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European Journal of Pharmacology 307 (1996) 291–298

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Role of tachykinin and bradykinin receptors and mast cells in gaseous formaldehyde-induced airway microvascular leakage in rats

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Received 13 November 1995; revised 6 March 1996; accepted 19 March 1996

Abstract

We have investigated the effects of CP-99,994 [(+)-(2*s*,3*s*)-3-(2-methoxybenzylamino)-2-phenylpiperidine], a tachykinin NK_1 receptor antagonist, HOE 140 (D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin), a bradykinin B_2 receptor antagonist, and ketotifen (4-(1-methyl-4-piperidylidene)4*H*-benzo[4,5]cycloheptal[1,2-*b*]thiophen-10(9*H*)-one hydrogen fumarate), a histamine H_1 receptor antagonist with mast cell-stabilizing properties, on microvascular leakage induced by gaseous formaldehyde. Extravasation of Evans blue dye into airway tissues was used as an index of airway microvascular leakage. Leakage of dye in the trachea and main bronchi increased significantly in a concentration-dependent fashion after 10 min inhalation of formaldehyde (5–45 parts per million (ppm)). The airway response induced by 10 min inhalation of 15 ppm formaldehyde (trachea: 119.5 ± 13.9 ng/mg, $n = 7$; main bronchi: 139.6 ± 7.9 ng/mg, $n = 7$) was abolished by the administration of CP-99,994 (3 and 6 mg/kg i.v.), but not by the administration of HOE 140 (0.65 mg/kg i.v.) nor ketotifen (1 mg/kg i.v.). The increase in vascular permeability induced by formaldehyde in the rat airway was mediated predominantly by NK_1 receptor stimulation. Activation of bradykinin receptors and mast cells did not appear to play an important role in this airway response.

Keywords: Bradykinin receptor; CP-99,994; Formaldehyde; Mast cell; Tachykinin receptor; Vascular permeability

1. Introduction

Formaldehyde, a common airborne pollutant in our modern environment (Samet et al., 1988), is irritating to the mucous membranes of the eyes and the upper respiratory tract. Formaldehyde is also considered to be a potential cause of inflammatory conditions of the lower airways (Bardana and Montanaro, 1991) because it is also a major constituent of cigarette smoke (Houlgate et al., 1989). Decreased lung function has been observed in individuals exposed to formaldehyde chronically (Alexandersson et al., 1982; Kilburn et al., 1985; Kriebel et al., 1993). Studies have shown that the inhalation of formaldehyde elicits bronchoconstriction in a small proportion of exposed individuals (Burge et al., 1985; Nordman et al., 1985). However, its mechanism of action is not clear.

Lundberg and Saria (1983) showed that the intratracheal administration of a formaldehyde solution increased vascular permeability in the rat trachea; capsaicin pretreatment, which depletes neuropeptides from sensory nerve endings in the airways, prevented the increase in vascular permeability induced by formaldehyde. In the present study, we investigated the effect of the inhalation of gaseous formaldehyde on vascular permeability and bronchoconstriction in rats. To further investigate the role of tachykinin release induced by formaldehyde, we evaluated the effect of a selective inhibitor of tachykinin NK_1 receptors, (CP-99,994) [(+)-(2*s*,3*s*)-3-(2-methoxybenzylamino)-2-phenylpiperidine] (McLean et al., 1993; Piedimonte et al., 1993), on the airway microvascular leakage induced by formaldehyde.

Capsaicin pretreatment (Lundberg and Saria, 1983) has been found to partly inhibit bradykinin-induced airway microvascular leakage in the rat (Saria et al., 1983), indicating that the effect of bradykinin, like the effect of formaldehyde, is mediated in part by tachykinin release.

