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Involvement of NK_2 receptors rather than NK_1 receptors in bronchial hyperresponsiveness induced by allergic reaction in guinea-pigs

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1 In this study, the role of neuropeptides in antigen-induced bronchoconstriction and bronchial responsiveness in guinea-pigs was evaluated by use of phosphoramidon, the inhibitor of neutral endopeptidases (NEP), the NK_1 receptor antagonist, FK888, and the dual NK_1/NK_2 receptor antagonist, FK224. The role of endogenous tachykinins in bronchial hyperresponsiveness induced by inhaled capsaicin was also observed with FK888 and FK224.

2 Allergic bronchoconstriction and bronchial responsiveness was evoked by inhalation of ovalbumin (OA), and increasing doses of methacholine were inhaled at 5-min intervals for 30 min after OA challenge in passively sensitized and artificially ventilated guinea-pigs. Animals were treated with a 30 s inhalation of phosphoramidon (10^{-3}M) or saline 10 min before the OA challenge. FK888 (1.0 or 10 mg kg^{-1}) or FK224 (1.0 or 10 mg kg^{-1}) was administered intravenously 5 min before the OA challenge.

3 Treatment with phosphoramidon did not alter the increase in the lateral pressure at the tracheal tube (P_{ao}) caused by OA inhalation or the increase in bronchial response to methacholine following the allergic reaction. Pretreatment with FK224 did not inhibit the increase in P_{ao} after antigen provocation but did significantly inhibit antigen-induced bronchial hyperresponsiveness in a dose-dependent manner, while FK888 did not affect either allergic bronchoconstriction or post-allergic bronchial hyperresponsiveness.

4 Histamine, 25, 50, 100 or 200 $\mu g\ ml^{-1}$ was inhaled for 20 s at 5-min intervals in non-sensitized guinea-pigs which were pretreated with inhalation of subthreshold dose of capsaicin (10^{-7} M). FK888 or FK224, each at a dose of 0.1 or 1.0 mg kg^{-1}, or vehicle was given to guinea-pigs intravenously 3 min before inhalation of capsaicin. The capsaicin inhalation significantly potentiated bronchial responsiveness to histamine, compared with control. The capsaicin-induced bronchial hyperresponsiveness was completely blocked by FK224 in a dose-dependent manner but not by FK888.

5 These results suggest that NK_2 receptors rather than NK_1 receptors may play an important role in bronchial hyperresponsiveness induced by antigen challenge as well as capsaicin while tachykinins do not play a primary role in the acute bronchospasm elicited by antigen challenge in passively sensitized guinea-pigs.

Keywords: FK224; FK888; NK_1 receptor antagonist; NK_1 and NK_2 dual antagonist; neuropeptides; antigen-induced bronchial hyperresponsiveness; phosphoramidon; capsaicin; guinea-pig airways

Introduction

Substance P (SP), neurokinin A (NKA) and related tachykinins have been implicated in several physiological functions in mammalian species and they may play a role in neurogenic inflammation (Martling, 1987). The characteristic features of bronchial asthma are prolonged nonspecific airway hyperresponsiveness and bronchoconstriction. While both tachykinin NK_1 and NK_2 receptor activation elicits airway smooth muscle contraction and thus bronchospasm in the guinea-pig, endogenously released tachykinins mediate their effects on airway smooth muscle primarily through activation of NK_2 receptors (Maggi *et al.*, 1991; Bertrand *et al.*, 1993). It has been postulated that tachykinins play a role in airway hyperresponsiveness in guinea-pigs after repeated antigen challenge because airway hyperresponsiveness is inhibited in animals pretreated with chronic administration of capsaicin, which is thought to deplete SP and other tachykinins (Matsuse *et al.*, 1991).

Selective inhibitors of neutral endopeptidase (NEP) such as phosphoramidon and thiorphan have been shown to potentiate tachykinin-induced bronchoconstriction in guinea-pigs *in vitro* (Shore *et al.*, 1988) and *in vivo* (Thompson & Sheppard, 1988). NEP is present in the airway and degrades tachykinins (Martins *et al.*, 1990). It is assumed that the activity of NEP decreases in the airway epithelium of asthma, because this tissue is destroyed and desquamated in asthmatic patients. It is still unknown whether inhibition of NEP potentiates bronchial responsiveness after allergic reaction in guinea-pigs.

