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Involvement of endogenous tachykinins in LTD_4-induced airway responses

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Involvement of endogenous tachykinins in LTD_4-induced airway responses. J. Ishikawa, M. Ichinose, M. Miura, N. Kageyama, H. Yamauchi, M. Tomaki, Y. Sasaki, K. Shirato. ©ERS Journals Ltd 1996.

ABSTRACT: Leukotriene D_4-(LTD_4) has been reported to cause tachykinin release from airway sensory nerves. However, the functional significance of endogenously released tachykinins in LTD_4-mediated airway responses has not been fully clarified.

The aim of this study was to investigate whether LTD_4-induced airway responses are due, in part, to tachykinin release in guinea-pigs. Airway plasma exudation and bronchoconstriction were assessed by measuring extravasation of Evans blue dye and by mean pulmonary resistance (R_L) in the presence of atropine (1 mg·kg^{-1} *i.v.*) and propranolol (1 mg·kg^{-1} *i.v.*), respectively.

LTD_4 (5 µg·mL^{-1} for 1 min) inhalation caused increase in plasma exudation and R_L. Capsaicin pretreatment of animals to deplete sensory neuropeptides significantly inhibited LTD_4-induced plasma exudation in the main bronchi, but not in the central (cIPA) and peripheral intrapulmonary airways (pIPA). Pretreatment with specific tachykinin neurokinin-1 (NK_1)-receptor antagonists, FK 888 (10 mg·kg^{-1} *i.v.*) and CP 96345 (4 mg·kg^{-1} *i.v.*), also significantly reduced LTD_4-induced plasma exudation in the main bronchi, and in the main bronchi and cIPA, respectively. However, these antagonists did not significantly affect the LTD_4-induced increase in R_L. In contrast, neurokinin-2 (NK_2)-receptor antagonist, SR 48968 (0.3 mg·kg^{-1} *i.v.*), significantly inhibited the bronchoconstriction after LTD_4-inhalation.

These results suggest that leukotriene D_4-induced bronchoconstriction and plasma exudation in guinea-pigs are, in part, due to tachykinin release from airway sensory nerves.

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Airway inflammation is recognized to be important in the pathogenesis of asthma and this phenomenon involves inflammatory cell-derived mediators, neural abnormality, and interactions between these two factors [1]. Among the inflammatory mediators, sulphidopeptide leukotrienes (LTs) are thought to play an important role in the pathogenesis of the disease, because recently developed receptor antagonists or synthesis inhibitors of LTs improve basal pulmonary function [2, 3] and protect antigen- and exercise-induced airway narrowing in asthmatic patients [4, 5]. Neuropeptides, especially tachykinins, released from airway sensory nerves are important, since these peptides cause airway smooth muscle contraction, microvascular leakage, and mucus secretion, which are compatible with the features of asthma [6].

There are several reports concerning the interaction between leukotriene D_4 (LTD_4) and tachykinins. In guinea-pig ileum, LTD_4 causes substance P (SP) release and the contractile response to LTD_4 is partially inhibited by SP antagonists, indicating that SP has a role in mediating the effect of LTD_4 in the longitudinal smooth muscle of the ileum [7]. A recent report showed that infusion of LTD_4 *via* the airways resulted in the enhanced recovery of SP- and neurokinin A (NKA)-like immunoreactivities from the lung [8], suggesting that LTD_4 also causes tachykinin release from airway sensory nerves. Further, bronchial smooth muscle contraction and vascular leakage by stimulation of capsaicin-sensitive sensory fibres are partially inhibited by LTD_4-antagonists, suggesting that tachykinins cause endogenous LT release [9]. However, the functional significance of endogenously released tachykinins in LTD_4-mediated airway responses has not been fully clarified.

Thus, in this study, using chronic capsaicin administration, which causes tachykinin depletion, and neurokinin (NK)-receptor antagonist pretreatment, it was demonstrated that LTD_4 causes airway responses *via* tachykinin release from airway sensory nerves. The airway inflammation was assessed by Evans blue dye leakage into airway tissues as a marker of microvascular leakage, and the bronchoconstrictor response was quantified by means of pulmonary resistance (R_L) measurements.