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PII S0014-2999(96)00285-3

Bradykinin is produced in the lung, presumably via the conversion of kininogens in plasma exudate by tissue or cellular kallikrein (Proud and Kaplan, 1988). These findings suggest that inhaled formaldehyde may induce airway microvascular leakage partly via the production of bradykinin in the airways. Bradykinin-induced airway microvascular leakage is mediated by a bradykinin B_2 receptor mechanism (Lembeck et al., 1991). Therefore, we also examined the effect of a selective bradykinin B_2 receptor antagonist, HOE 140 (D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) (Hock et al., 1991; Wirth et al., 1991), on formaldehyde-induced airway responses in rats. Finally, we investigated the role of mast cells in the plasma extravasation induced by formaldehyde in the airways of rats by using ketotifen (4-(1-methyl-4-piperidylidene)4*H*-benzo[4,5]cycloheptal[1,2-*b*]thiophen-10(9*H*)-one hydrogen fumarate), a histamine H_1 receptor antagonist that inhibits mast cell degranulation (Martin and Römer, 1978).

2. Materials and methods

The experimental procedures followed in this study adhered to the Principles in the Care and Use of Laboratory Animals approved by the Committee on Animal Care of Nagoya University (Nagoya, Japan).

2.1. Animal preparation

Specific pathogen-free male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 230–340 g were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg) and placed on a homeothermic blanket (KN-474, Natume, Tokyo, Japan) that maintained rectal temperature at about 37°C. An adequate level of anesthesia, which was indicated by the disappearance of the corneal reflex and the withdrawal response to pinching the paw, was maintained by additional injections of pentobarbital (30 mg/kg i.p.). A tracheal cannula (8 mm long with a 1.3 mm inner diameter) was inserted into the lumen of the cervical trachea through a tracheostomy and sutured snugly. A polyethylene catheter was inserted into the left carotid artery for monitoring of mean systemic blood pressure with a pressure transducer (TP-400T, Nihon Kohden, Tokyo, Japan). The right external jugular vein was cannulated for administration of intravenous drugs and solutions. Animals were placed in the supine position and the intratracheal cannula was connected to a constant volume mechanical ventilator (KN-55, Natume) set at a tidal volume of 8 ml/kg and a frequency of 90 strokes/min. Pulmonary insufflation pressure was measured using a pressure transducer (TP-400T, Nihon Kohden) connected to a side arm of the expiratory limb of the ventilation tubing. Pulmonary insufflation pressure and blood pressure signals from the transducers were amplified (AP-601G, Nihon Kohden) and recorded with a two channel recorder (RTA-1100, Nihon Kohden).

2.2. Measurement of microvascular leakage

Vascular permeability was quantified by the extravasation of Evans blue dye, which has been shown to correlate well with the extravasation of radiolabeled albumin in the airways (Rogers et al., 1989). The thoracic cavity was opened 5 min after inhalation of formaldehyde or its sham, and a cannula was inserted into the aorta through the left ventricle. Perfusion was performed with 100–150 ml of 0.9% saline at a pressure of 100–120 mm Hg. Blood and perfused liquid were expelled through incisions in the right atrium. The lower part of the trachea (6 mm in length) and the main bronchi were sectioned off at a point 2.5 mm distal to the carina, dissected, blotted dry and then weighed. Evans blue dye was extracted in 1 ml of formamide at 37°C for 48 h and was measured with a spectrophotometer (Model 450, Bio-Rad, Tokyo, Japan) at a wavelength of 620 nm. The tissue content of Evans blue dye was calculated by interpolation on a standard curve of dye concentrations in the range of 0.1–10 μg/ml and is expressed as nanograms per milligram of wet tissue.

2.3. Formaldehyde supply system

A dynamic standard gas generator (Permeater Model PD-1B, Gastec, Ayase, Japan) (Freeland, 1977) was used to generate constant concentrations of gaseous formaldehyde from a 37% (w/v) formaldehyde solution containing 13% methanol (formalin). Formalin (3 ml) was placed in D-10, D-20, D-30 and D-30 (1/2) diffusion tubes (Gastec) to yield 2, 5, 15 and 45 parts per million (ppm) formaldehyde, respectively; the four diffusion tubes were then fixed in the constant temperature (50°C) bath of the gas generator. The flow rate of the dilution gas (i.e., room air) was adjusted to 0.9, 1.3, 1.1 and 0.7 l/min to yield 2, 5, 15 and 45 ppm of formaldehyde, respectively. These conditions were determined by measurement of the formaldehyde concentration using the 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole method, as described previously (Dickinson and Jacobsen, 1970; Mimura et al., 1976). A sham gas corresponding to each concentration of formaldehyde was generated from 13% methanol, the vehicle for formalin, under the same conditions used for formaldehyde. The gases were delivered to the ventilatory circuit through the air inlet of the ventilator. The temperatures of formaldehyde and its sham at the port of the tracheal cannula were about 20°C, similar to room temperature. The concentrations of formaldehyde were confirmed by the 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole method before experiments.

