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Cigarette smoke increases mucosal permeability in guinea pig trachea via tachykinin NK_2 receptor activation

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Abstract

We investigated whether exposure to cigarette smoke increases the mucosal permeability in guinea pig trachea and if this effect could be mediated by tachykinin NK_2 receptor activation. Guinea pigs were exposed to either three different doses of cigarette smoke or room air. Mucosal permeability was measured by monitoring the rate of appearance in the circulation of horseradish peroxidase (HRP) that had been instilled into the isolated tracheal segment. Exposure to 20 and 30 puffs but not 10 puffs of cigarette smoke increased the tracheal mucosal permeability. Pretreatment with the tachykinin NK_2 receptor antagonist SR48,968 [(*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide] completely inhibited the increase in the permeability of the tracheal mucosa induced by exposure to cigarette smoke, whereas the tachykinin NK_1 receptor antagonist SSR240,600 [(*R*)-2-(1-{2-[4-{2-[3,5-bis(trifluoromethyl)phenyl]acetyl}-2-(3,4-dichlorophenyl)-2-morpholinyl]ethyl}-4-piperidinyl)-2-methylpropanamide] and the tachykinin NK_3 receptor antagonist SR142,801 [(*S*)-(*N*)-(1-[3-(1-benzoyl-3(3,4-dichlorophenyl)piperidine-3-yl)propyl]-4-phenylpiperidin-4-yl)-*N*-methyl-acetamide] had no effect. It is concluded that endogenous tachykinins via NK_2 receptor activation mediate the increase in the permeability of the tracheal mucosa induced by exposure to cigarette smoke in guinea pigs.

Keywords: Cigarette smoke; Tachykinins; Tachykinin NK receptors; SSR240,600; SR48,968; SR142,801

1. Introduction

Acute exposure to cigarette smoke is known to increase the airway mucosal permeability (inward permeability; substance movement from lumen to submucosa) in guinea pigs (Boucher et al., 1980). It has also been shown that the airway mucosal permeability is increased in smokers (Kennedy et al., 1984). In normal conditions, the airway epithelium serves as a barrier by restricting free passage of inhaled exogenous substances. However, when airway mucosal permeability is increased, it will favor the passage of the substances present in the lumen into the submucosa. Inhaled pollutants may cause increased stimulation to the irritant receptors, which are located just beneath the tight junctions, to result in coughing, bronchoconstriction and production of mucus. Inhaled bronchoconstrictors could also easily reach the underlying smooth muscle cells to cause bronchoconstriction. In addition, inhaled antigens are allowed to access the large amount of mast cells present in the submucosa. It is suggested that increased airway mucosal permeability will be involved in the pathogenesis of various airway inflammatory diseases.

Recently, we have shown that activation of tachykinin NK_2 receptors by endogenously released tachykinins mediate the increase in the permeability of the tracheal mucosa induced by exposure to ozone (Fu et al., 2002). Exposure to cigarette smoke causes the release of endogenous tachykinins, which induce neurogenic inflammation

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in the airways, including increased plasma extravasation (Lundberg and Saria, 1983), bronchoconstriction (Martling, 1987) and leukocyte adhesion to the vascular endothelium (Baluk et al., 1996) in rodent airways. Thus, it is possible that endogenous tachykinins also mediate the increase in the permeability of the airway mucosa induced by exposure to cigarette smoke via activation of tachykinin receptors.

The aim of the present study was to determine whether endogenous tachykinins mediate the increase in the permeability of the tracheal mucosa induced by exposure to cigarette smoke and to define the specific tachykinin receptor subtype involved in this response in guinea pigs.

2. Materials and methods

2.1. Animals and cigarette smoke exposure

The experimental protocol followed the 'Guiding Principles in the Care and Use of Animals' published by the council of the American Physiological Society and the 'Guide for the Care and Use of Laboratory Animals' published by NIH. The protocol was approved by the Committee on Animal Research of the Yokohama City University School of Medicine.