Methods

Animal preparations

Male Dunkin-Hartley guinea-pigs (n=70; Funabashi Farm, Funabashi, Japan) weighing 350–450 g were anaesthetized with an intraperitoneal injection of urethane (2 $g \cdot kg^{-1}$). Animals were placed on a heated pad (Deltaphase Isothermal Pad, model 39DP, Braintree Science Inc., UK) to maintain the body temperature at about 37°C. Both external jugular veins were exposed for administration of drugs. All animals were pretreated 30 min before experimentation with atropine and propranolol (1 $mg \cdot kg^{-1}$ *i.v.*, for both,) to block muscarinic and β-adrenergic receptor-mediated modification, respectively. The doses of atropine and propranolol were chosen according to previous studies [10, 11]. A tracheal cannula was inserted into the lumen of the cervical trachea, and this cannula was connected to a constant volume mechanical ventilator (Model SN-480-7; Shinano Seisakusho, Tokyo, Japan) at a frequency of 60 $strokes \cdot min^{-1}$ and at a tidal volume (V_T) of 1 $mL \cdot 100\ g^{-1}$.

Pulmonary resistance

Airflow (V') was determined by connecting a pneumotachograph (Model 00; Fleisch, Lausanne, Switzerland) to the tracheal cannula and measuring the pressure drop across the device with a differential pressure transducer (MP45, ±5 cmH_2O; Validyne Corp., Northridge, CA, USA). V_T was determined by electrical integration of the airflow signal. Pleural pressure (P_{pl}) was measured by means of an oesophageal balloon connected to the differential pressure transducer (MP45, ±100 cmH_2O). Transpulmonary pressure (P_{tp}) was obtained by electrical subtraction of P_{pl} from airway opening pressure (P_{ao}). Pulmonary resistance (R_L) was obtained by a subtraction method [12]. R_L change was monitored after LTD_4 inhalation for 5 min.

Measurement of airway plasma exudation

Vascular permeability was quantified by the extravasation of Evans blue dye, which correlates well with the extravasation of radiolabelled albumin in guinea-pig airways [13]. Evans blue dye (30 $mg \cdot kg^{-1}$, 30 mg in 1 mL saline) which was filtered using a 0.22 µm Millipore filter, was injected into the jugular vein 1 min before LTD_4 (5 $\mu g \cdot ml^{-1}$ for 1 min) or saline (for 1 min) inhalation. The inhalation challenge was performed using an ultrasonic nebulizer (mean particle size = 5 µm; manufacturer's specification) (NE-U11B; Omron, Tateishi Co., Tokyo, Japan) interposed between the inspiratory part of the ventilator and the tracheal cannula. After the induction of leakage (5 min after the LTD_4 challenge), the thorax was opened and a catheter was inserted into the aorta through a left ventriculotomy. Ventricles were cross-clamped, and blood was expelled through an incision in the right atrium at 80 mmHg pressure with about 100 mL saline (pH 5.5) until the perfusate became clear. The lungs were removed, and parenchyma was scraped off. The main bronchi and the intrapulmonary airways were separated from each other. The intrapulmonary airways were divided into central (cIPA, the proximal 3 mm portion) and peripheral (pIPA, the remaining distal portion) components as described previously [10, 11]. All tissues were blotted dry and weighed. Evans blue dye was extracted in 2 mL of formamide at 37°C incubation for 16 h. Dye concentration was quantified from light absorbance at 620 nm (Spectrophotometer 220A; Hitachi Ltd., Tokyo, Japan) and expressed as ng dye per mg wet weight of tissue, as calculated from a standard curve of dye concentrations in the range of 0.5–10 $\mu g \cdot mL^{-1}$.

Capsaicin pretreatment

Animals were anaesthetized with ketamine (50 $mg \cdot kg^{-1}$ *i.m.*) and xylazine (0.1 $mg \cdot kg^{-1}$ *i.m.*). Aminophylline (25 $mg \cdot kg^{-1}$ *i.p.*) and terbutaline (0.1 $mg \cdot kg^{-1}$ *s.c.*) were given 30 min before capsaicin administration to protect against bronchoconstriction. Capsaicin (50 $mg \cdot kg^{-1}$ *s.c.*) or vehicle for capsaicin (ethanol/Tween 80, 1 $mL \cdot kg^{-1}$ *s.c.*) was injected, and animals were studied 1 week after treatment.