2.4. Protocol

2.4.1. Effects of different concentrations of formaldehyde

Animals were divided into eight groups ($n = 5–8$) to investigate the effects of different concentrations of formaldehyde (2, 5, 15 and 45 ppm) on pulmonary insufflation pressure, blood pressure and vascular permeability in the trachea and main bronchi. Evans blue dye (20 mg/kg) was injected intravenously into rats for a 1 min period 10 min after rats were connected to the ventilator. 1 min later, formaldehyde, its sham or room air was administered by inhalation for 10 min. The room air used was also passed through the gas generator. Perfusion was performed to measure dye leakage 5 min after the challenge, as described above. The pulmonary insufflation pressure and blood pressure were monitored throughout the experiments. Baseline pulmonary insufflation pressure and blood pressure were determined just before inhalation of formaldehyde. The lowest concentration of formaldehyde studied is similar to the level sometimes used for bronchial provocation tests in humans (Frigas et al., 1984; Nordman et al., 1985).

2.4.2. Time course of formaldehyde-induced airway microvascular leakage

To study the time course of the response to formaldehyde, Evans blue dye (20 mg/kg i.v.) was injected into one group of animals ($n = 7$) 5 min after the end of 10 min inhalation of 15 ppm formaldehyde; perfusion was initiated 15 min later. Pulmonary insufflation pressure and blood pressure were recorded for 30 min after the start of formaldehyde inhalation.

2.4.3. Effects of CP-99,994, HOE 140 and ketotifen against formaldehyde-induced airway microvascular leakage

We also studied the effects of a tachykinin NK_1 receptor antagonist, CP-99,994; a bradykinin B_2 receptor antagonist, HOE 140; and a histamine H_1 receptor antagonist, ketotifen, on airway microvascular leakage induced by inhaled formaldehyde. Animals were divided into ten groups ($n = 4–7$). CP-99,994 (1, 3 or 6 mg/kg), HOE 140 (0.65 mg/kg) or their diluent (0.9% saline, 0.5 ml/kg) was injected intravenously 10 min after rats were connected to the ventilator. Evans blue dye (20 mg/kg i.v.) was administered 3 min later for 1 min; a 10 min period of inhalation of 15 ppm formaldehyde was initiated 1 min later. Ketotifen (1 mg/kg i.v.) was administered slowly over a 2 min period because its rapid injection caused a sustained fall in blood pressure, by up to 60%, in a preliminary study. Evans blue dye (20 mg/kg i.v.) was injected 10 min later and then formaldehyde (15 ppm, 10 min) was administered. Animals were perfused 5 min after the end of formaldehyde inhalation. Measurements of extravasated Evans blue dye, pulmonary insufflation pressure and blood pressure were performed as described above. The seventh group of animals, used as sham control, received the sham gas for 15 ppm formaldehyde for a 10 min period following pretreatment with 0.9% saline (0.5 ml/kg i.v.). The remaining three groups were used to evaluate the effects of HOE 140 (0.65 mg/kg i.v.) and ketotifen (1 mg/kg i.v.) on extravasation of Evans blue dye induced by 10 min inhalation of 5 ppm formaldehyde. These groups were pretreated with HOE 140 (0.65 mg/kg i.v.), ketotifen (1 mg/kg i.v.) or 0.9% saline (0.5 ml/kg i.v.), as described above, followed by the inhalation of formaldehyde. Animals were perfused 5 min after the challenge. Measurements of extravasated Evans blue dye, pulmonary insufflation pressure and blood pressure were performed as described above.