To evaluate the hypothesis that tachykinins are released by allergic reaction and contribute to both immediate allergic bronchoconstriction and airway hyperresponsiveness that develops after the allergic response and to ensure that endogenous tachykinins released by capsaicin-inhalation induce bronchial hyperresponsiveness via stimulation of tachykinin receptors, the effects of a selective NK_1 receptor antagonist,

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FK888 (Fujii *et al.*, 1992a), a NK_1 and NK_2 dual receptor antagonist, FK224 (Murai *et al.*, 1992) and the NEP inhibitor, phosphoramidon, on bronchoconstriction immediately after, and bronchial hyperresponsiveness to methacholine 30 min after antigen inhalation were studied in passively sensitized guinea-pigs; in addition the effects of FK888 and FK224 on bronchial hyperresponsiveness induced by capsaicin inhalation were examined in non-sensitized guinea-pigs *in vivo*.

Methods

Male albino Hartley strain guinea pigs (380–450 g) were used in this study.

Passive sensitization of animals

Guinea-pig homocytotropic antiserum was obtained according to the method of Santives *et al.* (1976). Briefly, 500 μg of ovalbumin (OA) was emulsified in Freund's complete adjuvant and injected intradermally (i.d.) into each guinea-pig at multiple sites. Boosting was carried out in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after boosting, pooled and kept frozen until use. The antibody titer of this serum was 1:12800, 1:6400 and 1:512 at 4 h, 24 h and 7 days respectively, as estimated by passive cutaneous anaphylaxis. Normal guinea-pigs were passively sensitized with 1.0 ml of antiserum kg^{-1} given intraperitoneally (i.p.).

Preparation of guinea-pigs

Seven to eight days after passive sensitization, the guinea-pigs were anaesthetized with sodium pentobarbitone (75 mg kg^{-1}, i.p.). They were placed in the supine position and a polyethylene tube (o.d. 2.5 mm; i.d. 2.1 mm) was inserted into the trachea and the left jugular vein was cannulated for administration of drugs. After surgery, each guinea-pig was artificially ventilated with a small animal respiratory pump (Model 1680, Harvard Apparatus Co., Inc., South Natick, MA) adjusted to a tidal volume of 10 ml kg^{-1} at a rate of 60 strokes min^{-1}. The change in lung resistance to inflation, the lateral pressure of the tracheal tube (pressure at the airway opening; P_{ao} (cmH_2O)), was measured with a pressure transducer (Model TP-603T, Nihon Koden Kogyo Co., Ltd., Tokyo) by the modified method of Konzett & Rossler (1940) as described by Jones *et al.* (1982). Since the change in P_{ao} following inhalation of leukotriene C_4(LTC_4) represented the average of the changes in pulmonary resistance (RL) and reciprocal dynamic lung compliance ($1/C_{dyn}$) (Fujimura *et al.*, 1983), we used P_{ao} as an overall index of bronchial response to bronchoactive agents. Before surgery, the animals were given diphenhydramine hydrochloride (60 mg kg^{-1},i.p.) to block the action of histamine simultaneously with sodium pentobarbitone, and the lungs were over-inflated with two times the tidal volume for two breaths by clamping the outlet port of the respirator to standardize the volume history of the lung (Santives *et al.*, 1976).

Bronchoconstriction and bronchial hyperresponsiveness induced by antigen challenge

Fifteen minutes after the preparation, when P_{ao} had stabilized, 8 passively-sensitized guinea-pigs were challenged with nebulized ovalbumin (OA) dissolved in physiological saline (1.0 mg ml^{-1}) under constant ventilation (OA group). The OA aerosol was generated for 30 s with an ultrasonic nebulizer developed for small animals at our institution (Minami *et al.*, 1983). The rate of aerosol production was 15.2 μl min^{-1} and 46.4% of the aerosol was deposited in the lung as measured by the radio-aerosol technique (Minami *et al.*, 1983). In 5 sensitized guinea-pigs, physiological saline was inhaled for 30 s as a substitute for OA (control group).