Experimental protocols

The effects of intravenously administered neurokinin-1 (NK_1) antagonist, FK 888, or LTD_4 antagonist, ONO-1078, on LTD_4-induced Evans blue dye exudation were studied in seven groups: Group I: dimethylsulphoxide (DMSO, vehicle for both antagonists, 0.1 $mL \cdot kg^{-1}$) and saline inhalation (n=5); Group II: FK 888 (10 $mg \cdot kg^{-1}$) and saline inhalation (n=5); Group III: ONO-1078 (200 $\mu g \cdot kg^{-1}$) and saline inhalation (n=5); Group IV: DMSO and LTD_4 inhalation (n=6); Group V: FK 888 (1 $mg \cdot kg^{-1}$) and LTD_4 inhalation (n=6); Group VI: FK 888 (10 $mg \cdot kg^{-1}$) and LTD_4 inhalation (n=7); Group VII: ONO-1078 (200 $\mu g \cdot kg^{-1}$) and LTD_4 inhalation (n=5). DMSO, FK 888 or ONO-1078 was administered 1 min before Evans blue dye injection (30 $mg \cdot kg^{-1}$ *i.v.*). LTD_4 or saline inhalation followed 1 min after the injection of Evans blue dye.

In a separate set of experiments, the effects of another NK_1-receptor antagonist, CP 96345 (4 $mg \cdot kg^{-1}$ *i.v.*), neurokinin-2 (NK_2)-receptor antagonist, SR 48968 (0.3 $mg \cdot kg^{-1}$ *i.v.*), or vehicle of both antagonists, DMSO (0.1 $mL \cdot kg^{-1}$ *i.v.*; n=5) on LTD_4-induced airway responses were studied. The doses of FK 888, CP 96345, and SR 48968 were chosen according to previous studies [14–16].

In a separate experiment, guinea-pigs chronically pretreated with capsaicin (n=8) or the vehicle of capsaicin (n=8) were challenged with LTD_4 inhalation to examine the effect of tachykinin depletion on the LTD_4-induced response.

Drugs and chemicals

FK 888 and ONO-1078 were kindly donated by Fujisawa Pharmaceutical Co. Ltd (Osaka, Japan) and Ono Pharmaceutical Co. Ltd (Osaka, Japan), respectively. CP 96345 was obtained from Yamanouchi Pharmaceutical Co. Ltd. (Ibaraki, Japan) and SR 48968 was kindly donated by X. Emonds-Alt (Sanofi Recherche, France). Evans blue dye was purchased from Aldrich Chemical Co. Inc.

(Milwaukee, WIS, USA). Substance P, urethane and xylazine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dimethylsulphoxide (DMSO), ethanol, formamide and Tween 80 were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Atropine sulphate was obtained from Tanabe Chemical Co. (Osaka, Japan). Propranolol hydrochloride was purchased from Imperial Chemical Industries plc (Macclesfield, UK). Five percent Glucose and saline (0.9% sodium chloride) were obtained from Otsuka Chemical Co. (Tokyo, Japan). Aminophylline was purchased from Eisai Co. Ltd (Tokyo, Japan). Ketamine was obtained from Sankyo Co. Ltd (Tokyo, Japan).

FK 888 and ONO-1078 were dissolved in 100% DMSO at the concentration of 100 and 2 $mg \cdot mL^{-1}$, respectively, and these antagonists were injected by microsyringe (MS-N50; Terumo, Tokyo, Japan) at a volume of 0.1 $mL \cdot kg^{-1}$. Capsaicin was diluted to a concentration of 50 $mg \cdot mL^{-1}$ in 10% ethanol, 10% Tween 80 and 80% saline.

Statistical analysis

Data are expressed as mean±SEM. Comparisons of the concentration of extractable Evans blue dye among groups were made with Student's unpaired t-test with Bonferroni correction. The degree of LTD_4-induced bronchoconstriction was analysed by two-way analysis of variance between groups, and Student's unpaired t-test. Probability values of less than 0.05 were considered significant.