Male Hartley strain guinea pigs (Japan SLC, Hamamatsu, Japan) weighing 320–380 g were used. The guinea pigs were exposed to cigarette smoke or room air as we previously reported (Nishikawa et al., 1990). In brief, the guinea pigs were individually exposed to cigarette smoke in an awake, restrained state and spontaneously breathing in the device, which was a modification of that described by Simani and coworkers (Simani et al., 1974). Using a 20-ml syringe, 20 ml of cigarette smoke (=1 puff) were drawn out of a conventional filter cigarette (Seven Stars®; Nihon Tobacco Ind., Tokyo, Japan) containing 14 mg of tar and 1.2 mg of nicotine per cigarette (Analysis by Nihon Tobacco Ind.), and then injected into the compartment in which the head of the guinea pig was secured. We chose this cigarette brand because we have used this in our previous study examining its effect on airway responsiveness and vascular permeability (Nishikawa et al., 1990). In that study, exposure to 20 puffs of cigarette smoke caused airway hyperresponsiveness without any increase in airway vascular permeability. The compartment had about a 1-l capacity and a smoke inlet hole (1 cm) on the front panel and four exhaust holes (1 cm) on the top panel. The smoke was delivered at a rate of 3 puffs/min. To determine the dose–response relationships, guinea pigs were divided into four groups of five guinea pigs each based on the amount of exposure to cigarette smoke: group A was exposed to 10 puffs (half of a cigarette), group B was exposed to 20 puffs (one cigarette), group C was exposed to 30 puffs (one and half of cigarettes), group D was the control, exposed to room air.

2.2. Measurement of the permeability of the tracheal mucosa

The permeability of the tracheal mucosa was measured by monitoring the appearance in the blood of horseradish peroxidase (HRP) that had been instilled into the isolated tracheal segment as previously reported (Nishiyama et al., 1998; Fu et al., 2002).

As soon as the animals had been exposed to cigarette smoke or room air, they were anesthetized by intraperitoneal injection of ketamine (60 mg/kg) and xylazine (7 mg/kg), and were placed in the supine position. Additional ketamine and xylazine were given as required to maintain anesthesia. A catheter (0.51 mm ID×0.94 mm OD; Dow Corning, Midland, MI, U.S.A.) filled with heparinized saline was inserted into the femoral vein of each guinea pigs for drawing blood. A tracheal segment was isolated in vivo between two polyethylene cannulae (Disposable Multipurpose Tube; ATOM, Tokyo, Japan) that were inserted by making tracheostomies. Twenty minutes after the exposure to cigarette smoke or room air, HRP solution (50 mg/ml) was instilled to fill the lumen of the isolated tracheal segment. Blood samples were drawn before and 10, 20, 30, 40 min after the instillation of HRP via the catheter in the femoral vein. The withdrawn blood was replaced with an equal volume of saline. Blood samples were immediately centrifuged, and the plasma was stored frozen at −20 °C until assayed. Plasma HRP levels were measured by enzyme-linked immunosorbent assay (ELISA) as previously described (Nishiyama et al., 1998; Fu et al., 2002).

2.3. Effect of tachykinin NK_1, NK_2 and NK_3 receptor antagonists on the cigarette smoke-induced increase in the permeability of the tracheal mucosa

The effect of a selective tachykinin NK_1 receptor antagonist SSR240,600 [(*R*)-2-(1-{2-[4-{2-[3,5-bis(trifluoromethyl)phenyl]acetyl}-2-(3,4-dichlorophenyl)-2-morpholinyl]ethyl}-4-piperidinyl)-2-methylpropanamide], a selective tachykinin NK_2 receptor antagonist SR48,968 [(*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl]benzamide] and a selective tachykinin NK_3 receptor antagonist SR142,801 [(*S*)-(*N*)-(1-[3-(1-benzoyl-3(3,4-dichlorophenyl)piperidine-3-yl) propyl]-4-phenylpiperidin-4-yl)-*N*-methyl-acetamide] on the cigarette smoke-induced increase in the permeability of the tracheal mucosa was examined. The dose of 20 puffs of cigarette smoke was used for this study, because this dose caused a significant increase in the mucosal permeability in the dose–response study. Guinea pigs were divided into five groups of five guinea pigs each based on the drugs administered and exposure to cigarette smoke or room air: group E was pretreated with a selective tachykinin NK_1 receptor antagonist SSR240,600 (3 mg/kg s.c.) and, 30 min later, exposure to cigarette smoke; group F was pretreated with SR48,968 (3 mg/kg s.c.) and, 30

