

Role of sensory neuropeptides in post-allergic propranolol-induced bronchoconstriction in guinea pigs *in vivo*

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Summary

Background Administration of propranolol can provoke bronchoconstriction in asthmatic patients. We hypothesized that such bronchoconstriction may result from the inflammatory mediators released by an allergic reaction. We recently developed a guinea pig model for propranolol-induced bronchoconstriction (PIB). Neuropeptides which are released from C-fibre nerve endings have been postulated to induce bronchial hyperresponsiveness through neurogenic inflammation.

Objective The purpose of this study was to examine whether sensory neuropeptides are involved in the development of PIB after allergic reaction.

Methods Propranolol at a concentration of 10 mg/ml was inhaled 20 min after antigen challenge in passively sensitized, anaesthetized and artificially ventilated guinea pigs. The animals were treated intravenously with a NK₁ and NK₂ dual antagonist, FK224, in a dose of 1 or 10 mg/kg or vehicle or a selective NK₁ antagonist, FK888, in a dose of 1 or 10 mg/kg or vehicle 10 min before or 15 min after antigen challenge.

Results Propranolol inhaled 20 min after antigen challenge caused bronchoconstriction. FK224 or FK888 administered 15 min after antigen challenge as well as 10 min before antigen challenge did not reduce the PIB.

Conclusion We conclude that neuropeptides such as neurokinin A and substance P do not directly contribute to the development of PIB after allergic reaction.

Keywords: propranolol-induced bronchoconstriction, guinea pigs, sensory neuropeptides, NK₁ receptors, NK₂ receptors.

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Introduction

Propranolol can cause bronchoconstriction in asthmatic patients, whether it is administered orally, intravenously, or by inhalation [1,2]. Propranolol challenge of asthmatic patients leads to bronchoconstriction usually within 10 min [3,4]. The reaction can be severe, prolonged, and difficult to reverse. We [5] recently reported that propranolol-induced bronchoconstriction (PIB)

developed within a few minutes when propranolol was inhaled 20 min after challenge with aerosolized antigen in passively sensitized and artificially ventilated guinea pigs. The pathogenesis of PIB is unclear. Some researchers [6] have proposed that cholinergic nerve activity may play a role. Mast cells in human lung possess β -receptors on their surface [7]. Beta-adrenoreceptor agonists have been shown to inhibit the anaphylactic release of mediators, including histamine, slow-reacting substance of anaphylaxis (SRS-A; leukotriene [LT] C₄, D₄, and E₄) and thromboxane A₂ [8]. Histamine was found to be involved in the PIB in asthmatic patients [9]. Our previous studies [5,10] showed that thromboxane A₂ and 5-lipoxygenase

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products are important in the PIB. These findings suggest that inflammatory mediators contribute to the development of PIB.

Airway sensory C-fibres provide an afferent limb for central neural reflex control of airway function and also synthesize and store neuropeptides, such as substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP), in granules found within their sensitive terminal varicosities. It has been shown that SP or NKA causes bronchoconstriction in animals [11] and in human asthmatic subjects [12–14] and potentiates bronchial responsiveness in animals [15–18]. We [19] recently showed that a NK₁ and NK₂ dual antagonist FK224 [20] but not a selective NK₁ antagonist FK888 [21] inhibited bronchial hyperresponsiveness to inhaled methacholine induced by allergic bronchoconstriction when these compounds were intravenously administered 5 min before the inhalation of antigen.

The purpose of this study was to examine whether sensory neuropeptides are involved in the PIB. We assessed the effect of FK224 and FK888 on the development of PIB following allergic reaction in guinea pigs.

Materials and methods

Animals

Male, albino, Hartley strain guinea pigs weighing 350 to 400 g were obtained from Sankyo Laboratory Service (Toyama, Japan). After arrival at the Institute of Animal Experiments in our university, they were kept in conventional animal housing facilities for 1 week before use. They were allowed to drink and feed *ad libitum*.

Passive sensitization of guinea pigs

Guinea-pig homocytotropic antiserum was obtained by the method of Santives *et al.* [22]. Briefly, 500 µg of ovalbumin (OA) was emulsified in Freund's complete adjuvant and injected intradermally into each guinea-pig at multiple sites. A booster dose was prepared and administered in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after the booster dose, pooled, and kept frozen until use. The antibody titre of this serum was 1:12 800, 1:6400, and 1:512, as estimated by passive cutaneous anaphylaxis at 4 h, 24 h, and 7 days, respectively. Normal guinea-pigs were passively sensitized with 1.0 ml antiserum/kg intraperitoneally.