Results

Effect of ONO-1078 on LTD_4-induced plasma exudation

The tissue content (mean±SEM) of Evans blue dye in response to saline inhalation challenge (basal plasma exudation) in main bronchi, cIPA and pIPA was 13.8±3.3, 18.9±8.8 and 17.4±4.6 $ng \cdot mg^{-1}$ tissue, respectively (fig. 1). LTD_4 inhalation significantly increased Evans blue dye exudation in main bronchi to 127.1±16.2, in cIPA to 124.9±8.4, and in pIPA to 178.3±18.2 $ng \cdot mg^{-1}$ tissue. ONO-1078 (200 $\mu g \cdot kg^{-1}$ *i.v.*) itself had no significant effect on the basal plasma exudation in any component of the airways, but almost completely inhibited the LTD_4-induced plasma exudation in all airways (fig. 1).

Effect of FK 888 on LTD_4-induced plasma exudation

FK 888 (10 $mg \cdot kg^{-1}$ *i.v.*) itself also had no significant effect on basal extravasation in any part of the airways (fig. 2). LTD_4 inhalation-induced plasma exudation was significantly reduced by intravenous injection of FK 888 at the dose of 10 $mg \cdot kg^{-1}$ in main bronchi but not in intrapulmonary airways (fig. 2).

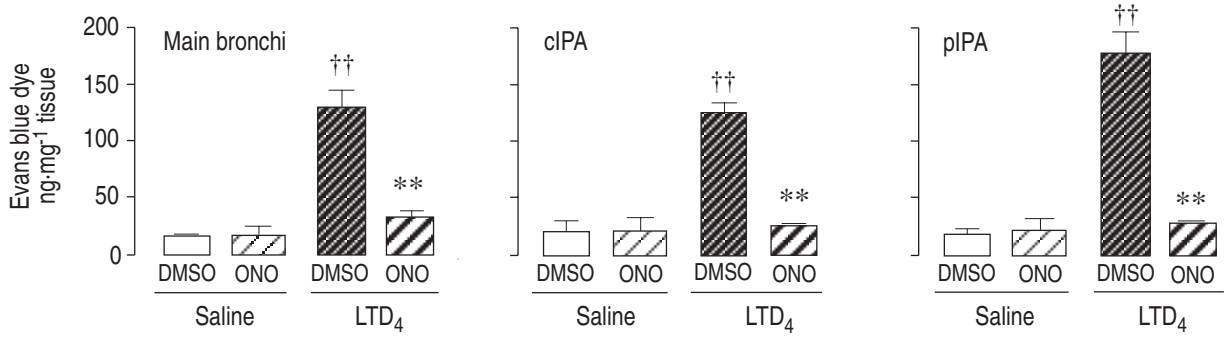

Fig. 1. – Histogram illustrating the degree of Evans blue dye exudation evoked by LTD_4 (5 $\mu g \cdot mL^{-1}$ in saline for 1 min) inhalation challenge in guinea-pig airways. ▭ : response to saline inhalation challenge (for 1 min) after *i.v.* injection of DMSO (vehicle for ONO-1078, 0.1 $mL \cdot kg^{-1}$, n=5). ▨ : response to saline inhalation after ONO-1078 (200 $\mu g \cdot kg^{-1}$, *i.v.*, n=5). ▩ : response to LTD_4 inhalation after *i.v.* injection of DMSO (n=6). ▩ : response to LTD_4 inhalation after ONO-1078. Values are presented as mean±SEM. cIPA and pIPA: central and peripheral intrapulmonary airways, respectively; LTD_4: leukotriene D_4; DMSO: dimethylsulphoxide; ONO: ONO-1078. ††: $p<0.01$, compared with values of saline inhalation after DMSO administration; **: $p<0.01$, compared with the value of LTD_4 inhalation after DMSO administration.

