kg, p.o.) inhibited plasma extravasation evoked by ovalbumin aerosol (5%, 2 min) by 77 ± 14.2% in the trachea and 65 ± 11.2% in the main bronchi. FR173657 (20 mg/kg, p.o.) did not affect the plasma extravasation caused by aerosolised capsaicin. These findings suggest that FR173657 is an orally active, promising anti-inflammatory agent for kinin-dependent inflammation following antigen challenge.

Keywords: Bradykinin; Bradykinin B_2 receptor antagonist; FR173657; Plasma extravasation; Airway; Antigen challenge

1. Introduction

Bradykinin, a nonapeptide produced by kallikrein, is a powerful proinflammatory autacoid (Regoli and Barabé, 1980). Bradykinin may cause inflammation by a direct action on bradykinin receptors, on effector cells, or via indirect mechanisms. For instance, part of the inflammatory response produced by locally applied bradykinin in certain tissues, such as the airways, is due to stimulation of the release of neuropeptides, including tachykinins, from peripheral endings of primary sensory neurons (Saria et al., 1988; Geppetti et al., 1988). There is growing evidence that endogenously released bradykinin also causes inflammation via sensory neuropeptide release (Bertrand and Geppetti, 1996; Lu et al., 1997). These and other findings from human studies have suggested the hypothesis that inflammatory responses induced by bradykinin may play a role in airway diseases such as asthma (Church and Levi-Schaffer, 1997).

Two subtypes of bradykinin receptors (B_1 and B_2 receptors) have been identified by pharmacological analysis and molecular cloning (Regoli and Barabé, 1980; Hess et al., 1992; Menke et al., 1994). Bradykinin B_2 receptors mediate most of the biological action of the kinins in the airway (Bhoola et al., 1992). HOE140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) is a highly potent and long-acting bradykinin B_2 receptor antagonist that has been widely used to inhibit bradykinin-induced inflammatory responses both in vitro and in vivo (Wirth et al., 1991). For instance, HOE140 blocks bradykinin-induced plasma extravasation in the guinea pig trachea and reduces that evoked by aerosolized antigen challenge in sensitized guinea pigs (Bertrand et al., 1993a). However, this antagonist is a peptide analog with limited therapeutic use because of its poor oral bioavailability. Especially when the antagonists are used as treatment for a chronic disease, oral activity is a prerequisite.

Recently, FR173657 (*E*)-3-(6-acetamido-3-pyridyl)-*N*-[*N*-[2,4-di-chloro-3-[(2-methyl-8-quinolinyl)oxymethyl]-phenyl]-*N*-methy-laminocarbonylmethyl] acrylamide), an orally active non-peptide bradykinin B_2 receptor antagonist has been developed (Asano et al., 1997). Oral adminis-

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tration of FR173657 inhibits bradykinin-induced bronchoconstriction in guinea pigs in vivo (Griesbacher et al., 1997). However, no information is available regarding the ability of FR173657 to inhibit microvascular leakage induced by bradykinin in the airways. The aim of the present study was two-fold. Firstly, we tested whether FR173657 modulates bradykinin-induced plasma extravasation. Secondly, we examined whether FR173657 inhibits antigen-induced plasma extravasation in sensitized guinea pig airways.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (Japan SLC) weighing 300–500 g at the time of housing, were used in this study. They were kept under constant temperature and humidity, and were deprived of food overnight before the experiments.

2.2. Sensitisation procedure

The animals were sensitised according to a protocol described previously (Dunn et al., 1988; Yoshihara et al., 1995) that consisted of injection of 70 mg ovalbumin (Grade V) in 1.5 ml 0.9% NaCl intraperitoneally twice with a 1-week interval between injections. The animals were studied 2 weeks after the second injection.

