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Effects of specific tachykinin receptor antagonists on citric acid-induced cough and bronchoconstriction in unanesthetized guinea pigs

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Abstract

We compared the effects of a tachykinin NK_1 receptor antagonist, FK888 (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide), and a tachykinin NK_2 receptor antagonist, SR48968 ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide]), on citric acid-induced cough and bronchoconstriction in conscious guinea pigs. FK888 and SR48968 inhibited the cough dose dependently. Combination of FK888 and SR48968 showed a small additive effect compared with that of FK888 or SR48968 alone. SR48968 but not FK888 inhibited the bronchoconstriction dose dependently. These results indicate that tachykinin NK_1 receptors as well as tachykinin NK_2 receptors are involved in the citric acid-induced cough response. The antitussive activity of the tachykinin NK_1 receptor antagonist appeared not to depend on the anti-bronchoconstrictor effects.

Keywords: FK888; SR48968; Citric acid; Bronchoconstriction; Cough; Tachykinin

1. Introduction

Tachykinins such as substance P and neurokinin A released from capsaicin-sensitive sensory nerve terminals can elicit smooth muscle contraction, airway mucus secretion and vascular extravasation in airways, and may play an important role in airway diseases (Barnes, 1986; Maggi, 1993). Recent and earlier data indicate that capsaicin-sensitive nerves or tachykinins are involved in the cough response, which is one of the most common symptoms of airway diseases (Collier and Fuller, 1984; Forsberg and Karlsson, 1986; Kohrogi et al., 1988). FK888, a tachykinin NK_1 receptor antagonist (Fujii et al., 1992), was shown to inhibit the cough response induced in guinea pigs by substance P or neutral endopeptidase inhibitors, indicating that tachykinin NK_1 receptors mediate cough responses induced by endogenous as well as exogenous tachykinins (Ujiie et al., 1993). In contrast, Advenier et al. (1993) reported that a tachykinin NK_2 receptor-selective antagonist, SR48968 (Emonds-Alt et al., 1992) inhibited the citric acid-induced cough response in guinea pigs. In the present study, we compared the effect of FK888 and SR48968 on the citric acid-induced cough response in unanesthetized guinea pigs. We also compared their effects on citric acid-induced bronchoconstriction which is predominantly mediated by tachykinin NK_2 receptors (Satoh et al., 1992), since cough and reflex bronchoconstriction have been considered closely related (Karlsson et al., 1988).

2. Materials and methods

2.1. General procedure

Male Hartley guinea pigs weighing 314–494 g (Japan SLC, Shizuoka, Japan) were used. FK888 (N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide, Fujisawa) and SR48968 ((*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide], Fujisawa) were dissolved in dimethyl sulfoxide (DMSO) and injected intravenously into the cephalic vein in a volume of 0.1 ml/kg 5 min before citric acid inhalation. The vehicle was injected in control groups. An unanesthetized animal was placed in a two-chambered, whole-body plethysmograph (model P, Buxco Electronics, CT, USA; Pennock et al., 1979), which is separated into a nasal chamber and a body chamber by thin rubber sheets at

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the neck. An aerosol of 20% citric acid-saline solution or saline generated by an ultrasonic nebulizer (NE-U10B, Omron, Tokyo, Japan) was introduced into the nasal chamber at 0.6 l/min for 2 min. Under these conditions, about 50 μl of citric acid solution was actually delivered into the nasal chamber as aerosol. We used 20% citric acid, since we obtained the best results at this concentration in our preliminary experiment with 2%, 7% or 20% citric acid (data not shown).

The bronchoconstriction experiment and the cough experiment were performed as separate experiments.

2.2. Cough

Our criteria for coughs were the characteristic high sound with the mouth opened, which was easily distinguished from a sneeze, and quick and large abdominal movement, which could be confirmed as a transient change in air flow in the body chamber.

