Increased secretion of glandular-kallikrein in the bronchial washings induced by intravenous injection of leukotriene C₄ in guinea-pigs

Hong Yong Jin, Makoto Katori,¹ Masataka Majima & *Noriyuki Sunahara

Department of Pharmacology, Kitasato University School of Medicine, Sagamihara, Kanagawa 228, Japan and *Dainippon Pharmaceutical Co., Enoki, Osaka 661, Japan

1 Intravenous administration of leukotriene C₄ (LTC₄) and LTD₄ (1–10 nmol kg⁻¹) caused a dose-dependent increase in secretion of glandular-kallikrein in the bronchial washings of guinea-pigs, as measured by cleavage of a synthetic substrate and the formation of kinin. LTC₄ was more potent than LTD₄ and pilocarpine was much less potent than peptide leukotrienes on a molecular basis.

2 The increases in levels of glandular-kallikrein in the bronchial washings that were induced by LTC₄ (3 nmol kg⁻¹, i.v.) were almost completely inhibited by pretreatment with an antagonist of leukotrienes (ONO-1078), with an antagonist of thromboxane (S-1452), with an inhibitor of thromboxane synthetase (OKY-046), with indomethacin, with atropine or with scopolamine. These results indicate that the LTC₄-induced increase in levels of glandular-kallikrein may have been mediated by the formation of thromboxane and the release of acetylcholine.

3 The increases in levels of glandular-kallikrein in the bronchial washings induced by STA₂ (20 pmol kg⁻¹, i.v.), a stable analogue of thromboxane A₂, were completely blocked by pretreatment with atropine, whereas increases induced by pilocarpine (41 µmol kg⁻¹, i.v.) were not blocked by pretreatment with indomethacin, although such increases were inhibited by atropine. This result indicates that secretion of kallikrein stimulated by LTC₄ may have been mediated by the successive formation of thromboxane A₂ and release of acetylcholine.

4 Intravenous administration of bradykinin (3–30 nmol kg⁻¹) caused a dose-dependent increase in levels of glandular-kallikrein in the bronchial washings. This increase was completely inhibited by pretreatment with atropine, with indomethacin or with an antagonist of thromboxane.

5 The increases in levels of glandular-kallikrein in the bronchial washings induced by LTC₄ (3 nmol kg⁻¹, i.v.) and pilocarpine (41 µmol kg⁻¹, i.v.) were significantly inhibited by pretreatment with an antagonist of bradykinin. These results suggest that intravenous LTC₄ may increase secretion of glandular-kallikrein via formation of thromboxane A₂ and release of acetylcholine in that order, and kinin released by kallikrein may enhance the rate of secretion of glandular-kallikrein.

Keywords: Leukotriene C₄; glandular-kallikrein; bronchial washing; thromboxane A₂; acetylcholine; asthma

Introduction

Bronchial asthma has long been recognized as a disease of reversible airway obstruction, which is induced not only by bronchoconstriction but also by increases in the secretion of mucus. Leukotrienes, released from lungs of sensitized guinea-pig, were reported to be potent bronchoconstrictors (Kellaway & Trethewie, 1940). Bronchoconstriction produced by leukotrienes was almost completely inhibited by pretreatment with indomethacin (Piper & Samhoun, 1981) and an inhibitor of thromboxane synthetase (Piper & Samhoun, 1981; Ueno *et al.*, 1982; Austen *et al.*, 1983). In addition to bronchoconstriction, leukotrienes have been reported to increase bronchial secretion of mucus in guinea-pigs and man (Peatfield *et al.*, 1982; Marom *et al.*, 1982; Johnson & McNee, 1983). As in the case of bronchoconstriction, the increase in bronchial secretion of mucus was blocked to a significant extent by pretreatment with indomethacin and with an inhibitor of thromboxane synthetase (Yanni & Smith, 1986).

Levels of glandular-kallikrein, a kinin-generating enzyme, increased in lavage fluid after provocation with allergen of the nasal (Proud *et al.*, 1983) and bronchial mucosa (Christiansen *et al.*, 1987) of atopic subjects. Here we show that intravenous injections of leukotrienes increase the levels of glandular-kallikrein in bronchial washings of guinea-pigs via the successive formation of thromboxane and release of acetylcholine.