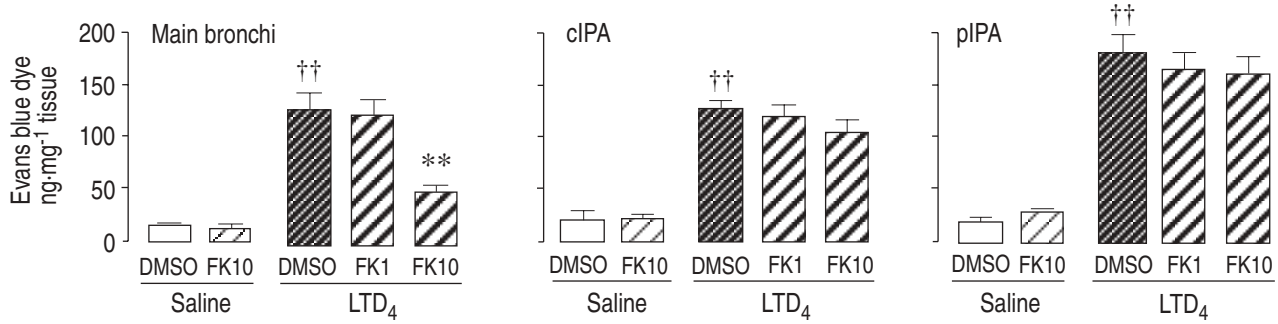

Fig. 2. – Effects of NK_1 receptor antagonist, FK 888 (FK), on LTD_4-induced airway plasma exudation in main bronchi, and central (cIPA) and peripheral intrapulmonary airways (pIPA). ▭ : response to saline inhalation (for 1 min) after *i.v.* injection of DMSO (vehicle for FK 888, 0.1 $mL \cdot kg^{-1}$, n=5). ▨ : response to saline inhalation after FK 888 (10 $mg \cdot kg^{-1}$, *i.v.*, n=5). ▩ : response to LTD_4 inhalation (5 $\mu g \cdot mL^{-1}$, for 1 min) after *i.v.* injection of DMSO (n=6). ▩ : response to LTD_4 inhalation after FK 888 (1 $mg \cdot kg^{-1}$, *i.v.*, n=6; and 10 $mg \cdot kg^{-1}$, *i.v.*, n=7). Values are presented as mean±SEM. ††: $p<0.01$, compared with values of saline inhalation after intravenous injection of DMSO. **: $p<0.01$, compared with the values of LTD_4 inhalation after intravenous injection of DMSO. FK1: FK 888 0.1 $mg \cdot kg^{-1}$; FK10: FK 888 10 $mg \cdot kg^{-1}$. For further abbreviations see legend to figure 1.

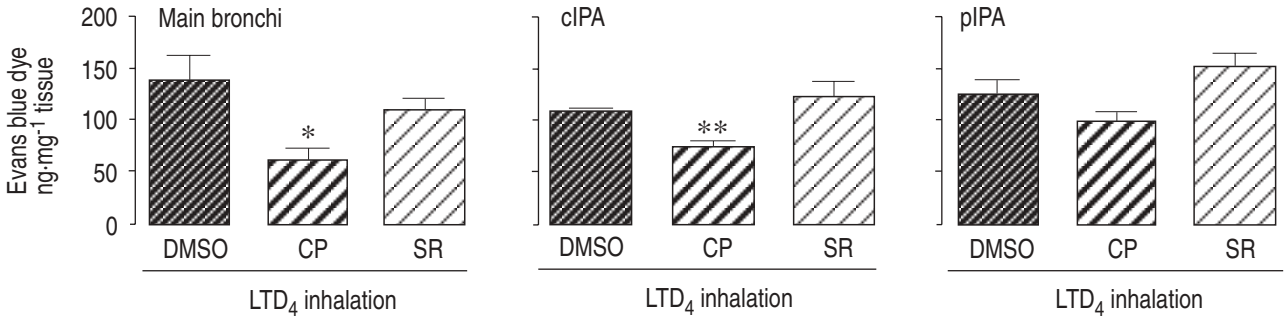

Fig. 3. – Effect of CP 96345 (CP) and SR 48968 (SR) on LTD_4-induced airway plasma exudation. Values are presented as mean±SEM. ▨ : response to LTD_4 inhalation (5 µg·mL^{-1}, for 1 min) after *i.v.* injection of DMSO (n=5); ▨ : response to LTD_4 inhalation after CP 96345 (4 mg·kg^{-1} *i.v.*, n=5); ▨ : response to LTD_4 inhalation after SR 48968 (0.3 mg·kg^{-1} *i.v.*, n=5). *: p<0.05; and **: p<0.01, compared with the values of DMSO (vehicle for CP and SR, n=5) pretreatment. For abbreviations see legend to figure 1.