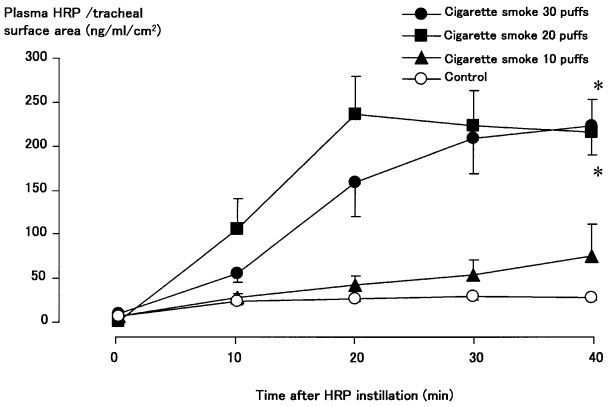

Fig. 1. Effect of acute exposure to cigarette smoke on the permeability of the tracheal mucosa. Shown are plasma levels of HRP during a 40-min sampling period in guinea pigs exposed to 10 puffs (n=5, closed triangles), 20 puffs (n=5, closed squares) and 30 puffs (n=5, closed circles) of cigarette smoke, and to room air (control, n=5, open circles). The time point 0 indicates the point of instillation of the HRP solution into the tracheal segment. *P<0.01, as compared with the control group.

min later, exposure to cigarette smoke; group G was pretreated with SR142,801 (3 mg/kg s.c.) and, 30 min later, exposure to cigarette smoke; group H was pretreated with the vehicle and exposed to cigarette smoke; and group I was pretreated with the vehicle and exposed to room air. The dose (3 mg/kg) of each tachykinin NK receptor antagonist was chosen since it has been shown that it shows selective inhibition of the corresponding tachykinin NK receptor in previous studies (Bolser et al., 1997; Yip and Chahl, 1997; Steinberg et al., 2002). In addition, higher doses of tachykinin NK receptor antagonists were examined if the dose (3 mg/kg) of tachykinin NK receptor antagonists showed no effect, and lower doses of tachykinin NK receptor antagonists were examined if the dose of tachykinin NK receptor antagonists showed complete inhibition. All drugs were dissolved in ethanol and stored at −20 °C. On the day of experiment, they were diluted further with filtered 0.9% NaCl.

2.4. Morphological analysis of tracheal mucosa

For histopathologic analysis, three guinea pigs per group (groups F, H and I) were evaluated. Trachea was removed and immersed in 10% phosphate-buffered formalin for 24 h and fixed in 70% histopathologic grade ethanol, and underwent routine processing for paraffin embedding. From these samples, 4-μm sections were cut and stained with hematoxylin and eosin for histopathologic analysis.

2.5. Statistical analysis

The mean values of HRP concentration in the plasma, normalized by the tracheal surface area (ng/ml/cm^2), were expressed as the arithmetic mean±S.E.M. Between-group differences in HRP concentration were assessed using two-way repeated-measure analysis of variance followed by the contrast method (means/regression coefficient comparisons). Probability values of P<0.05 were considered significant.

2.6. Drugs

The drugs used in this study were HRP (Zymed Laboratories, San Francisco, CA, U.S.A.) and rabbit anti-HRP polyclonal antibody (EY Laboratories, San Mateo, CA, U.S.A.). SSR240,600, SR48,968 and SR142,801 were kindly provided by Sanofi Recherche (Montpellier Cedex, France), respectively. All other drugs in this study not