Preparation of animals

Between 24 and 48 h after the passive sensitization, guinea-pigs were anaesthetized with sodium pentobarbital

(75 mg/kg intraperitoneally). They were placed in the supine position, and the trachea was cannulated with a polyethylene tube (outside diameter 2.5 mm, inside diameter 2.1 mm). The left jugular vein was cannulated for administration of drugs.

After surgery, each guinea-pig was artificially ventilated by a small animal respiratory pump (Model 1680, Harvard Apparatus Co. Inc., South Natick, MA). The tidal volume was 10 ml/kg and the rate was 60 strokes/min. The changes in lung resistance to inflation, defined as the lateral pressure of the tracheal tube or pressure at the airway opening [Pao (cmH₂O)] was measured using a pressure transducer (Model TP-603T, Nihon Koden Kogyo Co. Ltd, Tokyo, Japan). The modified method of Konzett and Rossler [23] described by Jones and co-workers [24] was used to measure these changes. Since we [25] demonstrated that the change in Pao following inhalation of leukotriene C₄ (LTC₄) represented the average of the changes in pulmonary resistance and reciprocal dynamic lung compliance, we used Pao as an overall index of the bronchial response to bronchoactive agents.

When all procedures were completed, the animals were administered diphenhydramine hydrochloride (60 mg/kg intraperitoneally) to block the action of histamine. By clamping the outlet port of the respirator, we over-inflated the animals by two times tidal volume for two breaths [25].

Propranolol-induced bronchoconstriction

Fifteen minutes after the preparation, when Pao had been stabilized, the animals were challenged with nebulized ovalbumin (OA) dissolved in saline (1.0 mg/ml) without interrupting the constant ventilation in passively sensitized animals. The OA aerosol was generated for 30 s with an ultrasonic nebulizer developed for small animals at our institution [26]. The amount of aerosol was 15.2 µl/min and 46.4% of the aerosol was deposited in the lung as measured by radio-aerosol technique [26]. Twenty minutes after the OA provocation, 10 mg/ml of propranolol was inhaled for 30 s.

Study 1: Effect of FK224 on the propranolol-induced bronchoconstriction

A: FK224 treatment before antigen challenge A NK₁ and NK₂ dual antagonist FK224 in a dose of 10 mg/kg (*n* = 7) dissolved in DMSO or the vehicle (DMSO) (*n* = 7) was administered intravenously 10 min before the OA challenge; 30 min before propranolol inhalation. As a negative control, saline was administered intravenously 10 min before the OA provocation and saline was inhaled 20 min after the challenge with OA (*n* = 7).

B: FK224 treatment after antigen challenge FK224 in a dose of 1 ($n = 7$) or 10 mg/kg ($n = 7$) or vehicle (DMSO) ($n = 7$) was given intravenously 15 min after the challenge with OA and then propranolol inhalation was performed 5 min later; 20 min after the OA challenge.

Study 2: Effect of FK888 on the propranolol-induced bronchoconstriction

A: FK888 treatment before antigen challenge A specific NK₁ antagonist FK888 in a dose of 10 mg/kg ($n = 7$) dissolved in DMSO or the vehicle (DMSO) ($n = 7$) was administered intravenously 10 min before the OA challenge; 30 min before propranolol inhalation.

B: FK888 treatment after antigen challenge FK888 in a dose of 1 ($n = 7$) or 10 mg/kg ($n = 7$) or vehicle (DMSO) ($n = 7$) was given intravenously 15 min after the challenge with OA and then propranolol inhalation was performed 5 min later; 20 minutes after the OA challenge.

Statistical analysis

All data are shown as mean $\pm$ standard error of the mean. Statistical differences were determined by non-parametric ANOVA among three groups and Mann-Whitney's U-test between two groups. Differences of time course curves for percentage increase in Pao from the baseline value after OA provocation and inhalation of propranolol were analysed among animals treated with FK224 (1 and 10 mg/kg) and vehicle or FK888 (1 and 10 mg/kg) and vehicle with 2-factor repeated ANOVA. A *P* value of 0.05 or less was considered to be significant.