The animals were continuously watched by a trained observer who counted the number of coughs. The sound of coughs was monitored using a microphone (ECM-202, Sony Corp., Tokyo, Japan) placed in the nasal chamber just below the mouth of the animal, a tape recorder (WM-RX77, Sony) and a stereo headphone (MDR-E565MP, Sony). The air flow in the body chamber was monitored with a pressure transducer (model DP45-14, Validyne Engineering Corp., CA, USA) connected to a pulmonary mechanics analyzer (Model 6, Buxco), a preamplifier (model Preamp/Val, Buxco) and a data logger (model LS-12, Buxco), and recorded on a pen recorder (Recti-Horiz-8K, Sanei, Tokyo, Japan). The number of coughs was counted during and after the aerosol inhalation, for a total of 15 min.

2.3. Bronchoconstriction

Pressure changes in the nasal and the body chambers were sensed by differential pressure transducers (model DP45-14, Validyne) connected to a non-invasive respiratory analyzer (Buxco). The analyzer was connected to a computer (PC9821Ap2, NEC, Tokyo, Japan) for data accumulation. Each chamber was calibrated by giving a known volume signal (3 ml air). Specific airway resistance was calculated as described (Pennock et al., 1979): $\tan\theta = W \times R \times C$, where, θ = phase difference between the body and nasal chamber flow signals = $2\pi \times$ time delay/total time; $W = 2\pi \times$ respiration rate = 2π/total time; R = airway resistance, and C = compressibility of lung volume = $V/(P\text{atm} - 47\ \text{mm Hg}) \times 1.36$, where, V = thoracic gas volume, Patm = barometric pressure in mm Hg and 1.36 = factor to convert mm Hg to cm H_2O. Specific airway resistance = $R \times V$ = total time/$2\pi \times (P\text{atm} - 47$ mm Hg) $\times 1.36 \times \tan\theta$. Forty-seven mm Hg is water vapor pressure at body temperature. The total time was computed by measuring the thoracic flow signal from the start of one inspiration to the start of the next. The time delay was measured from the moment the thoracic flow crossed zero at the end of inspiration to the moment the nasal flow crossed zero at the end of inspiration.

Initially, the animal was placed in the chamber and the baseline specific airway resistance value was measured for 1 min after the parameters had stabilized. The animal was taken out from the chamber, given a drug, and replaced in the chamber. After challenge with an aerosol, air was flushed for 2 min to drive out the remaining aerosol in the nasal chamber, and specific airway resistance was measured continuously for an additional 15 min.

2.4. Data analysis

For the cough study, the significance of differences was assessed by Kruskal-Wallis' h-test followed by Dunnett's multiple comparison.

For the bronchoconstriction study, the results are shown as means ± S.E.M. The data analyzer was programmed to give the specific airway resistance value at every breath. The specific airway resistance value at each minute was represented by an average of the last 20 values obtained just before the time point. Aberrant values due to animal movement were omitted. The areas under the curve (AUC) of specific airway resistance values plotted against time were also calculated using trapezoidal integration. Percent inhibition was calculated against vehicle control. The significance of differences was assessed by Dunnett's multiple comparison test following analysis of variance.

A P value less than 0.05 was always considered significant.

3. Results

Figs. 1 and 2 show the effects of FK888 and SR48968 on citric acid-induced cough. Both FK888 (0.0001–0.1

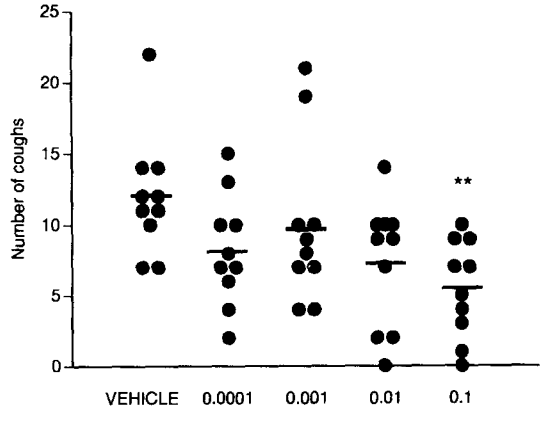

Fig. 1. Effect of FK888 (0.0001–0.1 mg/kg) on citric acid-induced cough in unanesthetized guinea pigs. Solid circles represent values for individual animals and bars indicate a mean value. $n = 10$ per group. Significant differences from vehicle group are indicated as: * $P < 0.05$; ** $P < 0.01$.