The doses of CP-99,994 used were based on the results of previous studies (Piedimonte et al., 1993). The dose of HOE 140 used has been shown to completely inhibit bradykinin (0.53 mg/kg, i.v.)-induced plasma extravasation in the trachea, duodenum and urinary bladder of anesthetized rats (Lembeck et al., 1991). In a preliminary study, a slight but significant increase in plasma exudation in the main bronchi and intrapulmonary airway was induced in rats by a dose of HOE 140 twice that used for pretreatment in the present study (data not shown). Similarly weak agonist activity of HOE 140 was observed in a previous study (Lembeck et al., 1991). We chose a single dose of 1 mg/kg i.v.of ketotifen because it largely inhibited extravasation of Evans blue dye in the trachea (98.8 ± 8.5 ng/mg, $n = 5$, to 64.9 ± 6.8 ng/mg, $n = 4$; $P < 0.05$) and main bronchi (92.2 ± 11.9 ng/mg, $n = 5$, to 38.3 ± 3.1 ng/mg, $n = 4$; $P < 0.05$) induced by aerosol administration of ovalbumin (10 mg/ml, 2 min) in passively ovalbumin-sensitized rats in a preliminary study; in the nonsensitized rats, the Evans blue dye concentration in the trachea and main bronchi was 47.6 ± 5.9 ng/mg ($n = 5$) and 35.2 ± 8.9 ng/mg ($n = 5$), respectively. Ketotifen alone (2 mg/kg i.v.) induced significant hypotension (data not shown).

Animals were divided into three groups to study the effects of CP-99,994 on the extravasation of plasma into the main bronchi induced by the sham gas. 3 min after the administration of CP-99,994 (6 mg/kg i.v., $n = 4$) or its vehicle (0.9% saline, 0.5 ml/kg, $n = 6$), Evans blue dye (20 mg/kg i.v.) was injected. 1 min later, a 10 min period of inhalation of the sham gas for 15 ppm formaldehyde was initiated. The remaining group ($n = 6$) received room air by inhalation for 10 min period following pretreatment with 0.9% saline (0.5 ml/kg i.v.). Animals were perfused 5 min after the challenge. Extravasation of Evans blue dye was measured as described above.

2.5. Drugs

CP-99,994, HOE 140 and ketotifen were donated by Pfizer (Groton, CT), Hoechst (Frankfurt, Germany) and Sandoz Pharmaceuticals (Basle, Switzerland), respectively. Evans blue dye, formalin and formamide were obtained

from Sigma Chemical Co. (St. Louis, MO); pentobarbital sodium from Abbott Laboratories (North Chicago, IL); methanol from Katayama Chemical Industries (Osaka, Japan); 0.9% saline from Otsuka Chemical Co. (Tokyo, Japan); and 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole from Wako Pure Chemical Industries (Osaka, Japan). Evans blue dye was dissolved in 0.9% saline (40 mg/ml) and passed through a 5 μm Millipore filter (Millipore Products Division, Bedford, MA).

2.6. Statistical analysis

All values are expressed as the means ± S.E.M. Differences between two independent groups with equal variance, which was assessed with the F test, were analyzed by the unpaired Student's t test (two-tailed). Otherwise, Welch's test (two-tailed) was used. One-way analysis of variance (ANOVA) and Williams' test were used for multiple comparisons with the control group. A level of P less than 0.05 was considered to be statistically significant.