Chemicals

These following chemicals were used: ovalbumin (Sigma, St Louis, USA), diphenhydramine hydrochloride (Sigma, St Louis, USA), sodium pentobarbital (Abbot Laboratories, North Chicago, USA), dl-propranolol hydrochloride (Wako Pure Chemical Ind. Ltd, Osaka, Japan), DMSO (Wako Pure Chemical Ind., Osaka, Japan), FK888 (N₂-[(4R)-4-hydroxy-1-(1-methyl-1H-indol-3-yl) carbonyl-L-prolyl]-N-methyl-N-phenylmethyl-3-(2-naphthyl)-L-alaninamide), FK224 (N-[N₂-[N-[N-[N-[2,3-didehydro-N-methyl-N-[N-[3-(2-pentylphenyl)-propionyl]-L-threnonyl]tyrosyl]-L-leucynyl]-D-phenylanyl]-L-allo-threonyl]-L-asparaginy]-L-serine-n-lactone) (Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan).

Results

Study 1: Effect of FK224 on the propranolol-induced bronchoconstriction

A: FK224 treatment before antigen challenge Pao values before ovalbumin (OA) challenge were 10.4 ± 0.5 and 10.1 ± 0.2 cmH₂O with pretreatment with 10 mg/kg of FK224 and vehicle (DMSO). There was no significant difference between these. Time courses of percentage increase in Pao from the baseline value after inhalation of propranolol following OA challenge in the two groups are shown in Fig. 1. Both the curves for antigen-induced bronchoconstriction (0 to 20 minutes after OA challenge) and propranolol-induced bronchoconstriction (20 to 35 min after OA challenge) were not altered by FK224. The peak values after OA challenge were 317 ± 43 and $277 \pm 34\%$ with vehicle and 10 mg/kg of FK224 and these value were not different. The maximum percent increase in Pao after propranolol inhalation from the pre-OA challenge value were 469 ± 42 and $457 \pm 35\%$ with vehicle and 10 mg/kg of FK224. There was not significant difference between these.

B: FK224 treatment after antigen challenge Pao values before OA provocation were 9.6 ± 0.2 , 9.6 ± 0.2 and 9.6 ± 0.2 cmH₂O in animals pretreated with 1 and 10 mg/kg of FK224 and vehicle. There were no significant differences among them. Time courses of percentage increase in Pao from the baseline value after inhalation of propranolol following OA challenge in the three groups are shown in Fig. 2. The peak values after OA challenge were 225 ± 39 , 303 ± 33 and $230 \pm 30\%$ with vehicle and 1 and 10 mg/kg of FK224 and the values did not differ from each other. Percentage increase in Pao immediately before propranolol inhalation (20 min after the OA challenge) were 177 ± 26 , 216 ± 31 and $191 \pm 30\%$ in the groups of vehicle, 1 and 10 mg/kg of FK224. These values were not significantly different.

The time courses of percentage increase in Pao after the inhalation of propranolol were not significantly different among the three groups. The maximum percentage increase in Pao after propranolol inhalation from the pre-OA challenge value were 404 ± 30 , 387 ± 39 and $379 \pm 47\%$ with vehicle and 1 and 10 mg/kg of FK224. The value was not significantly different among these three groups.

Study 2: Effect of FK888 on the propranolol-induced bronchoconstriction

A: FK888 treatment before antigen challenge Pao values before ovalbumin (OA) challenge were 9.9 ± 0.3 and

Fig. 1. Time course of percentage increase in Pao in passively sensitized guinea-pigs pretreated intravenously with vehicle (○) ($n = 7$) or 10 mg/kg of FK224 (●) ($n = 7$) 10 min before ovalbumin challenge. As a negative control, saline was administered intravenously 10 min before the OA provocation and saline was inhaled 20 min after the challenge with OA in passively sensitized animals (□) ($n = 7$). Vertical bars represent SEM.