2.3. Experimental design

On the day of the experiment, the guinea pigs (300–500 g) were anaesthetised with sodium pentobarbital (50 mg/kg, i.p.). A midline cervical incision was made to expose the larynx and upper trachea. The trachea was incised immediately below the larynx, and a cannula was inserted 4 mm into the trachea. The animals were then ventilated artificially at a frequency of 70 breaths/min with a tidal volume of 6 ml (Bertrand et al., 1993a) using a constant volume ventilator (Rodent Ventilator Model 683, Harvard Apparatus, Millis, MA, USA). The bradykinin B_2 receptor antagonist, FR173657 (5, 10 and 20 mg/kg) suspended in 0.5% methylcellulose solution or vehicle was administered through the oesophageal cannula 30 min before the inhalation of bradykinin, ovalbumin or capsaicin (Asano et al., 1997). Pretreatment was with HOE140 (0.1 μmol/kg) or vehicle injected 10 min before the inhalation of these aerosols (Hock et al., 1991; Wirth et al., 1991). Phosphoramidon (2.5 mg/kg, i.v.) dissolved in 0.9% NaCl (Piedimonte et al., 1993) was injected into the jugular vein over a 10-s period 5 min before the inhalation. We used Evans blue dye (3% solution in 0.9% NaCl) to measure plasma extravasation. Immediately after the injection of the dye (30 mg/kg, i.v. over 5 s) in the jugular vein, bradykinin (0.1 mM), ovalbumin (5%) or capsaicin (0.5 μM) aerosol was delivered for 2 min via the tracheal cannula, using an ultrasonic nebuliser, (Pulmo-Sonic model 25, De Vilbiss, Somerset, PA; aerosol delivery rate 0.2 ml/min). The vehicle of bradykinin and ovalbumin was 0.9% NaCl. The vehicle of capsaicin was 0.2 ml 99% ethanol and two drops of Tween 80 and the volume was adjusted to 10 ml with 0.9% NaCl. The chest was opened 10 min after injection of the tracer, a cannula was inserted into the ascending aorta through the left ventricle, and the circulation was perfused for 3 min with a phosphate buffer, PH 5, at a pressure of 120 mm Hg. The trachea and main bronchi were then removed, opened longitudinally along the ventral midline, blotted on absorbent paper, and weighed. The tissues were incubated in 3 ml of formamide at 50°C for 18 h to extract the extravasated Evans blue dye (Lundberg and Saria, 1983). The results were compared with those obtained from animals not treated with FR173657.

2.4. Measurement of plasma extravasation

Extravasation of the dye-labelled macromolecules was assessed by measuring the optical density of the formamide extracts at a wavelength of 620 nm with a spectrophotometer (model 220A, Hitachi, Japan). The amount of Evans blue dye extravasated from the tissue, expressed in ng/mg wet weight, was interpolated from a standard curve of Evans blue dye concentrations.

2.5. Drugs

Bradykinin, phosphoramidon and HOE140 were purchased from Peptide Institute (Osaka, Japan). Capsaicin was obtained from Wako Pure Chemicals (Osaka, Japan). FR173657 was supplied from Fujisawa Pharmaceutical (Tokyo, Japan). Ovalbumin was obtained from Sigma (St. Louis, MO).

2.6. Statistical analysis

All data are expressed as means ± S.E.M. Statistical comparisons were performed using a one-way analysis of variance and Dunnett's test or bilateral unpaired Student's *t*-test, when appropriate. In all cases, a P value of less than 0.05 was considered significant.

3. Results

3.1. Effect of FR173657 on bradykinin-induced plasma extravasation

The extravasation of Evans blue dye in the trachea and main bronchi of guinea pig after exposure to aerosolised