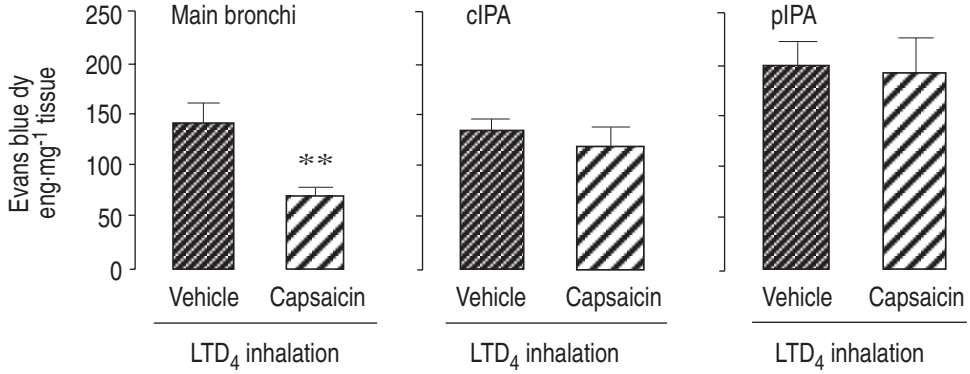

Fig. 4. – Effect of capsaicin pretreatment on LTD_4-induced airway plasma exudation in guinea-pig airways. ▨ : response to LTD_4 inhalation (5 µg·mL^{-1}, for 1 min) after pretreatment with vehicle for capsaicin (vehicle, n=8). ▨ : response to LTD_4 inhaltion after pretreatment with capsicin (50 mg·kg^{-1} *i.m.*, n=8). Values are presented as mean±SEM. **: p<0.01, compared with the value of vehicle pretreatment. For abbreviations see legend to figure 1.

Effect of CP 96345 and SR 48968 on LTD_4-induced plasma exudation

CP 96345 (4 mg·kg^{-1} *i.v.*) significantly inhibited LTD_4-induced airway plasma exudation in main bronchi and cIPA, but not in pIPA (fig. 3). SR 48968 (0.3 mg·kg^{-1} *i.v.*) did not have a significant effect on LTD_4-mediated plasma exudation in any airways (fig. 3).

Effect of the capsaicin pretreatment on LTD_4-induced plasma exudation

In the vehicle for capsaicin pretreated animals, LTD_4 inhalation-caused Evans blue dye exudation in main bronchi, cIPA and pIPA of 140.0±20.5, 133.5±12.1 and 197.3±23.0 ng·mg^{-1} tissue, respectively. This exudation was significantly reduced by capsaicin pretreatment in main bronchi but not in intrapulmonary airways (fig. 4).

Effect of ONO-1078 and FK 888 on the LTD_4-induced changes in pulmonary resistance

LTD_4 inhalation challenge significantly increased R_L at 1–5 min after the challenge (fig. 5). ONO-1078 (200 µg·kg^{-1} *i.v.*) significantly inhibited R_L changes evoked by LTD_4 challenge. On the other hand, FK 888 (10 mg·kg^{-1} *i.v.*) did not significantly affect the LTD_4-induced R_L changes (fig. 5).