Twenty minutes after challenge with OA or saline, the animals were overinflated with two times the tidal volume for two breaths by clamping the outlet port of the respirator, and 10 min later, 62.5 μg ml^{-1} of methacholine dissolved in saline was inhaled for 20 s and P_{ao} was measured for 5 min. The same procedure was repeated with aerosol challenge with 125, 250 and 500 μg ml^{-1} of methacholine at 5-min intervals.

Study 1: Effects of inhaled phosphoramidon on antigen-induced bronchoconstriction and bronchial hyperresponsiveness

The NEP inhibitor, phosphoramidon, dissolved in physiological saline (10^{-3} M) (P-OA group) or saline alone (Sa-OA group) was inhaled for 30 s from an ultrasonic nebulizer 10 min before OA provocation in sensitized guinea-pigs. P_{ao} was measured continuously for 20 min following the OA challenge and then bronchial responsiveness to inhaled methacholine was measured as described above.

Study 2: Effects of FK224 and FK888 on antigen-induced bronchoconstriction and bronchial hyperresponsiveness

An equipotent inhibitor of NK_1 and NK_2 receptors, FK224, and an NK_1-selective antagonist, FK888, were dissolved in 50% DMSO-diluted saline to produce solutions of 1.0 and 10 mg ml^{-1}. FK224 or FK888 at a dose of 1.0 or 10 mg kg^{-1} or vehicle was administered intravenously to passively sensitized guinea-pigs 5 min before inhalation of OA, 35 min before methacholine challenge.

Study 3: Effect of subthreshold dose of inhaled capsaicin on bronchial responsiveness

Capsaicin (30.5 mg) was dissolved in Tween 80 (1 ml) and ethanol (1 ml) and then in physiological saline (8 ml) to make a stock solution of 10^{-2} M. This solution was diluted with physiological saline to produce 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M capsaicin solutions. To determine a subthreshold concentration of capsaicin, the dose-response curve of capsaicin-induced bronchoconstriction was obtained in a preliminary study. Progressively increasing concentrations of the capsaicin solution were successively inhaled for 30 s at 5-min intervals in 6 non-sensitized animals. The subthreshold concentration of nebulized capsaicin that evoked negligible bronchoconstriction was determined to be 10^{-7} M from the dose-response curve.

The guinea-pigs were randomly assigned to three groups: those exposed to the subthreshold concentration of capsaicin (10^{-7} M; Capsaicin group); those given nebulized vehicle of capsaicin (Vehicle group); and those given saline (Control group). Each substance was inhaled for 30 s. Six minutes later histamine 25 μg ml^{-1} was inhaled for 20 s and P_{ao} was measured for 5 min. The same procedure was repeated with aerosol challenge with 50, 100 and 200 μg ml^{-1} of histamine.

Study 4: Effects of FK224 and FK888 on capsaicin-induced bronchidl hyperresponsiveness

FK888 or FK224, each at a dose of 0.1 or 1.0 mg kg^{-1} or vehicle was given to guinea-pigs intravenously 3 min before inhalation of the subthreshold concentration of capsaicin (10^{-7} M), and the bronchial response to inhaled histamine was observed as mentioned above.

Drugs

The following drugs were used: sodium pentobarbitone (Abbot Laboratories, North Chicago, U.S.A.); ovalbumin (Sigma, St. Louis, U.S.A.); diphenhydramine hydrochloride (Sigma, St. Louis, U.S.A.); methacholine, phosphoramidon (Wako Pure Chemical Ind., Osaka, Japan); capsaicin (Sigma Chemical Co., St. Louis, Mo, U.S.A.); FK888 (N_2-[(4**R**)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-prolyl]-N-methyl-N-phenylme-

thyl-3-(2-napthyl)-L-alaninamide) and FK224 (N-[N2-[N-[N-[N-[2,3-didehydro-N-methyl-N-[N-[3-(2-penthylphenyl)-propionyl]-L-threnonyl]tyrosyl]-L-leucynyl]-D-phenylalanyl]-L-allo-threonyl]-L-asparaginyl]-L-serine-n-lactone) (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan).

