Rhinology, 35, 63–66, 1997

Effects of tachykinin receptor antagonists, FK224 and FK888, in a guinea-pig model of nasal allergy*

Tatsuaki Katahashi, Akiyoshi Konno

Department of Otolaryngology, School of Medicine, Chiba University, Chiba, Japan

SUMMARY

The effects of two tachykinin receptor antagonists, FK888 (selective antagonist at the tachykinin NK-1 receptor) and FK224 (dual antagonist at NK-1 and NK-2 tachykinin receptors), on the frequency of sneezing, decrease of nasal patency, and increase of vascular dye leakage induced by antigen challenge upon the guinea-pig nasal mucosa were studied. The animals were sensitized with ovalbumin intraperitoneally. FK224 inhibited and FK888 tended to inhibit the decrease of nasal patency induced by antigen challenge. The increase of vascular dye leakage from nasal mucosa induced by antigen challenge tended to be inhibited by both FK224 and FK888. But both of them did not inhibit the increase of sneezing induced by antigen challenge. We conclude that in the guinea-pig model of nasal allergy, tachykinin receptors mediate plasma leakage and swelling of nasal mucosa induced by antigen challenge, but the participation of an axonal reflex via tachykinin receptors is rather small compared to the direct vascular effect of chemical mediators.

Key words: tachykinin receptor antagonist, axonal reflex, nasal mucosa, guinea pig, nasal allergy

INTRODUCTION

Axonal reflex via substance P (SP) and neurokinin A (NKA) containing fibers participates in the development of hyperreactive symptoms in bronchial asthma (Barnes, 1986). A rich supply of SP- and NKA-immunoreactive nerve fibers was identified in the nasal mucosa morphologically (Uddman et al., 1983; Brauniuk et al., 1991), but the participation of an axonal reflex in nasal allergy has not been clarified. The purpose of the present study was to evaluate the possible role of axonal reflex in the development of nasal hyperreactive symptoms in a guinea-pig model of nasal allergy.

SP and NKA are potent activators of NK-1 and NK-2 receptors, respectively (Regoli et al., 1988). We used the novel tachykinin antagonists, FK224 and FK888, because the former is a novel cyclopeptide tachykinin-receptor antagonist which exhibits dual inhibitory effects on both the NK-1 and NK-2 receptors (Morimoto et al., 1992), and the latter is a dipeptide tachykinin-receptor antagonist which is selectively potent against the NK-1 receptor (Fujii et al., 1992). They are more potent than other NK-1 or NK-2 antagonists.

MATERIAL AND METHODS

Animals and immunization method

Female guinea pigs (Dunkin-Hartley strain; body weight: 500-700 g) were used. The guinea-pig model of nasal allergy was made using the immunization method described by Konno et al. (1995).

The animals were injected intraperitoneally 7 times at 2-week intervals with 20 μg ovalbumin (OA) as antigen and 10 mg aluminum hydroxide as adjuvant. These were dissolved in 1 ml of normal saline. Nasal antigen challenge was performed with 2×10 μl of 5% OA once a day, for 7 days after the end of the immunization period.

Two applications each of 10 μl of tachykinin antagonist (1.5 mg/20 μl), FK224 and FK888, were instilled in both nasal cavities. Normal saline (2×10 μl) was applied to a control group.

Experimental protocols

In the first part of this study, we investigated the effect of the tachykinin antagonists, FK224 and FK888, on the frequency of sneezing by antigen challenge upon guinea-pig nasal mucosa. In this experiment the animals were challenged with 2×10 μl of 5%

* Received for publication June 24, 1996; accepted January 27, 1997

OA in normal saline in both nasal cavities. The sneezes occurring in the first 10 min folowing nasal challenge were counted. The data was evaluated by a comparison with the sneezes of control animals.

In the second part of the study, respiratory resistance was measured before and 10 min after nasal challenge by an oscillation method with a special apparatus (PMR-2, Shizume Medical Co, Tokyo) designed for measuring respiratory resistance in guinea pigs as reported by Konno et al. (1995). The degree of change in respiratory resistance was expressed by the rate of increase in respiratory resistance (post-challenge value minus pre-challenge value divided by pre-challenge value times 100). The data was evaluated by comparison with control animals. In addition, the change of respiratory resistance after antigen challenge might be affected by nasal secretion or nasopulmonary reflex via the parasympathetic nerve, so in another group of guinea pigs respiratory resistance was measured after blocking the parasympathetic reflex by the injection of atropine sulphate (1 mg/kg) 30 min before antigen challenge.

In the third part of this study, vascular permeability induced by nasal challenge with 2×10 μl of 5% OA was investigated following the method reported by Saria and Lundberg (1983). Twenty-five minutes after tachykinin antagonist administration, 20 mg/kg of Evans Blue in normal saline (20 mg/ml) was injected in the external jugular vein under local anaesthesia with 0.5 ml of 1% lidocaine. Five minutes after the injection with Evans Blue, 2 ×10 μl of 5% OA solution was challenged to both nasal cavities. Ten minutes later, the thorax was opened and the descending aorta was clamped. The vascular system of the head was perfused with 150 ml normal saline via the ascending aorta to expel intravascular dye. After decapitation, nasal mucosa was removed, blotted dry and weighed. The dye in the nasal mucosa was extracted by incubation in 4 ml formamide for 24 h at 50°C. After centrifugation, the dye concentration in the supernatant was quantified spectrophotometrically by measuring the absorbance at 620 nm (optical density). The data were compared to those of the control group, which had been administered 2 ×10 μl of normal saline to both nasal cavities instead of tachykinin antagonist.