Methods

Collection of bronchial washings

Male Hartley guinea-pigs (specific pathogen-free, weighing 450–550 g, from Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were anaesthetized with sodium pentobarbitone (50 mg kg⁻¹, s.c.) before the start of experimental procedures. Anaesthesia was maintained by occasional subcutaneous administration of sodium pentobarbitone (20 mg kg⁻¹). The right jugular vein was cannulated with a polyethylene cannula (PE-50, Clay Adams, Parsippany, NJ, U.S.A.). LTC₄ and other solutions of drugs were injected through this cannula. To avoid contamination by saliva of the bronchial washings during induction of bronchial secretion, the trachea was cannulated with a polyethylene cannula (size 6, Hibiki, Tokyo, Japan) through a short incision made just beneath the thyroidal cartilage bone, and a cotton ball containing silicon grease (High vacuum, Dow Corning, Midland, Mich., U.S.A.) was plugged into the oral side of the cut end of the trachea.

After completion of the experimental set-up, leukotriene C₄ (LTC₄, 3–30 nmol ml⁻¹ saline kg⁻¹), LTD₄ (3–30 nmol ml⁻¹ saline kg⁻¹), pilocarpine (12–120 µmol ml⁻¹ saline kg⁻¹), STA₂ (9,11-epithio-11,12-methanothromboxane A₂, 1–10 nmol ml⁻¹ saline kg⁻¹) or bradykinin (3–30 nmol ml⁻¹ saline kg⁻¹) was injected as a single bolus through the jugular vein. Twenty minutes later, each animal was killed by exsanguination and the lung and trachea with cannula were isolated. After washing the outside of the lung with chilled physiological saline, half of the peripheral portion of the lung

¹ Author for correspondence.

was removed by cutting with small scissors. Physiological saline (0.4 ml), injected through the cannula inserted into the trachea, was able to flow out through the cut surface of the lung. This washing procedure was repeated 6 times at 1 min intervals and washings were collected in plastic tubes. The samples of bronchial washings were kept on ice until measurements of levels of glandular-kallikrein.

Pretreatment with drugs

Guinea-pigs were pretreated with ONO-1078 ([8-(p-phenyl-butyloxy)benzoyl]amino-2-(tetrazol-5-yl)-4-oxo-4H-1-benzopyran hemihydrate, an antagonist of LTC₄, LTD₄ and LTE₄, 20–200 nmol ml⁻¹ saline kg⁻¹, i.p.) (Obata *et al.*, 1987), indomethacin (an inhibitor of cyclo-oxygenase, 8.3–83 µmol ml⁻¹ saline kg⁻¹, i.p.), OKY-046 ((E)-3-[4-(1-imidazolymethyl)phenyl]-2-propenoic acid hydrochloride monohydrate, an inhibitor of thromboxane synthetase, 10–100 µmol ml⁻¹ saline kg⁻¹, i.v.) (Nato *et al.*, 1984), S-1452 (an antagonist of thromboxane A₂, 0.3–1.0 µmol ml⁻¹ saline kg⁻¹, i.v.) (Hanasaki *et al.*, 1988), atropine (4.2–42 µmol ml⁻¹ saline kg⁻¹, i.v.), scopolamine (10–100 µmol ml⁻¹ saline kg⁻¹, i.v.) or an antagonist of bradykinin (Thi^{5,8}-D-Phe⁷-bradykinin, 100–1000 nmol ml⁻¹ saline kg⁻¹, i.v.). LTC₄, pilocarpine or STA₂ was administered intravenously 30 min after the intraperitoneal injection and 10 min after the intravenous injection of the above mentioned drugs.

Measurements of the activity of glandular-kallikrein

Immediately after the collection of bronchial washings, the activity of glandular-kallikrein was determined by use of a selective substrate for glandular-kallikrein. Fifty µl of bronchial washing was incubated with 950 µl of the solution of methylcoumaril amide (MCA) synthetic substrate (Pro-Phe-Arg-MCA, 2×10^{-4} M, in 50 mM Tris-HCl buffer that contained 0.1 M NaCl and 0.01 M CaCl₂, pH 8.0) at 37°C for 30 min. The reaction was terminated by addition of 2 ml of a 17% solution of acetic acid. The amount of amidomethylcoumarine (AMC) released was measured fluorometrically (excitation, 380 nm; emission, 460 nm) as described elsewhere (Morita *et al.*, 1977; Oh-ishi & Katori, 1979). A mean value from the six washings was calculated and taken as the activity of glandular-kallikrein in the bronchial washings.