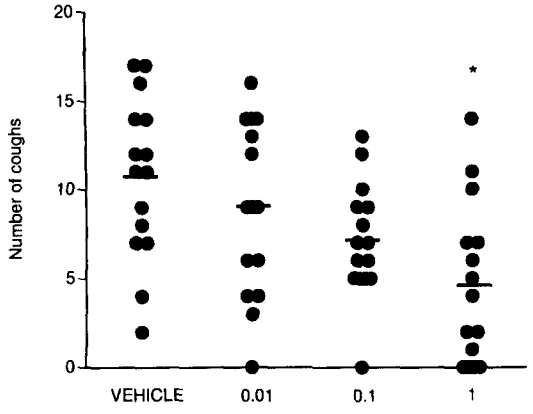

Fig. 2. Effect of SR48968 (0.01–1 mg/kg) on citric acid-induced cough in unanesthetized guinea pigs. Solid circles represent values for individual animals and bars indicate a mean value. $n = 15$ per group. Significant differences from vehicle group are indicated as: * $P < 0.05$; ** $P < 0.01$.

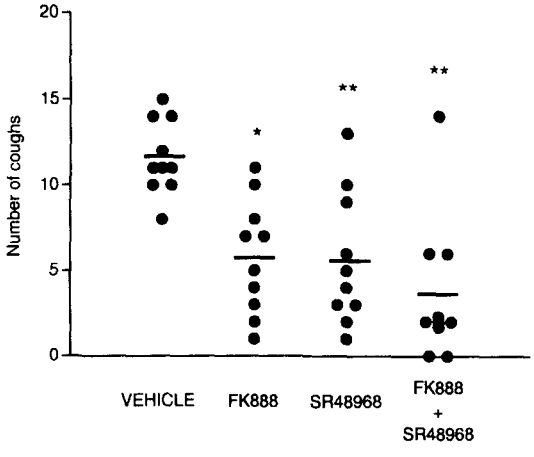

Fig. 3. Effect of FK888 (0.1 mg/kg, i.v.), SR48968 (1 mg/kg, i.v.) and their combination on citric acid-induced cough in unanesthetized guinea pigs. Solid circles represent values for individual animals and bars indicate a mean value. $n = 10$ per group. Significant differences from vehicle group are indicated as: * $P < 0.05$; ** $P < 0.01$.

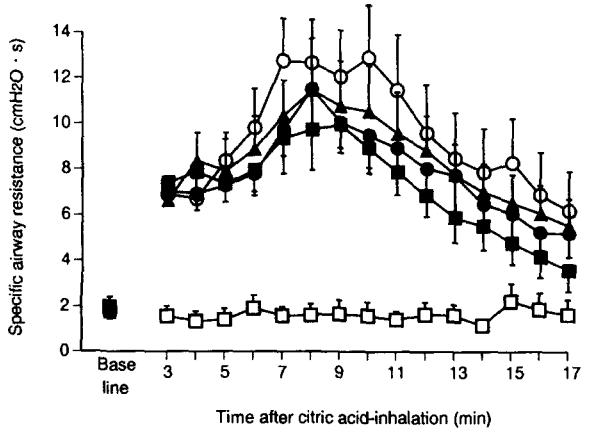

Fig. 4. Effect of FK888 0.001 mg/kg, i.v. (solid squares), 0.01 mg/kg, i.v. (solid circles) and 0.1 mg/kg, i.v. (solid triangles) on citric acid-induced bronchoconstriction in unanesthetized guinea pigs. Values for the saline-inhaling group are shown as negative controls (open squares). $n = 8–10$ per group. Significant differences from vehicle values (open circles) are indicated as: * $P < 0.05$; ** $P < 0.01$.