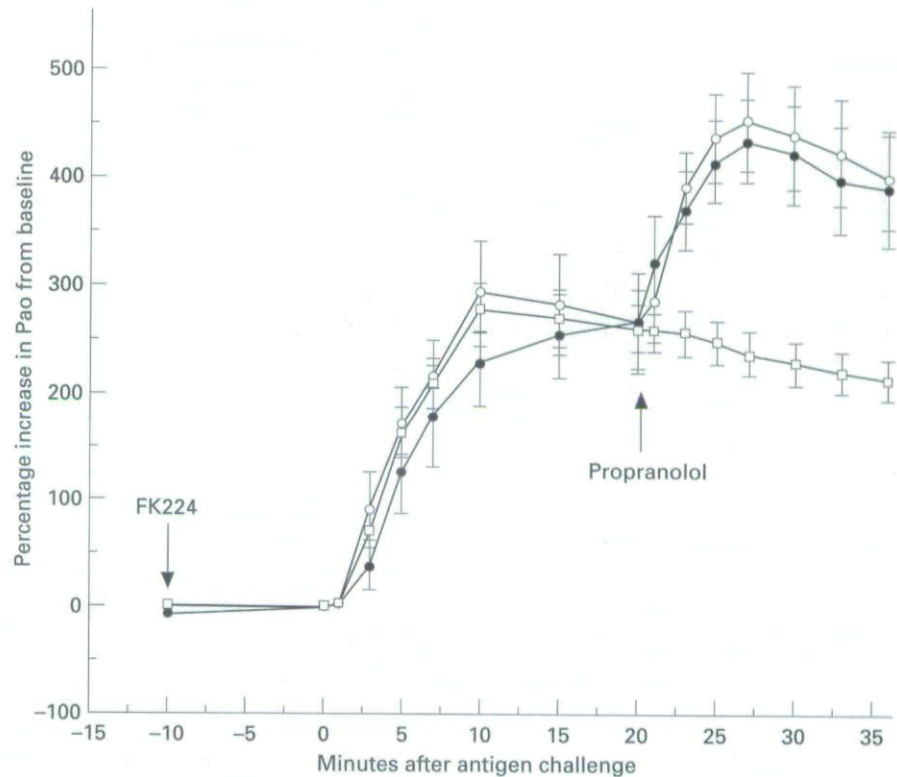
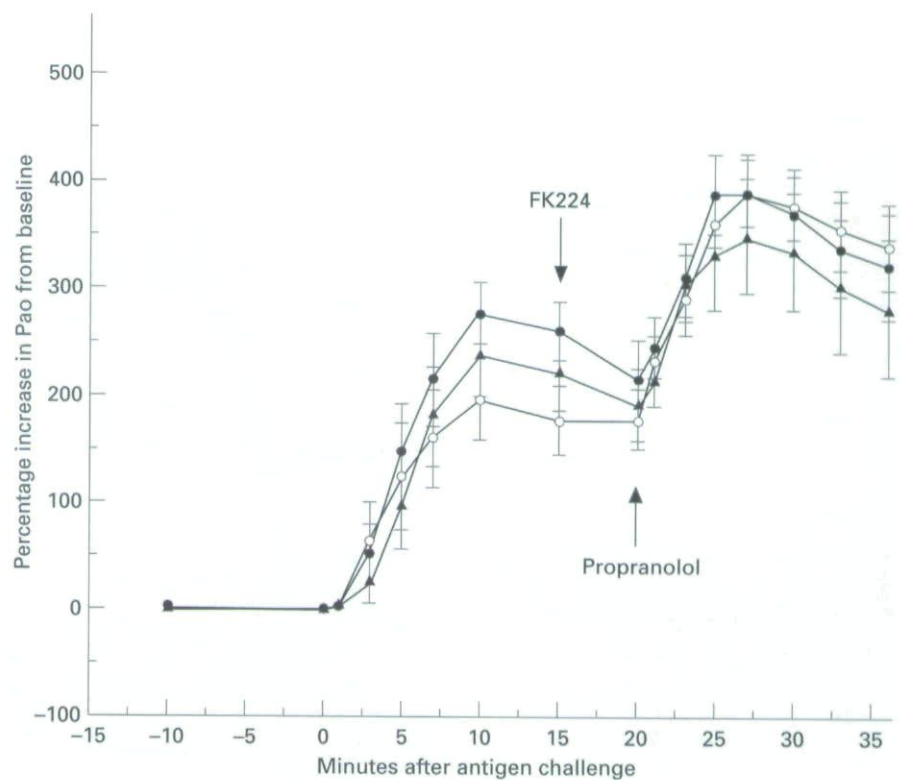


Fig. 2. Time course of percentage increase in Pao from the value before ovalbumin challenge in passively sensitized guinea-pigs treated intravenously with vehicle (○) ($n = 7$), 1 mg/kg of FK224 (●) ($n = 7$), or 10 mg/kg of FK224 (▲) ($n = 7$) 5 min before propranolol inhalation (15 min after the ovalbumin challenge). Vertical bars represent SEM.