Kinin-generating activity in bronchial washings

To assess the kinin-generating activity in the bronchial washings, purified low-molecular-weight (LMW) kininogen from bovine plasma, a natural substrate for glandular-kallikrein, was incubated with the bronchial washings. An aliquot of 300 µl of bronchial washings, 100 µl of 0.2 M Tris-HCl buffer (pH 8.0) and 100 µl of a solution of purified LMW kininogen (1 mg ml⁻¹, in physiological saline) were incubated together at 37°C for 60 min in the presence of an inhibitor of kininase I, DL-2-mercaptomethyl-3-guanidinoethylthiopropionic acid (10 µM) and an inhibitor of kininase II, captopril (30 µM) (Majima *et al.*, 1988). After addition of 100 µl of a 20% solution of trichloroacetic acid to the reaction mixture, the mixture was centrifuged at 1500 g for 15 min at 4°C. Four hundred µl of the supernatant were neutralized with the same volume of buffer B from an enzyme-immunoassay kit for bradykinin (Markit-A, Dainippon Pharmaceutical Co., Osaka, Japan). The amounts of bradykinin in the neutralized solution were determined with the enzyme-immunoassay kit, as previously described (Uchida *et al.*, 1986). Aprotinin (Vogel, 1979) was added to the incubation mixture (final concentration; 5000 KIU ml⁻¹) in order to ensure that the kinin-generating activity was due to glandular-kallikrein.

Drugs

Drugs used were as follows: sodium pentobarbitone (Nembutal, Abbot Lab., North Chicago, IL, U.S.A.), peptidyl

fluorogenic substrate, Pro-Phe-Arg-MCA (Peptide Institute Inc., Osaka, Japan), bradykinin (Peptide Institute Inc.), bradykinin B₂ receptor antagonist, Thi^{5,8}-D-Phe⁷-bradykinin (Peptide Institute Inc.), DL-2-mercaptomethyl-3-guanidinoethylthiopropionic acid (Calbiochem., La Jolla, CA, U.S.A.), pilocarpine, atropine and scopolamine (Wako Pure Chemical Ind., Osaka, Japan). LTC₄, LTD₄, STA₂ and ONO-1078 were generous gifts from Ono Pharmaceutical Co. Ltd., Osaka, Japan. Captopril was a gift from Sankyo, Co., Tokyo, Japan. S-1452 was a gift from Shionogi Pharmaceutical Co., Osaka, Japan. OKY-046 was a gift from Kissei Pharmaceutical Co., Matsumoto, Japan. Indomethacin was a gift from Merck, Sharp and Dohme, Rahway, NJ, U.S.A. Aprotinin was a gift from Bayer A.G., Leverkusen, Germany. Purified LMW kininogen was a gift from Seikagaku Kogyo, Tokyo, Japan. Other reagents were all of analytical grade and were obtained from commercial sources.

Statistical analysis

When variances were not heterogeneous, Student's *t* test was used to evaluate the significance of differences. When variances were heterogeneous, statistical analysis was performed by the Aspin-Welch method or by the Wilcoxon's rank sum test. A value of *P* of less than 0.05 was regarded as significant.

Results

Increases in glandular-kallikrein activity in bronchial washings caused by pilocarpine

The activity of glandular-kallikrein activity increased in a dose-dependent manner with increasing doses of pilocarpine (12–120 µmol kg⁻¹) (Figure 1a). The increase in the level of glandular-kallikrein caused by pilocarpine (41 µmol kg⁻¹) was inhibited by pretreatment with atropine in a dose-dependent manner (Figure 1b).