Statistical analysis

Data were expressed as mean±SD. Statistical analysis was performed by Wilcoxon test; $p<0.05$ was considered statistically significant.

RESULTS

Sneezing

The frequency of sneezing per 10 min by nasal OA challenge was 3.7±3.0 in the FK224-pretreated group, 4.0±3.9 in its control group, 5.2±5.5 in the FK888-pretreated group, and 5.0±6.3 in its control group. The frequencies of sneezing in both groups pretreated with FK224 and FK888 revealed no statistical difference between those of their respective control groups, signifying that tachykinin-receptor antagonist pre-treatment was not effective on the sneezing induced by antigen challenge (Figure 1).

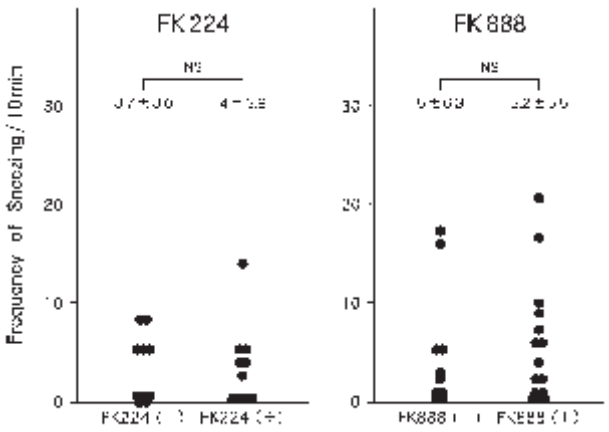

Figure 1. Effect of tachykinin receptor-antagonist pretreatment on the frequency of sneezing induced by nasal antigen challenge.

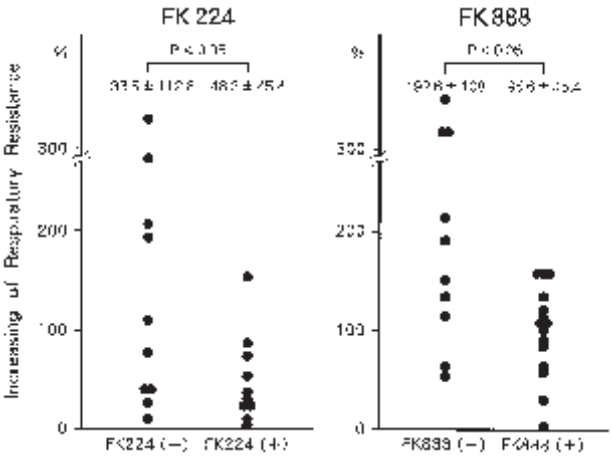

Figure 2. Effect of tachykinin receptor-antagonist pretreatment on the increase of respiratory resistance induced by nasal antigen challenge.

Respiratory resistance

The increase rates of respiratory resistance induced by antigen challenge were 48.3±45.4% in the FK888-pretreated group, 192.6±108% in its control, 95.6±45.4% in the FK224-pretreated group, and 133.5±112.8% in its control. Statistical differences ($p<0.05$) were noted between pairs of antagonist-pretreated and control groups (Figure 2).

When premedicated with atropine sulphate (1 mg/kg), the increase rates of respiratory resistance by nasal challenge were 65.3±78.0% in the FK224-pretreated group, 66.6±73.3% in the FK888-pretreated group, and 173.9±134.7% in the control group. There was also a statistical difference ($p<0.05$) between the FK224-pretreated group and the control, but not between the FK888-pretreated group and the control ($p<0.1$) although the respiratory resistance was reduced by, on average, 62% (Figure 3).

Vascular permeability

The amount of Evans Blue measured in nasal mucosa after antigen challenge was 3.5±2.3 μg/100 mg wet weight in the FK224-pretreated group, and 5.9±3.2 μg/100 mg wet weight in its control group. In the FK888-pretreated group, the amount of it was

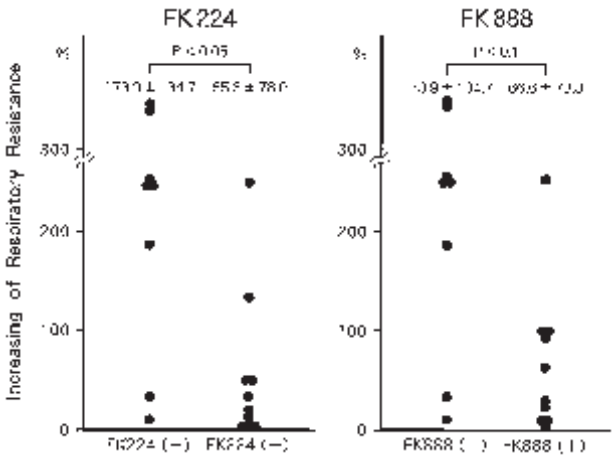

Figure 3. Effect of tachykinin receptor-antagonist pretreatment on the increase of respiratory resistance induced by nasal antigen challenge, after atropine sulphate injection.