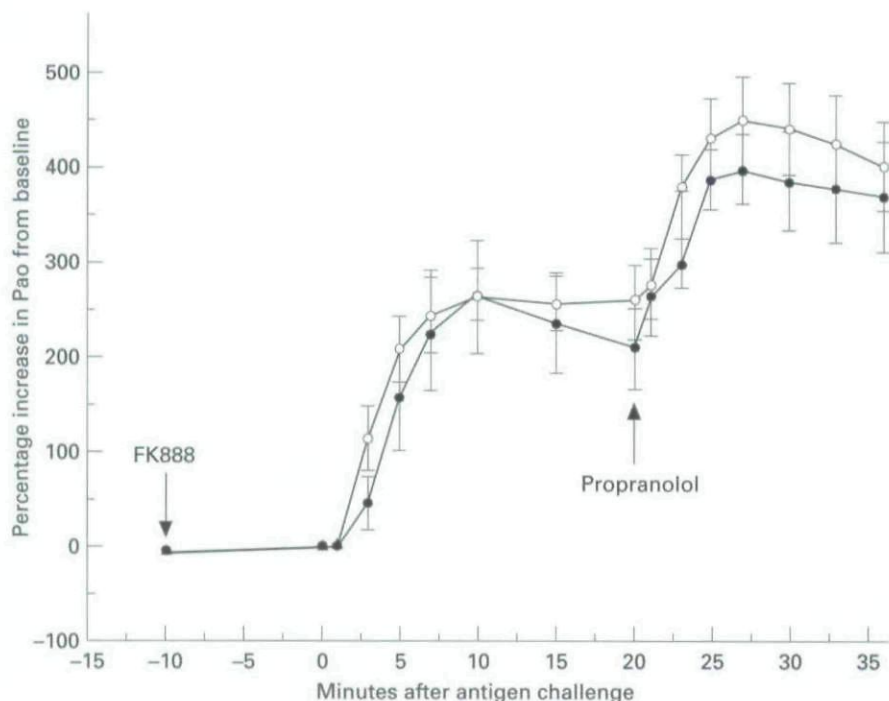


Fig. 3. Time course of percentage increase in Pao in passively sensitized guinea-pigs pretreated intravenously with vehicle (○) ($n = 7$) or 10 mg/kg of FK888 (●) ($n = 7$) 10 min before ovalbumin challenge. Vertical bars represent SEM.

$10.1 \pm 0.2 \text{ cmH}_2\text{O}$ with pretreatment with 10 mg/kg of FK888 and vehicle (DMSO). There was no significant difference between these. Time courses of percentage increase in Pao from the baseline value after inhalation of propranolol following OA challenge in the two groups

are shown in Fig. 3. Both the curves for antigen-induced bronchoconstriction (0 to 20 min after OA challenge) and propranolol-induced bronchoconstriction (20 to 35 min after OA challenge) were not altered by FK888. The peak values after OA challenge were 294 ± 27 and