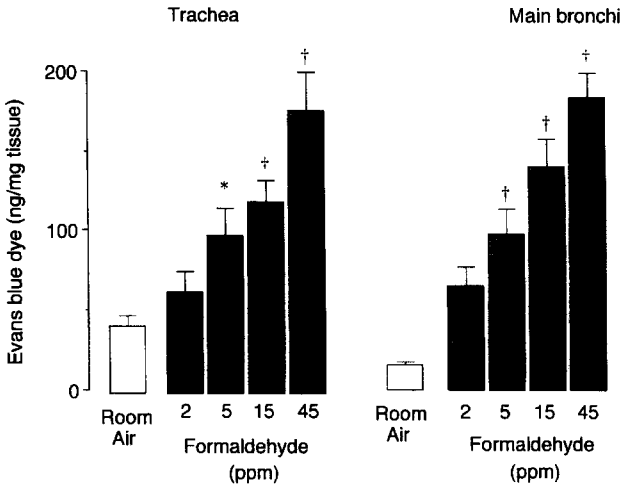

Fig. 1. Evans blue dye extravasation in the trachea and main bronchi induced by 10 min inhalation of four different concentrations (2, 5, 15, 45 ppm) of gaseous formaldehyde. Solid bars: formaldehyde ($n = 5$–7). Open bars: room air ($n = 7$). Values are the means ± S.E.M. * $P < 0.05$ and † $P < 0.01$ vs. room air-exposed group (Williams' test).

3. Results

3.1. Effect of different concentrations of gaseous formaldehyde

There were no significant differences in pulmonary insufflation pressure and blood pressure at baseline or 15 min after the start of formaldehyde inhalation among groups (Table 1). In concentrations of 5 ppm and above, formaldehyde significantly increased dye leakage in the trachea and main bronchi in a concentration-dependent manner compared with room air (Fig. 1) and the sham control. Sham gases, which contained barely detectable amounts of gaseous methanol (data not shown), induced a slight but significant increase in Evans blue dye extravasation in the main bronchi (5 ppm: 24.9 ± 7.0 ng/mg; 15 ppm: 26.3 ± 5.8 ng/mg; 45 ppm: 39.5 ± 8.3 ng/mg), but not in the trachea (5 ppm: 37.0 ± 11.0 ng/mg; 15 ppm: 36.9 ± 5.2 ng/mg; 45 ppm: 43.6 ± 10.4 ng/mg) compared with room air (trachea: 42.5 ± 14.7 ng/mg; main bronchi: 16.0 ± 4.2 ng/mg). In a concentration of 45 ppm, formaldehyde caused dye leakage into the parenchyma, as well as into the trachea and main bronchi, in two of seven rats. At a concentration of 15 ppm, formaldehyde caused a submaximal degree of Evans blue dye leakage at both airway levels without causing leakage of dye into the parenchyma. We therefore used a concentration of 15 ppm formaldehyde in subsequent studies.

3.2. Time course of formaldehyde-induced airway microvascular leakage

There was no significant increase in dye leakage in the trachea and main bronchi between 5 and 20 min after the cessation of formaldehyde inhalation (15 ppm, 10 min) (Fig. 2). Thus, although leakage of dye was increased 15 min after the start of formaldehyde inhalation, the increase was not maintained.

Table 1
Pulmonary insufflation pressure (PIP) and mean systemic blood pressure (BP) measured just before and 15 min after the start of formaldehyde inhalation

Stimulation	n	PIP (cm H_2O)		BP (mm Hg)	
		Before	After	Before	After
Room air	7	10.1 ± 0.3	10.8 ± 0.4	116.5 ± 11.0	122.2 ± 10.8
Formaldehyde (2 ppm)	5	10.8 ± 0.3	11.2 ± 0.4	118.9 ± 3.6	122.9 ± 5.6
Formaldehyde (5 ppm)	6	10.5 ± 0.4	11.6 ± 0.4	108.6 ± 11.6	119.0 ± 12.3
Sham	7	10.2 ± 0.2	11.6 ± 0.6	109.8 ± 7.8	109.8 ± 6.8
Formaldehyde (15 ppm)	7	10.9 ± 0.5	11.9 ± 0.5	124.3 ± 6.8	138.4 ± 7.2
Sham	6	9.8 ± 0.1	10.8 ± 0.2	125.4 ± 4.9	134.0 ± 1.9
Formaldehyde (45 ppm)	7	10.7 ± 0.4	11.7 ± 0.4	113.3 ± 10.3	115.2 ± 9.3
Sham	8	10.4 ± 0.2	11.5 ± 0.3	126.7 ± 6.8	127.9 ± 7.8

Values are the means ± S.E.M. There were no significant differences in baseline PIP and BP or in PIP and BP measurements 15 min after the start of formaldehyde inhalation among groups used in this study.