Statistical analysis

All data are shown as mean ± standard error of the mean (s.e.mean). Statistical differences were determined by nonparametric ANOVA among 3 or more groups and Mann-Whitney's U-test between 2 groups. Differences in time course curves for percentage increase in P_{ao} from the baseline value after OA or saline provocation were analysed by 2-factor repeated ANOVA. The time course curves after OA challenge were compared among animals treated with phosphoramidon and saline, FK224 (1.0 and 10 mg kg^{-1}) and vehicle, and FK888 (1.0 and 10 mg kg^{-1}) and vehicle, using 2-factor repeated ANOVA. A P value of 0.05 or less was considered significant.

Results

Evaluation of the baseline values for the P_{ao}

Mean values (±s.e.mean) of baseline P_{ao} immediately before challenge with aerosolized OA (OA group; $n=8$) or saline (control group; $n=5$), in passively sensitized guinea-pigs were not significantly different. Pre-methacholine challenge P_{ao} values were 20.0±1.8 and 10.2±0.2 cmH_2O, respectively; the value in the OA group was significantly ($P<0.01$) greater than that in the control group.

The P_{ao} values immediately before OA provocation in the guinea-pigs pretreated with aerosolized phosphoramidon (P-OA group; $n=8$) and saline (Sa-OA; $n=7$) were not significantly different. The values were not different among animals pretreated intravenously with 1 and 10 mg kg^{-1} of FK224 ($n=7$, respectively) and vehicle ($n=9$) or 1 and 10 mg kg^{-1} of FK888 ($n=6$ or $n=9$) and vehicle ($n=8$). Moreover, the percentage increase in P_{ao} from the pre-OA challenge value immediately before methacholine challenge also did not differ significantly.

Bronchial hyperresponsiveness induced by antigen challenge

Figure 1 shows the percentage increase in P_{ao} from the pre-methacholine challenge value that was induced by inhalation of increasing concentrations of methacholine 30 minutes after OA or saline challenge. The percentage increases in P_{ao} were 53.9±16.2, 92.2±28.1 and 126±34.1% respectively, with 62.5, 125 and 250 $\mu g\ ml^{-1}$ of methacholine in the OA group; these values were significantly ($P<0.01$, $P<0.01$ and $P<0.05$ respectively) greater than those in the control group (0.8±0.5, 8.2±1.7 and 30.2±12.0% respectively).

Study 1: Effects of inhaled phosphoramidon on antigen-induced bronchoconstriction and hyperresponsiveness The time course curves of percentage increase in P_{ao} induced by OA inhalation (0 to 20 min after OA challenge) did not significantly differ between the P-OA and the Sa-OA group. Peak values of the percentage increase in P_{ao} after OA challenge were 256±26 and 242±28%, respectively, in the P-OA and Sa-OA group at 7 min after OA provocation. The percentage increase in P_{ao} from the pre-methacholine challenge value that was induced by each concentration of inhaled methacholine was not significantly different between the two groups (Figure 2).

Study 2: Effects of FK224 and FK888 on antigen-induced bronchoconstriction and bronchial hyperresponsiveness The time courses of the percentage increase in P_{ao} after OA provocation did not differ significantly among animals pretreated with 1.0 and 10 mg kg^{-1} of FK224 and vehicle, the same as 1.0 and 10 mg kg^{-1} of FK888 and vehicle. The maximum values of the percentage increase in P_{ao} induced by inhalation of OA were 190±19, 221±13 and 244±18% with 1 and 10 mg kg^{-1} of FK224 and vehicle, respectively.

As shown in Figure 3, bronchial responsiveness to inhaled methacholine was significantly reduced by pretreatment with intravenous FK224 in a dose-dependent manner. The percentage increase in P_{ao} from the pre-methacholine challenge value that was induced by each concentration of inhaled methacholine was significantly ($P<0.05$) less with 10 mg kg^{-1} of FK224 than with vehicle.

The maximum values of percentage increase in P_{ao} after inhalation of OA were 244±13, 281±32 and 293±24% with 1 and 10 mg kg^{-1} of FK888 and vehicle, respectively (Figure 4); these values were not significantly different; also pretreatment with FK888 did not significantly alter the increased bronchial responsiveness to methacholine induced by OA challenge.