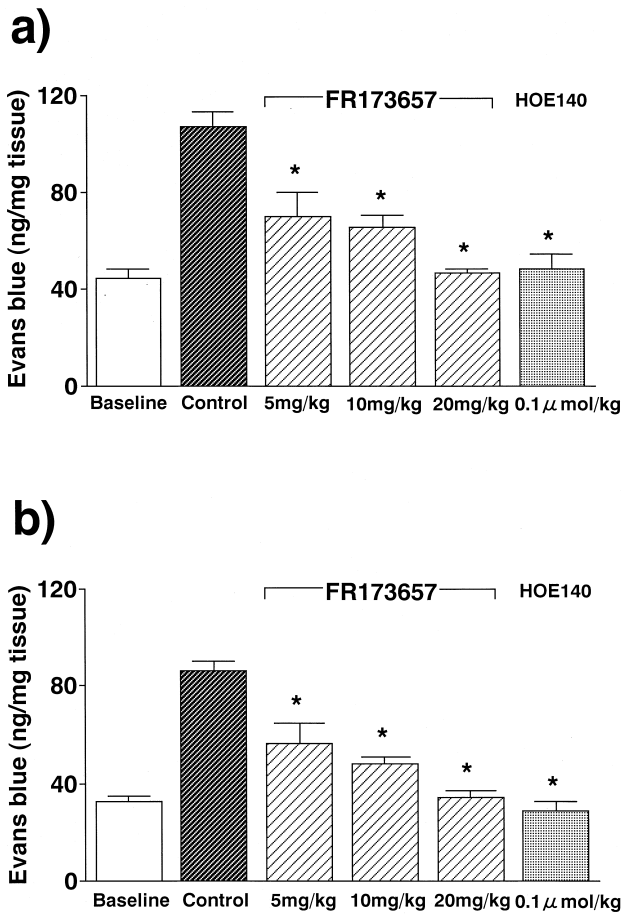

Fig. 1. Effect of FR173657 (5, 10 and 20 mg/kg = 4.2, 8.4 and 16.8 μmol/kg) or HOE140 (0.1 μmol/kg) on Evans blue extravasation in guinea pig trachea (a) and main bronchi (b) induced by inhalation of bradykinin (0.1 mM, 2 min) in presence of phosphoramidon (2.5 mg/kg, i.v., 5 min before exposure). Plasma extravasation was evaluated by measuring the amount of Evans blue dye extravasated in the trachea after 10 min. Values are means ± S.E.M; $n = 5$ per group. $^{*}P < 0.05$ vs. control (inhalation of OVA in presence of phosphoramidon) in both trachea and main bronchi.

vehicle of bradykinin and in the presence of phosphoramidon (2.5 mg/kg, i.v.) was 44.8 ± 3.5 ng/mg ($n = 5$, Fig. 1a) and 32.3 ± 2.2 ng/mg ($n = 5$, Fig. 1b), respectively. Injection of the vehicle for bradykinin (0.9% NaCl) did not affect baseline plasma extravasation (data not shown). Inhalation of 0.1 mM bradykinin for 2 min in the presence of phosphoramidon (2.5 mg/kg, i.v.) increased the extravasation of Evans blue dye significantly in both the trachea (104.3 ± 6.1 ng/mg, $n = 5$; Fig. 1a) and the main bronchi (85.9 ± 4.3 ng/mg, $n = 5$; Fig. 1b). Pretreatment with FR173657 (5, 10, and 20 mg/kg = 4.2, 8.4, and 16.8 μmol/kg, p.o.) 30 min before aerosol challenge, significantly and dose dependently reduced the bradykinin-induced plasma extravasation in the trachea (70.0 ± 9.9, 65.9 ± 5.0, and 46.8 ± 1.7 ng/mg, $P < 0.05$, $n = 5$ each; Fig. 1a) and in the main bronchi (56.2 ± 8.2, 47.8 ± 3.0, and 34.3 ± 2.9 ng/mg, $P < 0.05$, $n = 5$ each; Fig. 1b). Finally, pretreatment with HOE140 (0.1 μmol/kg, i.v.) 5 min before aerosol challenge, abolished the plasma extravasation in the trachea (48.5 ± 5.9 ng/mg, $n = 5$; Fig. 1a) and in the main bronchi (28.5 ± 3.7 ng/mg, $n = 5$; Fig. 1b). Inhibition of bradykinin-induced plasma extravasation caused by FR173657 (20 mg/kg = 16.8 μmol/kg, p.o.) was not significantly different from the inhibition caused by HOE140 (0.1 μmol/kg, i.v.) either in the trachea or main bronchi.