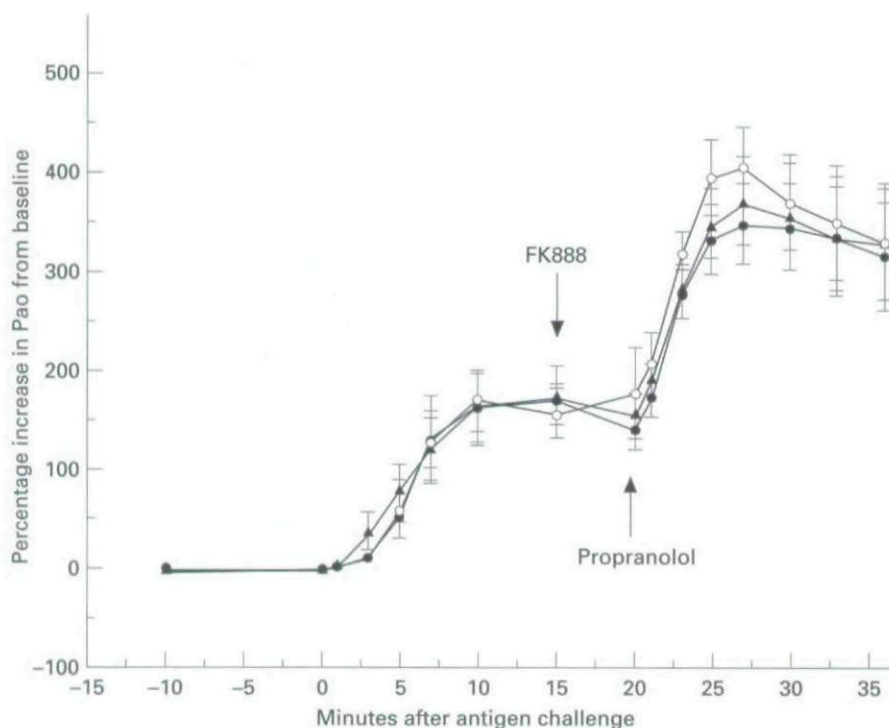


Fig. 4. Time course of percentage increase in Pao from the value before ovalbumin challenge in passively sensitized guinea-pigs treated intravenously with vehicle (○) ($n = 7$), 1 mg/kg of FK888 (●) ($n = 7$), or 10 mg/kg of FK888 (▲) ($n = 7$) 5 min before propranolol inhalation (15 min after the ovalbumin challenge). Vertical bars represent SEM.

321 ± 44% with vehicle and 10 mg/kg of FK888 and these values were not different. The maximum percentage increase in Pao after propranolol inhalation from the pre-OA challenge value were 468 ± 42 and 407 ± 51% with vehicle and 10 mg/kg of FK888. There was no significant difference between these.

B: FK888 treatment after antigen challenge Pao values before OA provocation were 9.9 ± 0.1, 10.4 ± 0.3 and 9.8 ± 0.2 cmH₂O in animals pretreated with 1 and 10 mg/kg of FK888 and vehicle. There were no significant differences among them. Time courses of percentage increase in Pao from the baseline value after inhalation of propranolol following OA challenge in the three groups are shown in Fig. 4. The peak values after OA challenge were 186 ± 25, 179 ± 32 and 191 ± 19% with vehicle and 1 and 10 mg/kg of FK888 and the values did not differ from each other. Percentage increase in Pao immediately before propranolol inhalation (20 min after the OA challenge) were 179 ± 44, 143 ± 17 and 160 ± 17% in the groups of vehicle, 1 and 10 mg/kg of FK888. These values were not significantly different.

The time courses of percentage increase in Pao after the inhalation of propranolol were not significantly different among the three groups. The maximum percentage increases in Pao after propranolol inhalation from the pre-OA challenge value were 431 ± 39, 391 ± 30 and 404 ± 47% with vehicle and 1 and 10 mg/kg of FK888. The value was not significantly different among these three groups.

Discussion

In this study a NK₁ and NK₂ dual antagonist FK224, or a selective NK₁ antagonist FK888, did not reduce inhaled propranolol-induced bronchoconstriction developed after antigen inhalation in passively sensitized guinea pigs.

Carpentiere *et al.* [9] reported that terfenadine, a histamine H₁-receptor antagonist, attenuated the airway responsiveness to inhaled propranolol, suggesting that histamine may be involved in PIB in asthmatics. Our previous study [5] revealed that thromboxane A₂ also may play an important role in the pathogenesis of this reaction. In addition, our preliminary study [10] showed that a 5-lipoxygenase inhibitor, AL3264, inhibited the propranolol-induced bronchoconstriction (PIB) induced by allergic reaction. These findings suggested the important role of inflammatory mediators in development of PIB which occurs specifically in asthmatic patients. As sensory neuropeptides cause neurogenic inflammation, the role of SP and NKA in the PIB was examined using FK224 and FK888 in our animal model.