Study 3: Effect of subthreshold dose of inhaled capsaicin on bronchial responsiveness Peak values of percentage increase in

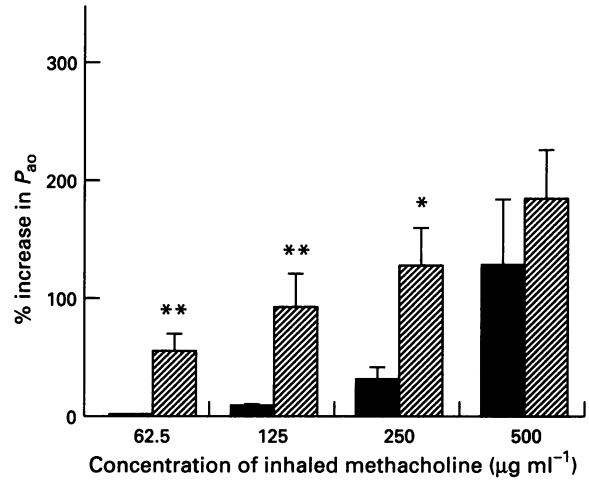

Figure 1 Percentage increase in P_{ao} from the pre-methacholine challenge value induced by inhalation of each methacholine dose 30 min after ovalbumin (OA) provocation (OA-Met group, hatched columns) or saline (Saline-Met group, solid columns) in sensitized guinea-pigs. Values are mean±s.e.mean. *$P<0.05$, **$P<0.01$ compared with the Saline-Met group.

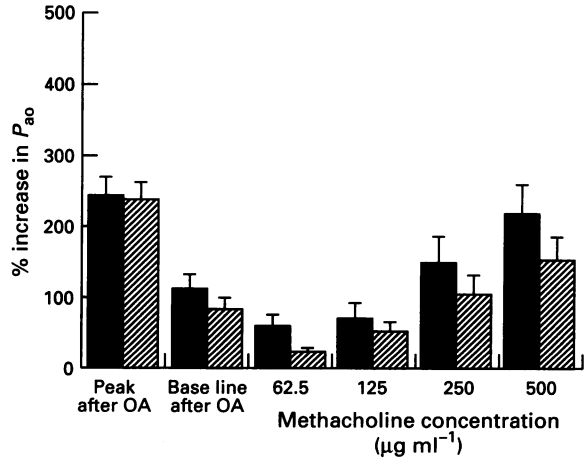

Figure 2 Effect of aerosolized phosphoramidon (Phos-OA-Met group, hatched columns) or saline (Sa-OA-Met group, solid columns) on maximal bronchoconstriction induced by ovalbumin (OA) and bronchial responsiveness to inhaled methacholine 30 min after OA provocation in sensitized guinea-pigs. Results are mean±s.e.mean.

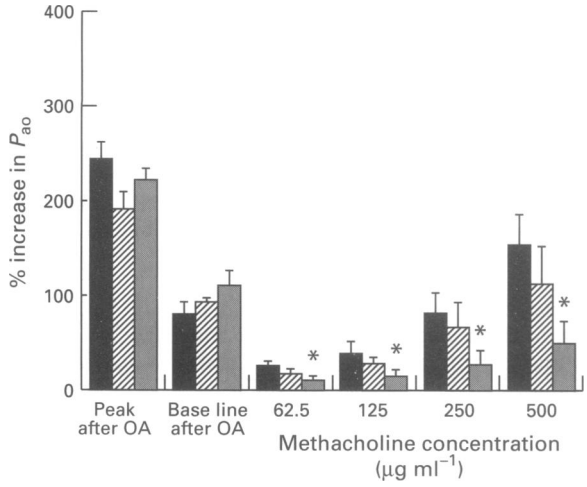

Figure 3 Effect of intravenous FK224 (1.0, hatched columns, and 10, stippled column, mg kg^{-1}) and vehicle of FK224 (solid column) on maximal bronchoconstriction induced by inhaled ovalbumin (OA) and bronchial responsiveness to inhaled methacholine 30 min after OA provocation in sensitized guinea-pigs. Each column represents mean ± s.e.mean. *$P<0.05$ compared with vehicle of FK224-treatment.