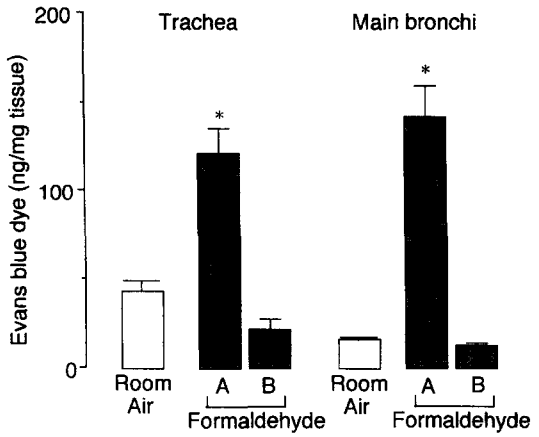

Fig. 2. Time course of extravasation of Evans blue dye in the trachea and main bronchi induced by inhalation of formaldehyde (15 ppm, 10 min) in the rats. Open bars represent the content of Evans blue dye extravasated for 15 min after the start of room air inhalation. Solid bars show the leakage of dye for 15 min (A), and between 15 and 30 min (B) after the start of formaldehyde inhalation. Data are expressed as the means ± S.E.M.; $n = 6$ rats/group. * $P < 0.01$ vs room air-exposed group (unpaired Student's t test or Welch's test).

3.3. Effects of CP-99,994, HOE 140 and ketotifen on formaldehyde-induced airway microvascular leakage

There were no significant differences among groups in pulmonary insufflation pressure and blood pressure before or after treatment with CP-99,994 (1, 3 and 6 mg/kg i.v.), HOE 140 (0.65 mg/kg i.v.) and ketotifen (1 mg/kg i.v.) (data not shown). There was no significant difference in leakage of dye in the trachea and main bronchi among the 3 and 6 mg/kg i.v. of CP-99,994-pretreated, formaldehyde-exposed groups and the 0.9% saline-pretreated, sham-stimulated group (Fig. 3), indicating that these doses of CP-99,994 completely abolished Evans blue dye extravasation in the trachea and main bronchi induced by 15 ppm formaldehyde. The lowest dose of CP-99,994 inhibited the airway response partly, but significantly (Fig. 3). HOE 140 and ketotifen had no effect on the airway response induced by 10 min inhalation of 15 ppm formaldehyde (Fig. 3) or of 5 ppm formaldehyde (trachea: 108.2 ± 8.3 ng/mg, $n = 6$; main bronchi: 96.3 ± 8.5 ng/mg, $n = 6$). CP-99,994(6 mg/kg i.v.) did not significantly inhibit the leakage of dye in the main bronchi induced by the sham gas for 15 ppm formaldehyde(data not shown).

4. Discussion

The inhalation of gaseous formaldehyde produced a concentration-dependent increase in vascular permeability in the rat trachea and main bronchi, whereas no bronchoconstrictor effect was observed. Wei and Kiang (1987) previously showed that an extremely high concentration of formalin vapor (713 ± 36 mg/m^3) induced microvascular leakage in the trachea in anesthetized rats. We determined the threshold concentration of formaldehyde that induces leakage above the baseline level in the airways of the anesthetized rat. The concentration of formaldehyde was similar to the level, namely above 3 ppm, sometimes detected in occupational settings (Occ. Health & Safety Letter, 1985, 15, 2).