Increases in glandular-kallikrein activity in bronchial washings caused by leukotriene

As shown in Figure 2, the activity of glandular-kallikrein in bronchial washing increased dose-dependently in response to 1, 3 and 10 nmol kg⁻¹ of LTC₄. The magnitude of increase caused by the 3 doses was 132%, 193% and 271% of the activity of the saline control respectively, whereas those induced by LTD₄ (1, 3 and 10 nmol kg⁻¹) were 121%, 152% and 155% of the saline control. Thus, LTC₄ was more potent than LTD₄ in enhancing the secretion of glandular-kallikrein into the bronchial washings.

Kinin-generating activity in the bronchial washings induced by pilocarpine and leukotriene C₄

To test whether the amidase activity in bronchial washings was due to kinin-generating activity, bronchial washings were incubated with low-molecular-weight kininogen (Figure 3). The kinin-generating activity in bronchial washings after injection of saline was 22 ± 6 pmol min⁻¹ ml⁻¹ washing (*n* = 6), which was increased to 500% after injection of pilocarpine and to 840% after injection of LTC₄. The addition of aprotinin to reaction mixtures caused marked inhibition of the kinin-generating activity.

Inhibition of leukotriene C₄-induced increases in the activity of glandular-kallikrein in washings by pretreatment with inhibitors

Pretreatment of guinea-pigs with an antagonist of leukotriene, ONO-1078 (20–200 µmol kg⁻¹) caused a dose-dependent decrease in the activity of glandular-kallikrein that was

Figure 1 Dose-dependent increases in levels of glandular-kallikrein in bronchial washings in guinea-pigs caused by pilocarpine and the inhibitory effect of atropine. The ordinate scale shows the levels of activity of glandular-kallikrein in bronchial washings: (○) and the shaded areas indicate the control levels when only saline was injected. Dosage of pilocarpine used in (b) was $41 \mu\text{mol kg}^{-1}$ (i.v.). The numbers beside each point show the numbers of experiments. Each point represents the mean value (s.e.mean shown by vertical bar) and is compared with the value of the saline control (a) and the value without atropine treatment (b). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

induced by LTC_4 (3 nmol kg^{-1}). The antagonist at $200 \mu\text{mol kg}^{-1}$ completely blocked the increase in the glandular-kallikrein activity.

The pretreatment of animals with indomethacin (an inhibitor of cyclo-oxygenase) or OKY-046 (an inhibitor of thromboxane synthetase) inhibited the increase in the activity of glandular-kallikrein in bronchial washings and complete inhibition was observed with the higher doses of both inhibitors, indicating the involvement of the formation of thromboxane A_2 in the stimulation of secretion of kallikrein by LTC_4 . The inhibition of the increase in the kallikrein activity caused by pretreatment with S-1452 (an antagonist of thromboxane) provided further evidence for the involvement of thromboxane A_2 in this process (Table 1).

When animals were pretreated with atropine or scopolamine, the increases in activity of glandular-kallikrein caused by LTC_4 were also inhibited by as much as 80% to 100%.

Inter-relationship between thromboxane A_2 and cholinergic nerves

In order to clarify the sequence of the actions of thromboxane A_2 and acetylcholine in the increased secretion of glandular-kallikrein into the bronchial washings caused by LTC_4 , guinea-pigs were pretreated with indomethacin ($83 \mu\text{mol kg}^{-1}$,

Figure 2 Dose-dependent increases in the activity of glandular-kallikrein in bronchial washings caused by leukotriene C_4 (LTC_4) and leukotriene D_4 (LTD_4) in guinea-pigs. The ordinate scale shows the levels of activity of glandular-kallikrein in bronchial washings: (■) and (●) show the levels of the activity of glandular kallikrein after administration of LTC_4 and LTD_4 , respectively; (○) indicates the control levels after injection of saline only. The numbers beside each point show the numbers of experiments. Each point represents the mean value (s.e.mean shown by vertical bar) and is compared with the value of the saline control. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 3 Kinin-generating activity in bronchial washings of guinea-pigs. The numbers beside each column show the numbers of experiments. Each column represents the mean value (s.e.mean shown by vertical bar). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

i.p.). As shown in Figure 4a, the increase in glandular-kallikrein induced by pilocarpine ($41 \mu\text{mol kg}^{-1}$, i.v.) was not inhibited by pretreatment with indomethacin.