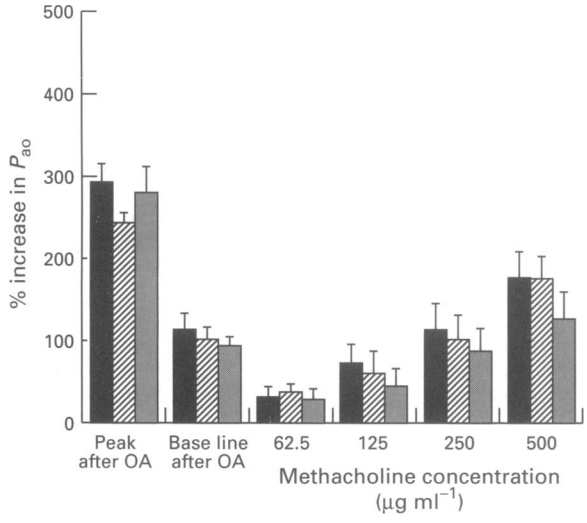

Figure 4 Effects of intravenous FK888 (1.0 mg kg^{-1}, hatched columns, and 10 mg kg^{-1}, stippled columns) and vehicle of FK888 (control, solid columns) on maximal bronchoconstriction induced by inhaled ovalbumin (OA) and bronchial responsiveness to inhaled methacholine 30 min after OA provocation in sensitized guinea-pigs. Vertical bars represent s.e.mean.

P_{ao} induced by each concentration of inhaled histamine in the capsaicin group ($n=7$), the control group ($n=7$) and vehicle group ($n=7$) were 15.6 ± 1.1, 14.4 ± 3.4 and 17.4 ± 1.7% at 25 μg ml^{-1} of histamine, 45.9 ± 7.1, 30.8 ± 5.6 and 30.4 ± 3.9% at 50 μg ml^{-1} of histamine, 219 ± 34, 93.8 ± 17 and 103 ± 31% at 100 μg ml^{-1} of histamine, and 616 ± 73, 356 ± 104 and 370 ± 154% at 200 μg ml^{-1} of histamine, respectively. The values were significantly greater in the capsaicin group, compared with the control and vehicle groups at 100 and 200 μg ml^{-1} of histamine ($P<0.05$).

Study 4: Effects of FK224 and FK888 on capsaicin-induced bronchial hyperresponsiveness The effects of FK888 and FK224 on capsaicin-induced bronchial hyperresponsiveness to

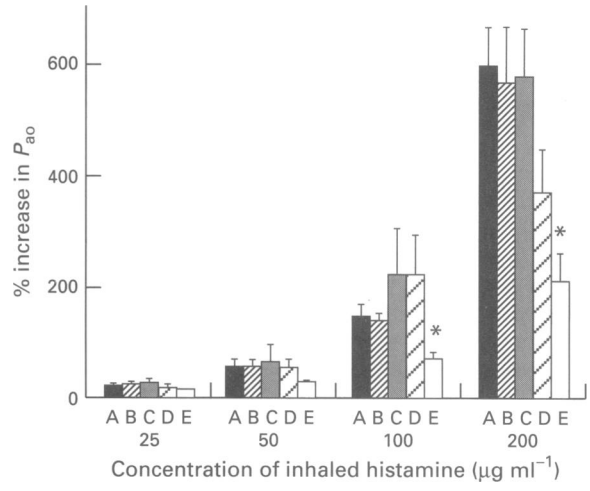

Figure 5 Effects of intravenous FK888 0.1 mg kg^{-1} (B) or 1.0 mg kg^{-1} (C) or FK224 at a dose of 0.1 (D) or 1.0 (E) mg kg^{-1} or vehicle (A) on bronchial hyperresponsiveness induced by subthreshold dose of inhaled capsaicin (10^{-7} M). Each column represents mean ± s.e.mean. *$P<0.05$ compared with vehicle of FK group.

histamine are shown in Figure 5 (each group: $n=7$). Treatment with 1.0 mg kg^{-1} of FK224 significantly inhibited the capsaicin-induced bronchial hyperresponsiveness to histamine at concentrations of 100 and 200 μg ml^{-1} ($P<0.05$) compared with treatment with vehicle. On the other hand, FK888 at a dose of 0.1 or 1.0 mg kg^{-1} did not significantly affect the bronchial hyperresponsiveness.