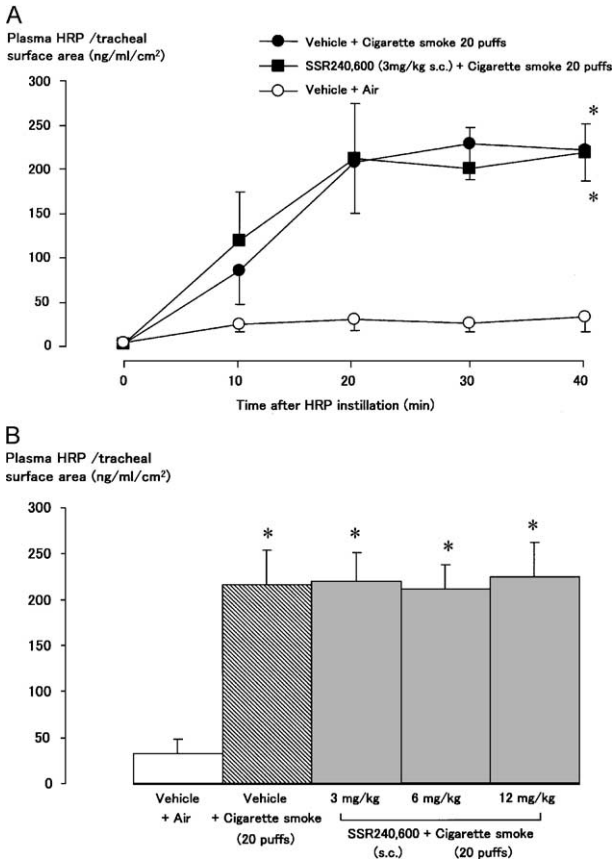

Fig. 2. Effect of the tachykinin NK_1 receptor antagonist, SSR240,600, on the cigarette smoke-induced increase in the permeability of the tracheal mucosa. (A) Plasma levels of HRP during a 40-min sampling period in guinea pigs pretreated with SSR240,600 (3 mg/kg s.c.) and then exposed to 20 puffs of cigarette smoke (n=5, closed squares), and in those pretreated with the vehicle and then exposed to 20 puffs of cigarette smoke (n=5, closed circles) or room air (n=5, open circles), are shown. The time point 0 indicates the point of instillation of the HRP solution into the tracheal segment. (B) Effect of increased doses of the tachykinin NK_1 receptor antagonist, SSR240,600, on the cigarette smoke-induced increase in the permeability of the tracheal mucosa. Plasma levels of HRP 40 min after the point of instillation of the HRP solution into the tracheal segment in guinea pigs pretreated with SSR240,600 (3 or 6 or 12 mg/kg s.c.) and then exposed to 20 puffs of cigarette smoke (n=5, respectively), and in those pretreated with the vehicle and then exposed to 20 puffs of cigarette smoke (n=5) or room air (n=5), are shown. *P<0.01, as compared with the group exposed to room air pretreated with vehicle.