By contrast, pretreatment with atropine ($14 \mu\text{mol kg}^{-1}$, i.v.) completely inhibited the increase in glandular-kallikrein induced by a bolus intravenous injection of STA_2 (20 pmol kg^{-1}) (Figure 4b).

Increased secretion of glandular-kallikrein into bronchial washings caused by bradykinin

As shown in Figure 5, intravenous administration of bradykinin ($3, 10, 30 \text{ nmol kg}^{-1}$) increased the levels of glandular-kallikrein in the bronchial washings in a dose-dependent

Table 1 Inhibitory effects of drug pretreatments on leukotriene C₄-induced increases in glandular-kallikrein in bronchial washings of guinea-pigs

	Pretreatment	Dose	Glandular-kallikrein activity (10 ⁻¹¹ mol min ⁻¹ ml ⁻¹ wash)	P	n
Saline	—	—	3.0 ± 0.2	—	6
	—	—	5.8 ± 0.4 [#]	—	5
	ONO-1078	20 nmol kg ⁻¹	4.1 ± 0.2	*	3
		60	3.7 ± 0.2	**	4
		200	3.1 ± 0.1	**	4
	Indomethacin	8.3 μmol kg ⁻¹	5.0 ± 0.5	NS	5
		25	3.9 ± 0.1	**	5
		83	3.0 ± 0.2	**	4
LTC ₄ 3 nmol kg ⁻¹	OKY-046	10 μmol kg ⁻¹	3.5 ± 0.2	*	3
		30	2.9 ± 0.1	**	3
		100	2.9 ± 0.2	**	3
	S-1452	0.3 μmol kg ⁻¹	3.9 ± 0.3	*	4
		1.0	2.7 ± 0.3	**	5
	Atropine	4.2 μmol kg ⁻¹	5.0 ± 0.5	NS	4
		13	3.8 ± 0.2	*	4
		42	3.8 ± 0.1	*	4
	Scopolamine	10 μmol kg ⁻¹	5.3 ± 0.4	NS	4
		30	4.1 ± 0.1	*	4
		100	3.1 ± 0.2	**	4

Each value represents the mean ± s.e.mean and is compared with the increased level [#] induced by leukotriene C₄ (LTC₄); * *P* < 0.05; ** *P* < 0.01, NS; not significant. *n*; numbers of animals. ONO-1078; a leukotriene antagonist. OKY-046; a thromboxane synthetase inhibitor. S-1452; a thromboxane antagonist.

Figure 4 Effects of indomethacin (a) and atropine (b) on the glandular-kallikrein activity induced by pilocarpine (a) and STA₂ (b) in guinea-pigs. The ordinate scales show the levels of glandular-kallikrein activity in bronchial washings. The numbers beside each column show the numbers of experiments. Each column represents the mean value (s.e.mean shown by vertical bar). *** *P* < 0.001.

Figure 5 The increases in glandular-kallikrein activity in bronchial washings by bradykinin in guinea-pigs. The ordinate scale shows the levels of glandular-kallikrein activity in bronchial washings. (○) indicates the control level when only saline was injected. The numbers besides each point show the numbers of experiments. Each point represents the mean value (s.e.mean shown by vertical bar) and is compared with saline control (saline). ** *P* < 0.01.

manner to 118%, 133% and 198% of the levels in saline controls, respectively. Indomethacin (83 μmol kg⁻¹, i.p.) completely inhibited the increase in the levels of the glandular-kallikrein that was induced by bradykinin (30 nmol kg⁻¹). S-1452, a selective antagonist of thromboxane A₂ (0.3–1.0 μmol ml⁻¹ saline kg⁻¹, i.v.) also inhibited the increase induced by bradykinin in a dose-dependent manner. Pretreatment with atropine (14 μmol kg⁻¹, i.v.), also completely inhibited this increase in the glandular-kallikrein (Figure 6).