Discussion

The present study showed that pretreatment with aerosolized phosphoramidon had no effect on either allergic bronchoconstriction or bronchial hyperresponsiveness to methacholine which developed 30 min after antigen challenge. Further, the NK_1 and NK_2 receptor dual antagonist, FK224, significantly inhibited antigen-induced bronchial hyperresponsiveness in a dose-dependent manner but did not alter allergic bronchoconstriction. In contrast, the selective NK_1 receptor antagonist, FK888, had no effect on allergic bronchoconstriction or antigen-induced bronchial hyperresponsiveness in passively sensitized and artificially ventilated guinea-pigs. Joos *et al.* (1987) reported that inhalation of NKA caused bronchoconstriction in asthmatic subjects but not in normal volunteers, and that bronchoconstrictor activity was stronger with NKA than with SP. It appears that impaired activity of an endogenous neutral endopeptidase in asthmatic airways is responsible for the different effects of NKA in asthmatic versus nonasthmatic subjects (Nadel & Borson, 1991).

FK888 preferentially inhibits SP (10^{-8} M)-induced contraction with an IC_{50} 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 (Fujii *et al.*, 1992b). In *in vivo* experiments, intravenous administration of FK224 and FK888 inhibits SP (13.5 μg kg^{-1}, i.v.)-, NKA (1.1 μg kg^{-1}, i.v.)- and capsaicin (3.1 μg kg^{-1}, i.v.)-induced plasma extravasation of guinea-pig airways with ED_{50} values of 0.14 and 0.011 mg kg^{-1}, 0.29 and 0.0063 mg kg^{-1}, and 0.30 and 0.018 mg kg^{-1} respectively (Murai *et al.*, 1992). Our observation (unpublished data) demonstrated that bronchoconstriction elicited by intravenous NKA (10^{-6} mol kg^{-1}) was significantly inhibited by pretreatment with intravenous FK224 at concentrations of 1 mg kg^{-1} and 10 mg kg^{-1} in a dose-dependent manner, but not FK888 at concentrations of 1 mg kg^{-1} and 10 mg kg^{-1}, and that FK888 at doses of 1 and

10 mg kg^{-1} and FK224 at a dose of 10 mg kg^{-1} inhibited bronchoconstriction induced by intravenous substance P (10^{-8} mol kg^{-1}) in non-sensitized guinea-pigs *in vivo*.

Although Ingenito *et al.* (1991) and Lai, (1991) suggested that tachykinins do not contribute to the bronchoconstriction evoked by antigen challenge in sensitized guinea-pigs, Bertrand *et al.* (1993) found that tachykinins released from sensory nerves play a significant role in antigen-induced bronchoconstriction. The latter group investigated the bronchoconstrictor effect of inhaled ovalbumin (OA) at low and high concentrations after pretreatment with phosphoramidon, NK$_1$ and NK$_2$ receptor antagonists in sensitized guinea-pigs *in vivo*. They found that the increase in pulmonary resistance caused by antigen inhalation was potentiated by phosphoramidon and the increase in bronchoconstriction was attenuated by an NK$_2$ receptor antagonist, (SR-48968) but not by an NK$_1$ receptor antagonist, (CP-96,345), whereas bronchoconstriction was maximal and was not enhanced by phosphoramidon when high doses of inhaled antigen were used. They suggested that, with high doses of antigen, other mediators (e.g. histamine) played a predominant role in bronchoconstriction, and that the early phase of the response, especially with high doses of antigen, may be due to mediators other than tachykinins. The differences between our data and theirs may have resulted from the differences in the animal models used. Although actively sensitized guinea-pigs were pretreated with atropine in their study, passively sensitized animals were pretreated with diphenhydramine in our study. In our model, allergen-induced bronchoconstriction had been shown to be almost completely inhibited by leukotriene receptor antagonists (FPL-55712 and AS-35) (Fujimura, 1983; Saito *et al.*, 1993). Kawano *et al.* (1993) also showed that phosphoramidon significantly potentiated the increasing pulmonary resistance caused by inhaled OA in sensitized guinea-pigs and that the potentiating effect of phosphoramidon on antigen-induced bronchoconstriction was reduced in guinea-pigs pretreated repeatedly with capsaicin.