FK224 is an antagonist for NK₁ and NK₂ receptors [20] and FK888 is a selective antagonist for NK₁ receptors [21]. Fujii *et al.* [27] have shown that FK888 preferentially inhibits SP (10⁻⁸ M)-induced contraction with an IC₅₀ value of 3.2 × 10⁻⁸ M compared with a value of 3.8 × 10⁻⁶ M for NKA (10⁻⁹ M)-induced contraction of guinea pig isolated trachea. They [28] have also shown that FK888 and FK224 displace ³H-SP (10⁻⁹ M) binding to guinea pig lung membrane preparation with IC₅₀ values of 2.7 × 10⁻⁹ M and 1 × 10⁻⁷ M, respectively. FK888 inhibits SP-induced contraction of guinea pig ileum with an IC₅₀ value of 5.4 × 10⁻¹⁰ M but the value of FK224 is 3.7 × 10⁻⁸ M. On the other hand, The IC₅₀ values of FK888 and FK224 against contraction of rat vas deferens by NKA are 1.1 × 10⁻⁵ M and 7.2 × 10⁻⁸ M. These data show that FK888 is a very potent and selective inhibitor of the NK₁ receptors and FK224 is an equipotent inhibitor of NK₁ and NK₂ receptors *in vitro*.

In *in vivo* experiments, intravenous administration of FK224 and FK888 inhibits SP (13.5 µg/kg, i.v.), NKA (1.1 µg/kg, i.v.)- and capsaicin (3.1 µg/kg, i.v.)-induced plasma extravasation of guinea pig airways with ED₅₀ values of 0.14 and 0.011 mg/kg, 0.29 and 0.0063 mg/kg and 0.30 and 0.018 mg/kg, respectively [27]. Hirayama *et al.* [29] have also reported that FK888 is only 5–10 times more potent than FK224 in inhibiting SP-induced plasma exudation in guinea pigs *in vivo*. In our previous study [30], FK888 in doses of 1 and 10 mg/kg strongly and dose-dependently inhibited bronchoconstriction induced by NKA but not by SP. On the other hand, FK224 in doses of 1 and 10 mg/kg inhibited both NKA- and SP-induced bronchoconstriction in a dose-dependent manner. These findings ensure that FK224 and FK888 are a NK₁ and NK₂ dual antagonist and a selective NK₁ antagonist, respectively, and indicate that the doses (1 and 10 mg/kg) of FK224 and FK888 used in this study are appropriate and enough to examine the involvement of neuropeptides in the PIB.

Although we did not measure heart rate or blood pressure in this study, it has been shown that 10 mg/kg of FK224 has no cardiovascular effects [31] and 10 mg/kg of FK888 has transient hypotensive effect within 5 min when these are intravenously administered to guinea pigs (personal communication from M Karino, Department of Pharmacology, Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan).

In this study neither FK224 nor FK888 in a dose of 10 mg/kg inhibited the PIB developed after allergic reaction. It has been shown that SP and NKA cause bronchoconstriction in animals [11] and in human asthmatic subjects [12–14] and potentiate bronchial responsiveness in animals [15–18]. Tamura and coworkers [16]

reported that the bronchoconstrictor response to inhaled methacholine was augmented in Japanese monkeys for at least 4 weeks after a single 2-min exposure to NKA aerosol, while no such change was evident after similar exposure to SP. Sheppard and Scypinsky [17] demonstrated that tachykinins are involved in the development of bronchial hyperresponsiveness following toluene diisocyanate exposure in guinea pigs. Omini and colleagues [18] showed that SP potentiates acetylcholine-induced bronchoconstriction in guinea pigs and this effect is abolished by vagotomy.

We also discovered that inhaled antigen-induced bronchial hyperresponsiveness was significantly inhibited by pretreatment with 0.1 and 1 mg/kg of FK224 in a dose-dependent manner, but not with 1 mg/kg of FK888 in guinea pigs [19]. It is speculated that if neuropeptides released from bronchial C-fibres by allergic reaction are involved in the PIB, NKA might be a major contributor to the development of PIB. However, our results showing that sufficient doses of FK224 or FK888 did not inhibit the PIB indicate no direct involvement of neuropeptides in the post-allergic PIB in guinea pigs. This view may coincide with Okayama and *et al.*'s report [32] that there was no relationship between degree of sensitivity to inhaled propranolol and bronchial responsiveness to methacholine in asthmatics.

To clarify the involvement of neuropeptides in PIB in human asthmatics further studies are needed in which the effects of selective antagonists for SP or NKA, such as FK224 and FK888, on PIB in asthmatics should be examined.

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