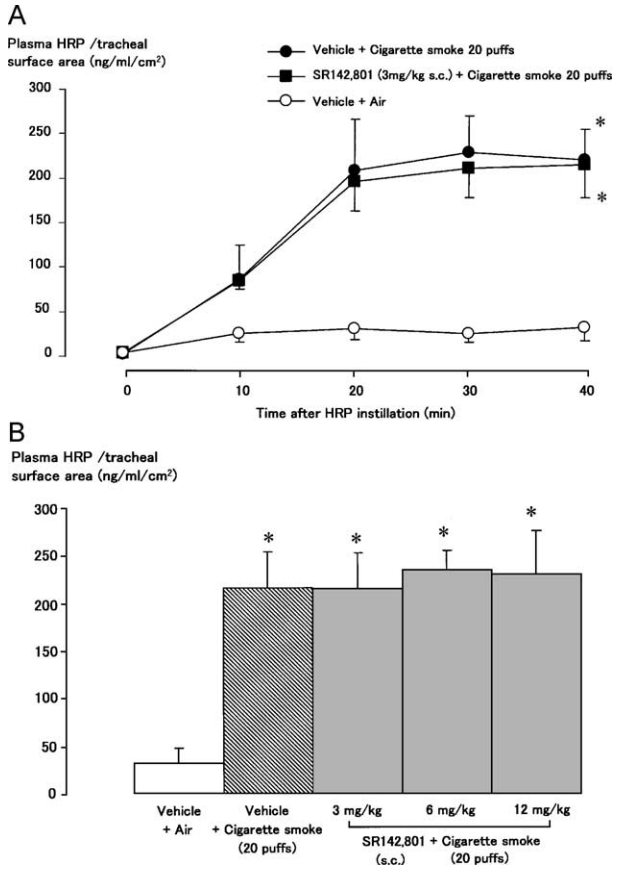

Fig. 3. Effect of the tachykinin NK_3 receptor antagonist, SR142,801, on the cigarette smoke-induced increase in the permeability of the tracheal mucosa. (A) Plasma levels of HRP during a 40-min sampling period in guinea pigs pretreated with SR142,801 (3 mg/kg s.c.) and then exposed to 20 puffs of cigarette smoke (n=5, closed squares), and in those pretreated with the vehicle and then exposed to 20 puffs of cigarette smoke (n=5, closed circles) or room air (n=5, open circles), are shown. The time point 0 indicates the point of instillation of the HRP solution into the tracheal segment. (B) Effect of increased doses of the tachykinin NK_3 receptor antagonist, SR142,801, on the cigarette smoke-induced increase in the permeability of the tracheal mucosa. Plasma levels of HRP 40 min after the point of instillation of the HRP solution into the tracheal segment in guinea pigs pretreated with SR142,801 (3 or 6 or 12 mg/kg s.c.) and then exposed to 20 puffs of cigarette smoke (n=5, respectively), and in those pretreated with the vehicle and then exposed to 20 puffs of cigarette smoke (n=5) or room air (n=5), are shown. *P<0.01, as compared with the group exposed to room air pretreated with vehicle.

mentioned above were purchased from Sigma (St. Louis, MO, U.S.A.).

3. Results

3.1. Increase in the permeability of the tracheal mucosa induced by acute exposure to cigarette smoke

Acute exposure to 10 puffs of cigarette smoke did not cause any measurable increase in the permeability of the tracheal mucosa (Fig. 1). In contrast, exposure to 20 or 30 puffs of cigarette smoke significantly increased the permeability of the tracheal mucosa. Therefore, the dose of 20 puffs was used for the subsequent studies to determine the involvement of the tachykinin receptors in the increase in the mucosal permeability induced by acute exposure to cigarette smoke in the trachea of guinea pigs.

3.2. Effect of tachykinin NK_1, NK_2 and NK_3 receptor antagonists on the cigarette smoke-induced increase in the permeability of the tracheal mucosa

Pretreatment with the three different tachykinin receptor antagonists did not affect baseline permeability in the