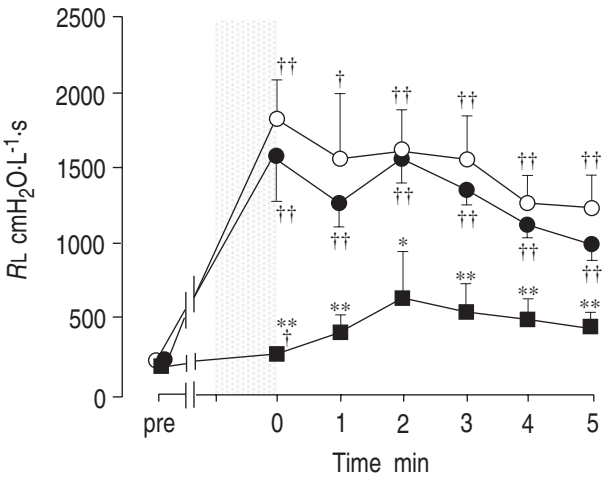

Fig. 5. – Effect of FK 888 and ONO-1078 on pulmonary resistance (R_L) changes evoked by LTD_4 inhalation (5 µg·mL^{-1}, for 1 min) challenge in guinea-pig airways. The shaded area indicates the period of LTD_4 inhalation. —○— : response to LTD_4 inhalation after intravenous injection of DMSO (vehicle for ONO-1078 and FK 888) (n=6); —●— : response to LTD_4 inhalation after FK 888 (10 mg·kg^{-1} *i.v.*, n=7); —■— : response to LTD_4 inhalation after ONO-1078 (200 µg·kg^{-1} *i.v.*, n=5). Values are presented as mean±SEM. †: p<0.05; and ††: p<0.01, compared with the values of preinhalation (pre); *: p<0.05; and **: p<0.01, compared with the values of LTD_4 inhalation after intravenous injection of DMSO. R_L: pulmonary resistance. For further abbreviations see legend to figure 1.

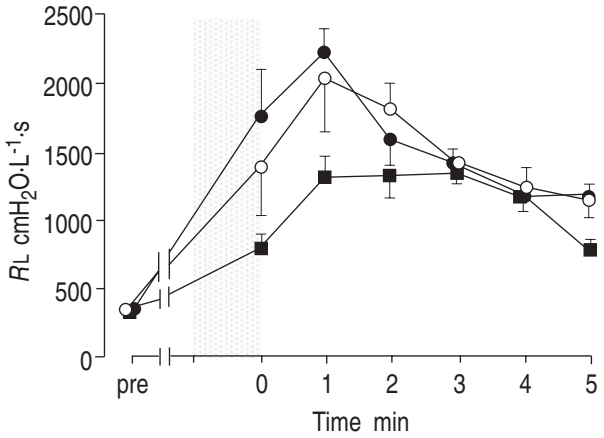

Fig. 6. – Effect of CP 96345 (—●—) and SR 48968 (—■—) on pulmonary resistance (R_L) changes evoked by LTD_4 inhalation. The shaded area indicates the period of LTD_4 inhalation (for 1 min). —○— : response to LTD_4 inhalation after DMSO pretreatment (n=5). Values are presented as mean±SEM. All points after LTD_4 inhalation were significantly different from the preinhalation (pre) values ($p<0.05$). At 1 and 2 min after LTD_4 inhalation, R_L values of SR 48968 pretreatment were significantly lower than those of DMSO pretreatment ($p<0.05$). There was no significant difference between the values of CP 96345 and DMSO pretreatment. R_L: pulmonary resistance. For further abbreviations see legend to figure 1.

Effect of CP 96345 and SR 48968 on the LTD_4-induced changes in pulmonary resistance

SR 48968 (0.3 mg·kg^{-1} *i.v.*) pretreatment significantly inhibited the LTD_4-induced R_L increase at 1 and 2 min after the inhalation (fig. 6). In contrast, CP 96345 (4 mg·kg^{-1} *i.v.*) had no significant effect on the LTD_4-mediated R_L increase (fig. 6).

Discussion

In the present study, it was shown that LTD_4 inhalation causes microvascular leakage from central to peripheral airways. The LTD_4 (and LTC_4) receptor antagonist ONO-1078 almost completely blocked the LTD_4-induced airway microvascular leakage, indicating that this response is LT receptor specific. In guinea-pigs, airway microvascular leakage by LTD_4 has been demonstrated using Evans blue dye [17, 18] and ^{125}I-bovine serum albumin [19] as a tracer for plasma exudation, and the results were compatible with our data.