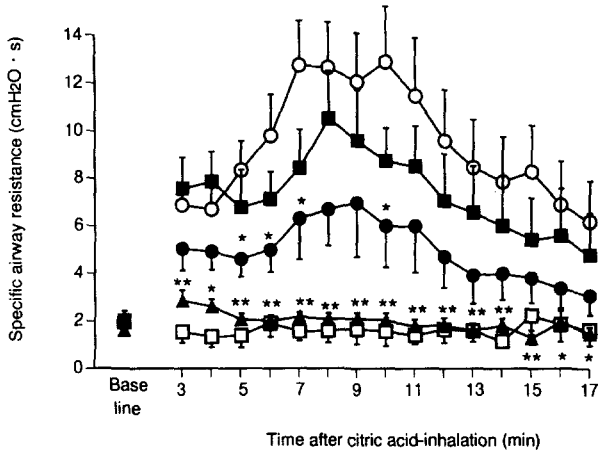

Fig. 5. Effect of SR48968 0.01 mg/kg, i.v. (solid squares), 0.1 mg/kg, i.v. (solid circles) and 1 mg/kg, i.v. (solid triangles) on citric acid-induced bronchoconstriction in unanesthetized guinea pigs. Values for the saline-inhaling group are shown as negative controls (open squares). $n = 7–10$ per group. Significant differences from vehicle values (open circles) are indicated as: * $P < 0.05$; ** $P < 0.01$.

mg/kg, i.v.) and SR48968 (0.01–1 mg/kg, i.v.) inhibited the cough response in a dose-dependent manner. FK888 (0.1 mg/kg) and SR48968 (1 mg/kg) inhibited the cough by 54% and 57%, respectively.

In another experiment, we tested the effect of combination treatment with FK888 and SR48968 (Fig. 3). FK888 (0.1 mg/kg, i.v.) and SR48968 (1 mg/kg, i.v.) inhibited the response by 50% and 52%, respectively. The combination treatment significantly inhibited the response by 69%, indicating a small additive effect, although the effect was not significantly different from that obtained with each drug alone.

Figs. 4 and 5 show the effects of FK888 (0.001–0.1 mg/kg, i.v.) and SR48968 (0.01–1 mg/kg, i.v.) on citric

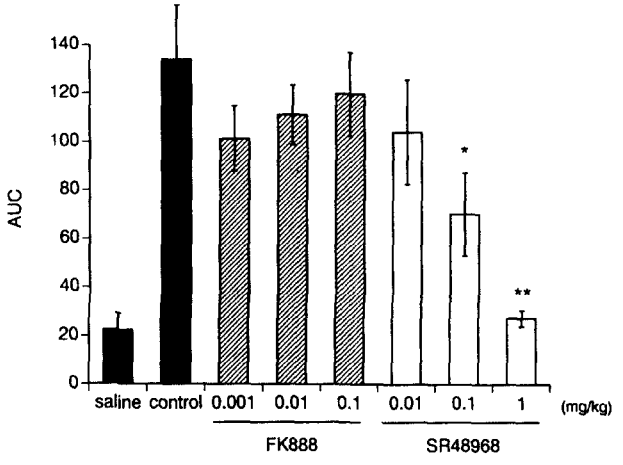

Fig. 6. Effect of FK888 (0.001–0.1 mg/kg, i.v.) and SR48968 (0.01–1 mg/kg, i.v.) on citric acid-induced bronchoconstriction in unanesthetized guinea pigs shown as areas under the curve (AUC). Values for the saline-inhaling group are shown as negative controls (saline). $n = 7–10$ per group. Significant differences from vehicle values (control) are indicated as: * $P < 0.05$; ** $P < 0.01$.

acid-induced bronchoconstriction. The peak response was usually observed about 10 min after citric acid inhalation. FK888 exerted only slight inhibition, while SR48968 inhibited the bronchoconstriction in a dose-dependent manner and almost abolished the response at 1 mg/kg. In AUC, FK888 showed no significant effect, while SR48968 significantly inhibited the response by 96% at 1 mg/kg (Fig. 6).

4. Discussion

Advenier et al. (1993) reported that tachykinin NK_2 receptors are involved in the citric acid-induced cough response in guinea pigs. In the present study, we demonstrated that tachykinin NK_1 receptors as well as tachykinin NK_2 receptors are involved in the citric acid-induced cough response.

Our results are not consistent with those of Girard et al. (1995), where only SR48968 but not a tachykinin NK_1 receptor antagonist, SR140333, inhibited citric acid-induced cough in guinea pigs. The reason for the discrepancy is not clear, but it may be attributed to the difference in experimental conditions. We used 20% (about 1 M) citric acid to induce cough, a higher concentration than the concentration (0.4 M) used by Girard et al. (1995). It is unlikely that FK888 acted on tachykinin NK_2 receptors to inhibit citric acid-induced cough, since FK888 had no significant effect on citric acid-induced bronchoconstriction, which is predominantly mediated via tachykinin NK_2 receptors as discussed below.