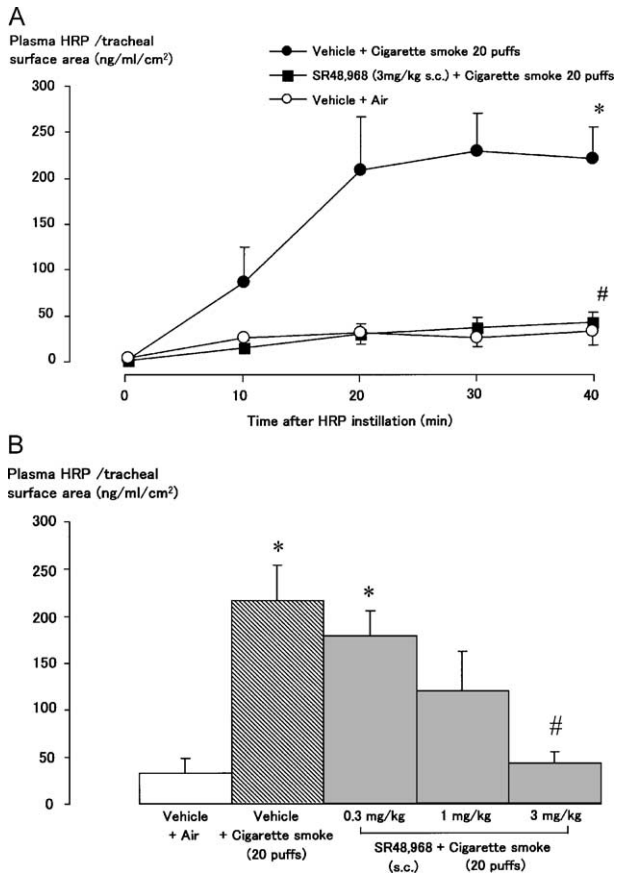

Fig. 4. Effect of the tachykinin NK_2 receptor antagonist, SR48,968, on the cigarette smoke-induced increase in the permeability of the tracheal mucosa. (A) Plasma levels of HRP during a 40-min sampling period in guinea pigs pretreated with SR48,968 (3 mg/kg s.c.) and then exposed to 20 puffs of cigarette smoke (n=5, closed squares), and in those pretreated with the vehicle and then exposed to 20 puffs of cigarette smoke (n=5, closed circles) or room air (n=5, open circles), are shown. The time point 0 indicates the point of instillation of the HRP solution into the tracheal segment. (B) Effect of decreased doses of the tachykinin NK_2 receptor antagonist, SR48,968, on the cigarette smoke-induced increase in the permeability of the tracheal mucosa. Plasma levels of HRP 40 min after the point of instillation of the HRP solution into the tracheal segment in guinea pigs pretreated with SR48,968 (0.3 or 1 or 3 mg/kg s.c.) and then exposed to 20 puffs of cigarette smoke (n=5, respectively), and in those pretreated with the vehicle and then exposed to 20 puffs of cigarette smoke (n=5) or room air (n=5), are shown. *P<0.01, as compared with the group exposed to room air pretreated with vehicle. $^{\#}P$<0.01, as compared with the group exposed to 20 puffs of cigarette smoke pretreated with vehicle.

trachea of guinea pigs induced by exposure to room air (data not shown). Pretreatment with the tachykinin NK_1 receptor antagonist SSR240,600 (3 mg/kg s.c.) and tachykinin NK_3 receptor antagonist SR142,801 (3 mg/kg s.c.) did not affect the increase in the permeability of the tracheal mucosa induced by acute exposure to cigarette smoke (Figs. 2A and 3A). Moreover, even in higher doses (6 and 12 mg/kg s.c.) of SSR240,600 and SR142,801 had no effect (Figs. 2B and 3B). In contrast, pretreatment with the tachykinin NK_2 receptor antagonist SR48,968 (3 mg/kg s.c.) completely inhibited cigarette smoke-induced increase in the permeability of the tracheal mucosa (Fig. 4A). However, in lower doses (0.3 and 1 mg/kg s.c.) of SR48,968 had no effect (Fig. 4B).

3.3. Morphological analysis of tracheal mucosa

Light-microscopic observation of tracheal mucosa showed that there was neither desquamation of the epithelial layer or subsequent denudation of the basement membrane in tracheal tissue from all groups, irrespective of exposure and pretreatments (Fig. 5).

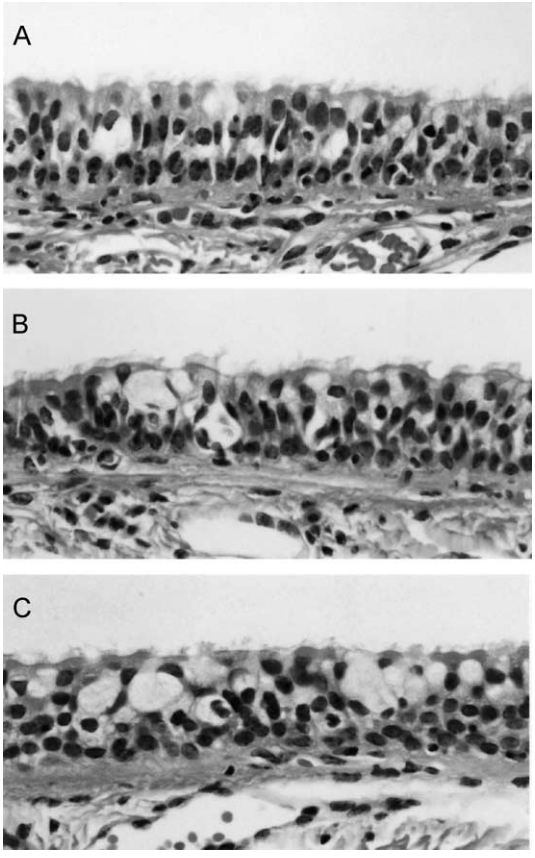

Fig. 5. Light micrographs of tracheal epithelium from isolated tracheal segments (hematoxylin and eosin; original magnification: ×400). (A) Tracheal epithelium removed from a guinea pig pretreated with the vehicle and then exposed to room air. (B) Tracheal epithelium removed from a guinea pig pretreated with the vehicle and then exposed to cigarette smoke. (C) Tracheal epithelium removed from a guinea pig pretreated with SR48,968 and then exposed to cigarette smoke.