Our results showing that phosphoramidon failed to enhance the antigen-induced bronchoconstriction or the increase in bronchial responsiveness induced by the allergic reaction suggest one of the following: (1) NEP may be rapidly inactivated by the allergic reaction; or (2) The ability of endogenously released tachykinins to induce bronchial hyperresponsiveness in passively sensitized guinea-pigs may not be prevented by NEP. Our earlier study (unpublished observations) demonstrated that pretreatment with aerosolized phosphoramidon at the same dose potentiated the bronchoconstriction induced by inhaled SP in a dose-dependent manner in normal guinea-pigs. Kohrogi *et al.* (1991) reported that in guinea-pig bronchi *in vitro*, phosphoramidon (10^{-5} M) significantly prolonged contraction following the peak reaction after antigen challenge. Accordingly, the dose of inhaled phosphoramidon used in this study possibly inhibited the activity of NEP. It seems likely that NEP may be rapidly inactivated by the allergic reaction; however, it is speculation, without biochemical assessment of NEP activity and further pharmacological analysis.

Pretreatment with FK224 before antigen challenge significantly reduced bronchial hyperresponsiveness to methacholine 30 min after inhalation of antigen, whereas FK888 did not affect the bronchial hyperresponsiveness. These findings indicate that post-allergic bronchial hyperresponsiveness is partially produced by endogenous neuropeptides released by allergic reaction and that the relative contribution of NK$_2$ receptors is greater than that of NK$_1$ receptors. Similarly, Maggi *et al.* (1991) showed that NK$_2$ receptors have a greater effect on the noncholinergic bronchoconstriction in guinea-pig isolated bronchi. In addition as the tachykinin NK$_2$ receptor antagonist, SR48968, prevented antigen-induced airway hyperresponsiveness in sensitized guinea-pigs, whereas the NK$_1$ receptor antagonist, SR140333 did not, it is possible that the NK$_2$ receptor may be stimulated in the development of antigen-induced airway hyperresponsiveness (Boichot *et al.*, 1995).

Umeno *et al.* (1992) reported that endogenous neuropeptides, especially tachykinins such as substance P, evoked by aerosolized capsaicin potentiated bronchial responsiveness to intravenous histamine *in vivo* in guinea-pigs. However, no conflicting results have been obtained regarding the participation of different tachykinin receptors that contribute to bronchial hyperresponsiveness induced by neuropeptides released endogenously. Therefore, we investigated the effects of FK224 and FK888 on bronchial hyperresponsiveness to histamine induced by subthreshold dose of inhaled capsaicin in guinea-pigs. Our data showed that subthreshold dose of capsaicin increased the contractile response to each concentration of inhaled histamine and that this potentiating effect of capsaicin was inhibited by intravenous FK224 (1 mg kg^{-1}) but not FK888 (0.1 and 1 mg kg^{-1}). These data suggest that subthreshold dose of inhaled capsaicin, which is assumed to release endogenous neuropeptides, enhances bronchial responsiveness and that this enhancement is dependent on NK$_2$ receptors.

In summary, phosphoramidon did not affect bronchoconstriction caused by antigen inhalation or bronchial hyperresponsiveness induced by antigen challenge, while the NK$_1$ and NK$_2$ receptor antagonist, FK224, but not the NK$_1$-selective antagonist, FK888, had an inhibitory effect on antigen-induced bronchial hyperresponsiveness. Subthreshold dose of inhaled capsaicin also enhanced bronchial responsiveness and this enhancement was dependent on the NK$_2$ receptor preferentially. These findings suggest that although the level of neuropeptides released by antigen challenge may be too low to cause bronchoconstriction even if NEP is inactivated, it may be enough to develop bronchial hyperresponsiveness, and that NK$_2$ rather than NK$_1$ receptors may be stimulated in the process of bronchial hyperresponsiveness after allergic reaction in passively sensitized guinea-pigs as is capsaicin-induced bronchial hyperresponsiveness through endogenous tachykinin release.

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