3.2. Effect of FR173657 on ovalbumin-induced plasma extravasation

The extravasation of Evans blue dye in the trachea and main bronchi of guinea pig after exposure to aerosolised vehicle of ovalbumin and in the presence of phosphoramidon (2.5 mg/kg, i.v.) was 40.5 ± 5.8 ng/mg ($n = 5$, Fig. 2a) and 23.2 ± 3.8 ng/mg ($n = 5$, Fig. 2b), respectively.

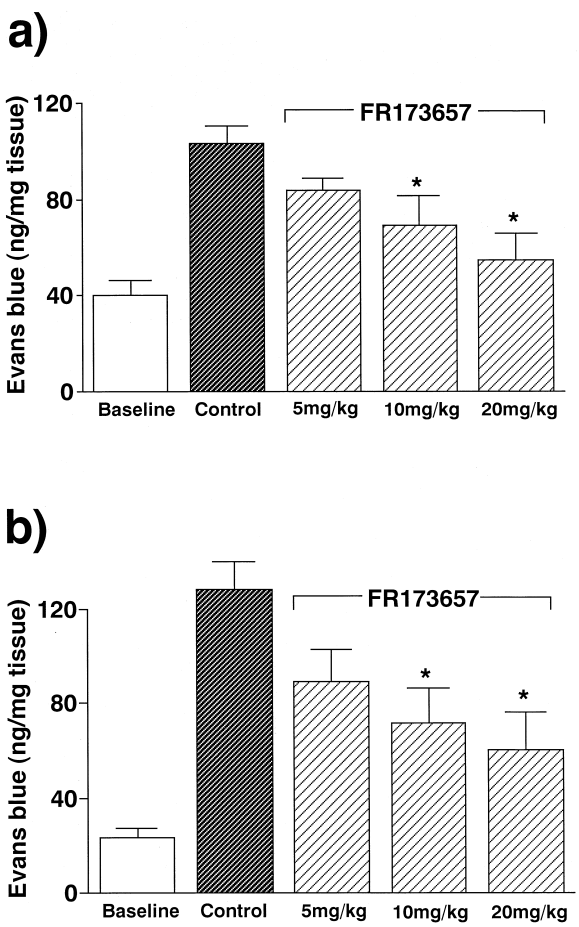

Fig. 2. Effect of FR173657 (5, 10 and 20 mg/kg = 4.2, 8.4 and 16.8 μmol/kg) on Evans blue extravasation in guinea pig trachea (a) and main bronchi (b) induced by inhalation of ovalbumin (OVA; 5% for 2 min) in presence of phosphoramidon (2.5 mg/kg, i.v., 5 min before exposure). Plasma extravasation was evaluated by measuring the amount of Evans blue dye extravasated in the trachea after 10 min. Values are means ± S.E.M; $n = 5$ per group. $^{*}P < 0.05$ vs. control (inhalation of OVA in presence of phosphoramidon) in both trachea and main bronchi.

Inhalation of 5% ovalbumin for 2 min in the presence of phosphoramidon (2.5 mg/kg, i.v.) increased the extravasation of Evans blue dye significantly in both the trachea (103.3 ± 6.9 ng/mg, $n = 5$; Fig. 2a) and the main bronchi (128.5 ± 11.9 ng/mg, $n = 5$; Fig. 2b). Pretreatment with FR173657 (5, 10, and 20 mg/kg = 4.2, 8.4, and 16.8 μmol/kg, p.o.) 30 min before aerosol challenge significantly and dose dependently reduced the antigen-induced plasma extravasation in the trachea (83.3 ± 5.0, 68.9 ± 12.7, and 54.8 ± 10.8 ng/mg, $P < 0.05$, $n = 5$ each; Fig. 2a) and in the main bronchi (89.1 ± 13.9, 71.7 ± 14.7, and 60.1 ± 16.1 ng/mg, $P < 0.05$, $n = 5$ each; Fig. 2b). FR173657 vehicle did not affect the ovalbumin-, bradykinin (see 3.1)- or capsaicin (see 3.3)-induced plasma extravasation (data not shown).