CP-99,994, a selective NK_1 receptor antagonist (McLean et al., 1993; Piedimonte et al., 1993), completely abolished the formaldehyde-induced airway response, indicating that formaldehyde-induced airway microvascular leakage was mediated by stimulation of tachykinin NK_1 receptors. Our finding is consistent with the results of a

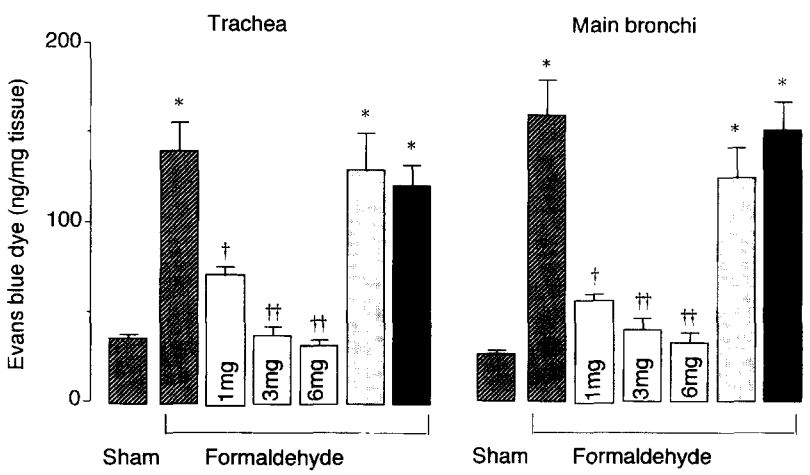

Fig. 3. Effect of CP-99,994, HOE 140 and ketotifen on Evans blue dye extravasation in the trachea and main bronchi induced by inhalation of gaseous formaldehyde (15 ppm, 10 min). Rats were treated with 1, 3 or 6 mg/kg i.v. CP-99,994 (open bars), 0.65 mg/kg i.v. HOE 140 (hatched bars), 1 mg/kg i.v. ketotifen (solid bars) or their vehicle (0.5 ml/kg i.v. 0.9% saline; shaded bars) before formaldehyde challenge. Sham: animals exposed to the sham gas for 15 ppm formaldehyde (10 min) after pretreatment with 0.9% saline (0.5 ml/kg i.v.). Data are the means ± S.E.M.; $n = 6$–7 rats/group. * $P < 0.05$ vs. sham-stimulated group(unpaired Student's t test or Welch's test), † $P < 0.05$, †† $P < 0.01$ vs. 0.9% saline-pretreated, formaldehyde-exposed control group (Williams' test).

previous study showing that NK_1-selective agonists dose dependently increase vascular permeability in the rat tracheo-bronchial region, but that NK_2- and NK_3-selective agonists are inactive (Abelli et al., 1991). Morimoto et al. (1992) demonstrated that FR 113680, a selective NK_1 receptor antagonist, completely prevents plasma extravasation in the rat trachea induced by substance P and neurokinin A. NK_1 receptors have been found to have a similar effect on vascular permeability in guinea pig airways (Murai et al., 1992; Sakamoto et al., 1993). Although we did not use an enantiomer of CP-99,994 in the present study, CP-99,994 has previously been shown to inhibit the increase in vascular permeability in the rat trachea induced by intravenous administration of substance P and inhalation of hypertonic saline in a stereoselective manner (Piedimonte et al., 1993).

The vehicle gas for formaldehyde, which contained gaseous methanol, induced less plasma extravasation in the main bronchi than formaldehyde did in the present study. CP-99,994 had no effect on this airway response, suggesting that NK_1 receptors may not be stimulated by very low concentrations of gaseous methanol.

NK_1 receptors are stimulated by the tachykinins substance P and neurokinin A (Regoli et al., 1988). These mediators are released from sensory nerve endings in the trachea and main bronchi, in which substance P-immunoreactive sensory nerves are densely distributed (Lundberg et al., 1984; Baluk et al., 1992). Thus, gaseous formaldehyde and formalin (Lundberg and Saria, 1983) can cause airway microvascular leakage by inducing the release of tachykinins from airway sensory nerves in the rat. The present results indicate that formaldehyde-induced plasma extravasation has a very short half-life and is not detected if the tracer is given too late after the start of the stimulation. This finding is consistent with that of a recent study showing that substance P-induced plasma leakage into the rat trachea peaks within 2 min and then declines rapidly (Bowden et al., 1994). Delay-Goyet and Lundberg (1991) have shown that cigarette smoke-induced airway microvascular leakage is also due to NK_1 receptor-mediated mechanisms. Cigarette smoke contains significant quantities of formaldehyde (Houlgate et al., 1989), suggesting that formaldehyde may play an important role in the airway response induced by cigarette smoke.