4. Discussion

In the present study, we have demonstrated that acute exposure to cigarette smoke increases the permeability of the tracheal mucosa without any overt damage to the airway epithelium in guinea pigs in vivo. Selective antagonist of tachykinin NK_1 receptor, SSR240,600, and for tachykinin NK_3 receptor, SR142,801, did not affect the increase in the permeability induced by exposure to cigarette smoke in the guinea pig trachea. However, we found that the selective tachykinin NK_2 receptor antagonist, SR48,968, completely inhibited the increase in the permeability of the tracheal mucosa induced by acute exposure to cigarette smoke. These findings indicate that endogenous tachykinins are involved in the mucosal permeability of the trachea induced by exposure to cigarette smoke, via the activation of the tachykinin NK_2 receptor subtype.

The most important finding of the present study is the confirmation that tachykinin NK_2 receptors play a major role in tachykinin-mediated increase in the mucosal permeability in the airways. Tachykinins are well known as important mediators for the increase in the vascular permeability (outward permeability; substance movement from submucosa to lumen) produced by a variety of agents. This key inflammatory response that occurs in postcapillary venules by the opening of gaps between endothelial cells, due to exogenous or endogenous tachykinins, is invariably mediated by activation of tachykinin NK_1 receptors (Lundberg et al., 1983; Piedimonte et al., 1993). However, the airway mucosal permeability (inward permeability; substance movement from lumen to submucosa) of the trachea induced by two different stimuli, ozone (Fu et al., 2002) and cigarette smoke was mediated by the activation of tachykinin NK_2 receptors, suggesting that tachykinins NK_2 receptors are involved in the increasing airway mucosal permeability regardless of the causes. It is reasonable that two different tachykinin receptors, NK_1 and NK_2, are responsible for the two quite opposite directional permeabilities, vascular and mucosal permeability, respectively.

The association of cigarette smoking with pulmonary diseases, such as chronic obstructive pulmonary disease is well documented (Fletcher and Peto, 1977; Davis and Novotny, 1989; Barnes, 2003). Cigarette smoke has also been considered to play a role in the pathogenesis of asthma and as a trigger for acute symptoms (Bowler and Crapo, 2002). Stimulation of airway sensory nerve endings by exposure to cigarette smoke is known to trigger the release of tachykinins (Hong et al., 1995; Lee et al., 1995). Tachykinins have an ability to mimic many features of bronchial asthma, including bronchoconstriction, edema of bronchial mucosa due to increased plasma extravasation, hypersecretion of tracheobronchial mucous glands and airway hyperresponsiveness, which is the hallmark of bronchial asthma (Lundberg and Saria, 1983; Rogers et al., 1988; Boichot et al., 1995). The present study showed that exposure to cigarette smoke increased airway mucosal

permeability by releasing endogenous tachykinins that, in turn, activate tachykinin NK_2 receptors. This additional proinflammatory effect of tachykinins may contribute to the symptoms of airway inflammatory diseases.

Regarding the distribution of tachykinin NK_2 receptors in the airways that may justify the role of this receptor subtype in the regulation of mucosal permeability, it has been indicated that tachykinin NK_2 receptors are present on airway epithelial cells in both human and guinea pigs (Kim et al., 1995). In that study, migration of guinea pig tracheal epithelial cells and human bronchial epithelial cells was stimulated by the tachykinin NK_2 agonist, and was inhibited by the selective tachykinin NK_2 receptor antagonist. In addition, it has been suggested that airway macrophages possess tachykinin NK_2 receptors in guinea pigs (Brunelleschi et al., 1990). In another study using human airway tissue, the presence of tachykinin NK_2 receptors was detected in lymphocytes, mast cells and macrophages present in the airway mucosa (Mapp et al., 2000). It is thus possible that airway mucosal permeability increases via activation of tachykinin NK_2 receptors on airway epithelial cells and macrophages/inflammatory cells.

In summary, the present study in guinea pigs has demonstrated that endogenous tachykinins are involved in the increased mucosal permeability of the trachea induced by exposure to cigarette smoke, via the activation of the tachykinin NK_2 receptor subtype. It is suggested that preventing an increase in the airway mucosal permeability by administering an tachykinin NK_2 receptor antagonist might be of clinical benefit in the treatment of airway inflammatory diseases, such as bronchial asthma and chronic obstructive pulmonary disease.

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