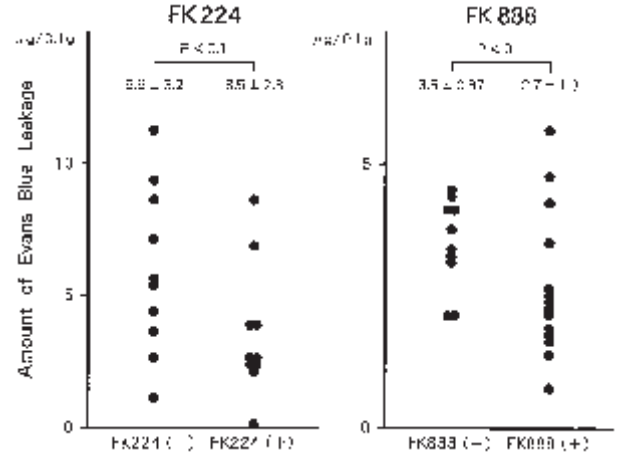

Figure 4. Effect of tachykinin receptor-antagonist pretreatment on vascular leakage of Evans Blue induced by nasal antigen challenge.

2.7±1.7 μg/100 mg wet weight, and 3.5±0.87 μg/100 mg wet weight in its control. Although FK224 and FK888 premedications reduced challenge-induced vascular permeability by 41% and 23%, respectively, neither was statistically different from the control ($p<0.1$; Figure 4).

DISCUSSION

Tachykinins include SP, NKA, neurokinin B (NKB) and their precursors. SP and NKA are believed to be present in type-C nociceptive sensorimotor neurons. Immunohistochemically, they are found in the vascular walls, glands, near the basement membrane and in the epithelium of the nasal mucosa (Regoli et al., 1987). NKB has not yet been identified in the airway mucosa.

Three subtypes (NK-1, NK-2, NK-3) of tachykinin receptors have been reported. Furthermore, SP, NKA and NKB are the most potent natural ligands at NK-1, NK-2 and NK-3 receptors, respectively (Regoli et al., 1988).

Oedema and vasodilation of airway mucosa is induced by SP more severely than by NKA (Rogers et al., 1988). On the other hand, NKA is more potent than SP in causing bronchoconstriction (Joos et al., 1987).

It has been reported that FK224 injected intravenously inhibited airway oedema and airway constriction induced by SP, NKA, and sensory nerve stimulation in guinea pigs (Murai et al., 1992). Similarly, the administration of FK888 inhibited SP-induced airway constriction as well as plasma extravasation induced by SP, NKA and capsaicin (Murai et al., 1993). Moreover, the intratracheal application of FK888 was about 100 times more potent than its intravenous application (Murai et al., 1993).

Previous studies showed that NK1-receptor mediates vasodilation in rat nasal mucosa (Piedimonte et al., 1993). In allergic rhinitis, Braunstein et al. (1991) reported that nasal obstruction leading to vasodilation is mediated through specific activation of NK-1 receptors, and microvascular leakage was increased by the activation of both NK-1 and NK-2 receptors.

In this study, we examined the role of axonal reflex in the development of hyperreactive nasal symptoms in a guinea-pig model of nasal allergy by way of the transnasal administration of FK224 and FK888.

Neither FK224 nor FK888 pretreatment inhibited sneezing induced by antigen. However, capsaicin pretreatment, which depletes tachykinin in the nasal mucosa, significantly inhibited the sneezing induced by antigen. Therefore, it can be considered that sneezing is a respiratory reflex via the central nervous system and its centripetal way is the tachykinin-immunoreactive fibers. Increase in respiratory resistance induced by antigen was inhibited by pretreatment with FK224 or FK888. After blocking parasympathetic reflex by injection of atropine sulphate, FK224 pretreatment significantly inhibited and FK888 pretreatment tended to inhibit the increase in respiratory resistance. Vascular permeability induced by antigen tended to be reduced by pretreatment with FK224 and FK888.

For the reasons mentioned above, the axonal reflex via NK-1 and NK-2 receptors was assumed to be involved in development of vascular response of the nasal mucosa in nasal allergy. But the blockage of NK-1 or NK-2 receptor inhibited the vascular response of the nasal mucosa induced by antigen challenge only partially in a guinea-pig model of nasal allergy. Therefore, the contribution of an axonal reflex in nasal vascular responses is rather small compared to the large, direct vascular effects of chemical mediators released from basophilic cells in the nasal mucosa.

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Tatsuaki Katahashi, MD
Department of Otolaryngology
School of Medicine
Chiba University
1-8-1 Inohana, Chuo-ku
Chiba City
Chiba 260
Japan