Both SR48968 and FK888 only partially inhibited the cough response. We cannot entirely exclude the possibility that the distribution of the two drugs to relevant sites was insufficient to inhibit the cough response, when administered intravenously. However, the dose of SR48968 used should have been enough to block the tachykinin NK_2 receptors in airways, since SR48968 at 1 mg/kg almost abolished citric acid-induced bronchoconstriction. This result indicates that the response is predominantly mediated via tachykinin NK_2 receptors, which is consistent with a previous report of work with anesthetized guinea pigs (Satoh et al., 1992). It is also unlikely that the maximal dose of FK888 used (0.1 mg/kg) was too low to give a greater inhibition of the cough response, since the effect of 1 mg/kg of FK888 was not different from that of 0.1 mg/kg in our preliminary experiment (data not shown). Advenier et al. (1993) also reported partial inhibition of the citric acid-induced cough by SR48968. Thus, it is most likely that both tachykinin NK_1 receptors and tachykinin NK_2 receptors are partially involved in the citric acid-induced cough response, at least in airways.

The effects of FK888 and SR48968 on the central nervous system may make some contribution to their antitussive effect. However, the effect of FK888 could be attributed to its peripheral action. It has been reported that i.c.v. injection of a tachykinin NK_1 receptor agonist induced foot tapping in gerbils, which could be inhibited by a central nervous system-penetrant tachykinin NK_1 receptor antagonist, CP-99,994, but not by a non-penetrant tachykinin NK_1 receptor antagonist (Rupniak and Williams, 1994). In our hands, FK888 did not inhibit substance P (i.c.v. injection)-induced foot tapping in gerbils even at 10 mg/kg, i.v., whereas CP-99,994 (1 mg/kg, i.v.) inhibited the response significantly (data not shown). Thus, FK888 may penetrate poorly into central nervous system.

Combination treatment with FK888 and SR48968 exerted a small additive inhibitory effect on the citric acid-induced cough response compared with effects obtained with FK888 or SR48968 alone. This could be attributed to the differences in the mechanism of the antitussive activity of FK888 and SR48968 as discussed below. More interestingly, the data indicate that much of the antitussive activity of FK888 and SR48968 in this animal model overlaps in spite of the clear difference in their tachykinin receptor selectivities.

The precise mechanism(s) of the antitussive activities of the tachykinin receptor antagonists cannot be elucidated from the present study. One possible explanation is that they inhibit bronchoconstriction to suppress the subsequent cough response. Cough may be secondary to other lung actions including bronchoconstriction (Karlsson et al., 1988). We cannot exclude this possibility, especially for SR48968, since it inhibited both bronchoconstriction and cough, although Girard et al. (1995) reported that the antitussive activity of SR48968 may not be related to the inhibition of bronchoconstriction. In the present study, cough experiments and bronchoconstriction experiments were performed separately, so that further experiments observing both cough and bronchoconstriction simultaneously should be done to elucidate more precisely the relationship between the antitussive effect and the anti-bronchoconstrictor effect of SR48968. In contrast, FK888 exerted no significant inhibitory effect on bronchoconstriction, whereas it showed an inhibitory effect on the cough response comparable to that of SR48968. This suggests that FK888 inhibited the cough response by a mechanism other than an anti-bronchoconstrictor action. Sekizawa et al. (1995) reported that a β_2-agonist inhibited the cough responses induced by histamine, acetylcholine or antigen at doses which also had a bronchodilator action, whereas FK888 inhibited the cough responses without apparent bronchodilating action. Together, the various findings suggest that the role of tachykinin NK_1 receptors in the cough response may be irrelevant to bronchoconstriction itself. This does not exclude the possibility that bronchoconstriction may cause a subsequent tachykinin NK_1 receptor-mediated cough response. In this context, it is very interesting that lidocaine, a local anesthetic, inhibits effectively the citric acid-induced cough response but not the bronchoconstriction (Forsberg et al., 1992; our unpublished observation). Tachykinins released from sensory nerve ter-

minals by citric acid might stimulate cough receptors sensitive to lidocaine to induce a cough.

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