To investigate the possible involvement of endogenously released tachykinins in LTD_4-induced airway microvascular leakage, two different kinds of strategies were employed, *i.e.* capsaicin or NK_1-receptor antagonist pretreatment. Capsaicin has selective effects on peptide-containing C-fibres [20–22], and systemic administration of this compound may result in temporary or permanent depletion of neuropeptides from these nerves - a process known as capsaicin desensitization [23]. Capsaicin-pretreated animals fail to develop neurogenic airway microvascular leakage upon sensory nerve stimulation [21, 24–26]. On the other hand, SP is about eight times more potent than neurokinin A or B (NKA or NKB), suggesting that NK_1 receptors are most important for the tachykinin-mediated airway microvascular leakage in guinea-pigs [27]. NK_1 receptors have been localized to postcapillary venules which are the leaky sites [28, 29]. Further, the NK_1-receptor antagonist, FK 888, has been reported to abolish sensory nerve stimulation [30] or exogenous SP [31]-mediated responses. In the present study, both pretreatments (capsaicin desensitization and NK_1-receptor antagonist pretreatment) significantly inhibited LTD_4-induced airway microvascular leakage in central airways, suggesting that, in this portion, the LTD_4-mediated airway microvascular leakage is, in part, mediated *via* tachykinin release from sensory nerves.

Administration of LTD_4 into the airways has been shown to enhance the recovery of SP- and NKA-like immunoreactivities from the lung, suggesting that LTD_4 causes the tachykinin release from airway sensory nerves [8]. However, conflicting results have been reported on the role played by tachykinins in the LTD_4-mediated responses in different tissues. In guinea-pig ileum, the LTD_4-mediated contractile response is partially inhibited by a neurotoxin, tetrodotoxin. This inhibitory effect is abolished by capsaicin desensitization or pretreatment with an SP antagonist, indicating that SP is released from neurones by LTD_4 in guinea-pig ileum [7]. In contrast, MANZINI and MEINI [32] have reported that capsaicin desensitization does not affect the bronchial contraction elicited by LTD_4 in guinea-pigs. The results of our present investigation are compatible with the former study.

In the present study, both capsaicin desensitization and NK_1-receptor antagonist pretreatment inhibited the LTD_4-induced airway microvascular leakage in central but not in peripheral airways, suggesting that tachykinin-mediated mechanisms are involved in LTD_4-induced inflammation in central rather than peripheral airways. SP-immunoreactive nerves are distributed in all airways [33], but sensory nerve-stimulation-induced airway microvascular leakage is more predominant in central and mid-airways than in distal airways [34, 35]. Taken together, LTD_4 inhalation may cause airway sensory nerve stimulation and result in the release of endogenous SP mainly in the central airways. Thus, in central airways, the LTD_4-induced airway microvascular leakage is due to the activation both of NK_1- and LTD_4-receptors on the endothelium of airway postcapillary venules. In contrast, in peripheral airways, LTD_4-mediated response may be due to LTD_4- but not NK_1-receptors. A similar effect of endogenous tachykinins in central airways after bradykinin administration into the airways has also been reported [14].

In the present study, we observed significant inhibitory effects of FK 888 only in the high dose range compared with the former studies [30, 31]. Therefore, the nonspecific effect of this antagonist may influence our results. However, another NK_1-receptor antagonist, CP 96345, also showed a similar inhibitory effect on LTD_4-induced responses. Thus, we believe that the effect of FK 888 observed in this study was a NK_1-receptor specific response.

In the present study, LTD_4 inhalation caused significant bronchoconstriction. In contrast to the plasma leakage, NK_1-receptor antagonists, FK 888 or CP 96345, did

not affect the bronchoconstrictor response elicited by LTD_4 administration. However, NK_2-receptor antagonist, SR 48968, significantly inhibited the LTD_4-induced R_L elevation. These results are in keeping with observations in humans *in vivo*, showing a slight increase in maximal bronchoconstriction to LTD_4 following pretreatment with an inhibitor (thiorphan) or tachykinin-degrading enzyme, neutral endopeptidase [36]. Because tachykinins have been reported to cause airway smooth muscle contraction *via* NK_2-receptors [6, 27], the LTD_4-induced R_L elevation observed in the present study seems to be due to the airway smooth muscle contraction rather than airway plasma leakage and subsequent airway wall oedema.

In summary, we have shown that LTD_4 inhalation causes airway microvascular extravasation and bronchoconstriction. Both chronic capsaicin administration, which causes tachykinin depletion, and NK_1-receptor antagonist pretreatment partially but significantly reduced the plasma exudation in central airways. Furthermore, NK_2-receptor antagonist significantly inhibited the LTD_4-induced bronchoconstrictor response. These results suggest that the LTD_4-induced airway responses are, in part, mediated *via* tachykinin release from airway sensory nerves.

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