3.3. Effect of FR173657 on capsaicin-induced plasma extravasation

The extravasation of Evans blue dye in the trachea and main bronchi of guinea pig after exposure to the aerosolised vehicle for capsaicin in the presence of phosphoramidon (2.5 mg/kg, i.v.) was 38.3 ± 5.0 ng/mg ($n = 5$, Fig. 3a) and 22.9 ± 3.0 ng/mg ($n = 5$, Fig. 3b), respectively. Inhalation of 0.5 μM capsaicin for 2 min in the presence of phosphoramidon (2.5 mg/kg, i.v.) increased the extravasation of Evans blue dye significantly in both the trachea (59.6 ± 5.9 ng/mg, $n = 5$; Fig. 3a) and the main bronchi (81.6 ± 7.3 ng/mg, $n = 5$; Fig. 3b). Pretreatment with FR173657 (20 mg/kg = 16.8 μmol/kg, p.o.) 30 min before capsaicin aerosol challenge, did not affect the increase in plasma extravasation in the trachea (59.5 ± 7.1 ng/mg, $n = 5$; Fig. 3a) or in the main bronchi (83.3 ± 8.7 ng/mg, $n = 5$; Fig. 3b).

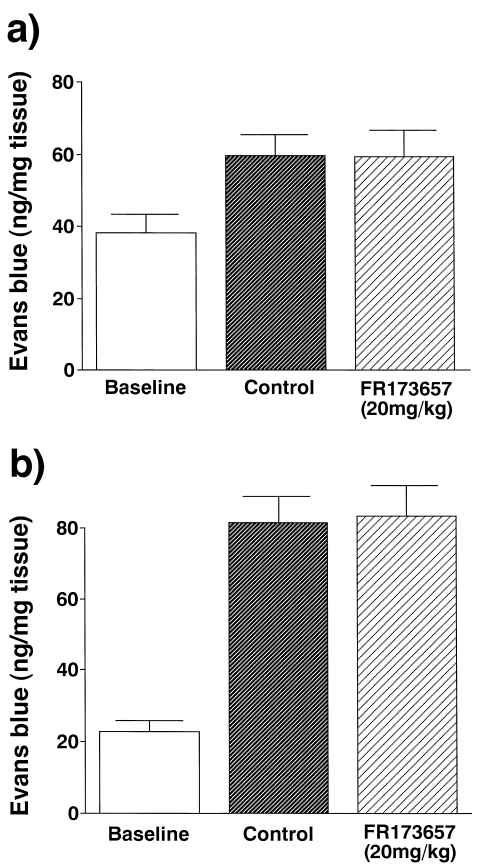

Fig. 3. Effect of FR173657 (20 mg/kg = 16.8 μmol/kg) on Evans blue extravasation in guinea pig trachea (a) and main bronchi (b) induced by inhalation of capsaicin (0.5 μM, 2 min) in presence of phosphoramidon (2.5 mg/kg, i.v., 5 min before exposure). Plasma extravasation was evaluated by measuring the amount of Evans blue dye extravasated in the trachea after 10 min. Values are means ± S.E.M; $n = 5$ per group.

4. Discussion

The pathophysiological role of kinins in various models of inflammatory diseases has been determined with various methods including the use of receptor antagonists. In this respect, one of the most successful compounds has been the potent and selective antagonist, HOE140 which binds with high affinity to bradykinin B_2 receptors. For instance, HOE140 was shown to inhibit antigen-induced bronchoconstriction and plasma extravasation in sensitized guinea pigs (Bertrand et al., 1993b; Bertrand and Geppetti, 1996). However, this compound is a peptide analog, and its possible therapeutic use is limited because of its poor oral availability. Antagonists of a non-peptide nature may overcome this problem. The first non-peptide bradykinin B_2 receptor antagonist, WIN64338, showed low affinity for bradykinin B_2 receptors in some species (Salvino et al., 1993).