There are some similarities in the effects of formaldehyde and bradykinin in rat airways. The intratracheal administration of bradykinin induces plasma extravasation and release of endogenous tachykinins in rat airways (Lundberg and Saria, 1983). Studies suggest that plasma exudation in the airways may cause secondary generation of bradykinin (Proud and Kaplan, 1988; Chung et al., 1990). Bertrand et al. (1993) showed that allergen-induced plasma extravasation in guinea pig airways was inhibited by the bradykinin B_2 receptor antagonist HOE 140. However, HOE 140 had no effect on formaldehyde-induced airway microvascular leakage in the present study, although the dose of HOE 140 we used has previously been shown to effectively inhibit similar degrees of bradykinin-induced airway microvascular leakage (Lembeck et al., 1991). Thus, formaldehyde appeared to cause tachykinin release, without bradykinin mediation. However, a minor contribution of bradykinin cannot be completely ruled out because we used only a single, high dose of HOE 140.

Previous studies on isolated rat peritoneal mast cells (Mazurek et al., 1981; Devillier et al., 1989) suggest that tachykinins, such as substance P, induce mast cell degranulation without binding to a specific tachykinin receptor. Rat lung mast cells have been shown to release histamine in response to high doses of substance P in vitro (Ali et al., 1986). In the present study, we used ketotifen to examine the role played by mast cells in formaldehyde-induced airway leakage resulting from tachykinin release. Ketotifen has previously been shown to be a potent inhibitor of allergen-induced increases in airway resistance in the sensitized rat, an effect that is probably due to both antagonism of histamine H_1 receptors and mast cell-stabilizing properties (Martin and Römer, 1978). We also found that ketotifen induced a strong inhibition of airway microvascular leakage mediated by activation of mast cells (see Section 2.4.3, second paragraph). However, ketotifen had no effect on the airway responses to formaldehyde. Thus, the release of tachykinin after the inhalation of formaldehyde did not appear to accompany mast cell activation. In addition, formaldehyde is unlikely to have stimulated mast cells directly. The effect of higher doses of ketotifen on this response was not evaluated, since weak agonist activity has been observed at doses above 1 mg/kg i.v.

Exogenous tachykinins produce bronchoconstriction (Joos et al., 1994) and hypotension in the rat (Maggi et al., 1987; Lembeck et al., 1992). In the present study, however, the formaldehyde-induced release of endogenous tachykinins did not induce these effects. The reason for this discrepancy is not clear. It is possible that the site of tachykinin release may be distant from the airway and vascular smooth muscles. Inhaled formaldehyde is likely to induce the release of tachykinins from sensory nerve endings in the epithelium, where most of the tachykinin-containing sensory nerves in the airways are located (Baluk et al., 1992). Released tachykinins may diffuse and be actively degraded by several peptidases, including neutral endopeptidase and angiotensin-converting enzyme (Dusser et al., 1988; Lötvall et al., 1991), before they reach their site of action. Airway microvascular leakage may increase the thickness of the airway wall due to edema, leading to a narrowing of the airway lumen (Persson, 1986). Formaldehyde may have caused a small increase in wall thickness that did not alter lung function in the present study.

In conclusion, the formaldehyde-induced microvascular leakage of the airways in the rat resulted from the release of tachykinins from sensory nerve endings, which suggests that formaldehyde may cause neurogenic inflammation in asthmatic airways.

Acknowledgements

We are grateful to Professor Kazuyoshi Watanabe of the Department of Pediatrics, Nagoya University School of Medicine (Nagoya, Japan) for his kind support. We thank Pfizer Inc. (Groton, CT), Hoechst AG (Frankfurt, Germany) and Sandoz Pharmaceutical Ltd. (Basle, Switzerland) for their gifts of CP-99,994, HOE 140 and ketotifen, respectively.

This study was supported in part by a grant for Health and Welfare Programs of the Ministry of Health and Welfare, and by funds from the Aichi Health Promotion Foundation, Japan.

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