More recently, the discovery of a selective non-peptide receptor antagonist, FR173657, which blocks with high affinity bradykinin B_2 receptors in a variety of mammalian species has been reported (Asano et al., 1997). Its activity to block bradykinin-mediated responses, including bradykinin-induced bronchoconstriction even after oral administration, has been proved (Griesbacher et al., 1997). The major finding of the present study was that orally administered FR173657 abolished another important sign of inflammation induced by bradykinin, the extravasation of plasma proteins in the guinea pig trachea and main bronchi. The inhibitory effect of FR173657 was dose-dependent and, at the maximum dose used, was complete and similar to the inhibition obtained with an intravenous dose of HOE140.

The selectivity of the inhibitory action of the maximum dose of FR173657 for bradykinin-mediated responses was demonstrated by experiments in which capsaicin was used as a stimulus. Capsaicin stimulates directly the terminals of peptide-containing primary sensory neurons, via the activation of a recently cloned vanilloid receptor/channel

(Caterina et al., 1997). This stimulation leads to the release of sensory neuropeptides, including the tachykinins, substance P and neurokinin A, that stimulate tachykinin NK_1 receptors on postcapillary endothelial cells (Bowden et al., 1994), inducing leakage of plasma proteins from the lumen to the interstitial space. Both capsaicin-induced stimulation of the vanillod receptor/channel and the activation by tachykinins of endothelial tachykinin NK_1 receptors are phenomena independent of bradykinin generation and/or bradykinin B_2 receptor stimulation. Thus, the observation that FR173657 did not affect the plasma extravasation response to a moderate dose of capsaicin indicates selectivity of the oral dose of FR173657 used in the present study.

Another important finding from the present work was the observation that FR173657 decreased in a dose-dependent manner the plasma extravasation produced by antigen challenge in sensitized guinea pigs. The involvement of bradykinin in the anaphylactic response in the guinea pig airways has been reviewed recently (Bertrand and Geppetti, 1996). Bradykinin is likely released in a first phase of the inflammatory response to antigen and subsequently may stimulate sensory nerves. Tachykinins released from airways sensory nerves following bradykinin stimulation cause leakage of plasma proteins and should be considered as the final mediators of this inflammatory cascade (Bertrand and Geppetti, 1996). Indeed, bradykinin has been found markedly increased in the bronchoalveolar lavage fluid from sensitized guinea pigs 10 min after antigen challenge (Erjefält et al., 1993), and the role of tachykinins in the anaphylactic airway plasma extravasation and bronchoconstriction in guinea pigs has now been confirmed (Kudlacz et al., 1996). The present findings further support the view that kinins released during anaphylaxis play a major role to increase plasma extravasation in the guinea pig airways. The observation that oral FR173657 caused a marked reduction in plasma extravasation in the trachea ($77 \pm 14.2\%$) and main bronchi ($65 \pm 11.2\%$) indicates the substantial role of bradykinin, in particular when the catabolism of tachykinins and kinins by neutral endopeptidase is inhibited.

Kininogenase activity and kinin immunoreactivity are increased in bronchoalveolar lavage fluid of asthmatic patients (Christiansen et al., 1992). Bradykinin has been shown to cause bronchoconstriction in asthmatic patients (Fuller et al., 1987). Bradykinin-induced bronchoconstriction is markedly increased in moderate asthmatics by inhibition of the L-arginine nitric oxide pathway (Ricciardolo et al., 1996) or in severe asthma under baseline conditions (Ricciardolo et al., 1997), when, possibly, the release of protective NO is impaired and the activity of peptidases that metabolize proinflammatory peptides is down-regulated. The precise role of kinins in asthma remains to be determined. However, the pharmacodynamic and pharmacokinetic characteristics of FR173657 propose this compound as a suitable tool to clarify this point and as a possible new therapeutic agent in asthma.

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