Figure 6 Inhibition of the bradykinin-induced increase in glandular-kallikrein activity by indomethacin, thromboxane antagonist (S-1452) and atropine in guinea-pigs. The ordinate scale shows the levels of glandular-kallikrein activity in bronchial washings. The numbers beside each column show the numbers of experiments. Each column represents the mean value (s.e.mean shown by vertical bar) and is compared with the increased levels induced by bradykinin (BK). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Inhibitory effects of a bradykinin antagonist on the increases in glandular-kallikrein activity induced by leukotriene C₄ and pilocarpine

As shown in Figure 7, intravenous administration of a bradykinin antagonist, $\text{Thi}^{5,8}\text{-D-Phe}^7\text{-bradykinin}$ ($100\text{--}1000\text{ nmol kg}^{-1}$), 10 min before stimulation, caused a dose-dependent inhibition (by 55–80%) of the increases in levels of glandular-kallikrein caused by LTC_4 . The increases in levels of glandular-kallikrein activity induced by pilocar-

Figure 7 Inhibitory effects of bradykinin antagonist (BK antagonist) on the increases in glandular-kallikrein activity induced by leukotriene C₄ (LTC_4) and pilocarpine in guinea-pig. The ordinate scale shows the levels of the glandular-kallikrein activity in bronchial washings. The numbers beside each column show the numbers of experiments. Each column represents the mean value (s.e.mean shown by vertical bar) and is compared with the increased levels induced by leukotriene C₄ and pilocarpine, respectively. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

pine were also inhibited by 81% by pretreatment with this bradykinin antagonist (300 nmol kg^{-1}).

Discussion

The kallikrein-kinin system in the body can be divided into two separate systems: the plasma kallikrein-high-molecular-weight (HMW) kininogen and glandular-kallikrein-low-molecular-weight (LMW) kininogen. Both systems operate independently *in vitro* and *in vivo* (Uchida & Katori, 1985; Katori *et al.*, 1988; 1989; Majima *et al.*, 1990). The plasma kallikrein-HMW kininogen system is activated via the activation of factor XII (Hageman factor) and activated plasma prekallikrein (plasma kallikrein) can release bradykinin from its substrate, HMW-kininogen. Glandular-kallikrein, present in the tissues of exocrine glands and their secretions, can act on its substrate, LMW kininogen, from which kallidin (Lys-bradykinin) is released (Katori *et al.*, 1988).

In the present study, intravenous injection of LTC_4 was found to induce an increase in the level of glandular-kallikrein in bronchial washings in guinea-pigs. This finding suggests that the increased activity of the glandular kallikrein-LMW kininogen system and, hence, increased formation of kinin in the airway might be involved in the pathogenesis of some kinds of airway disease, such as bronchial asthma, airway infection, stimulation by cold and dry breathing air, via the secretion of glandular-kallikrein.

Slow reacting substance of anaphylaxis (SRS-A) was originally reported to be released from lungs of sensitized guinea-pig (Kellaway & Trethewie, 1940) and it has potent bronchoconstrictor activity in this species (Adams & Lichtenstein, 1979). LTC_4 , LTD_4 and LTE_4 , chemical principles of SRS-A (Murphy *et al.*, 1979; Samuelsson & Hammarstrom, 1980), share this bronchoconstrictive activity (Ueno *et al.*, 1982). Furthermore, it was also reported that from guinea-pig isolated lung, SRS-A releases rabbit aorta contracting substance (RCS), which is now known to be mainly thromboxane A₂ (Piper & Vane, 1969). Similarly, an intravenous challenge with SRS-A also produces significant increases in tracheal mucus gel thickness and this increase is inhibited by pretreatment with indomethacin or imidazole (Yanni & Smith, 1986).

It is reported that in an isolated preparation of human bronchial mucosa, methacholine induced secretion both of glycoprotein from submucosal glands and goblet cells and of lysozyme, a selective indicator of the secretion from serous cells of submucosal glands, whereas LTC_4 and LTD_4 induced only the secretion of glycoprotein from submucosal glands (Coles *et al.*, 1981; 1983). An immuno-histochemical study for the localization of kallikrein in the gastrointestinal tracts indicated that goblet cells, not chief cells, in the mucosa express immunoreactive glandular-kallikrein intensely (Schachter *et al.*, 1983). Glandular-kallikrein may have an analogous location in the airway.

It is noteworthy that the enhancement of secretion by LTD_4 was much lower than that by LTC_4 in the present experiments, although the activity of LTD_4 in bronchoconstriction is three times that of LTC_4 in guinea-pigs (Ueno *et al.*, 1982). This difference may indicate a difference between the activities of the two leukotrienes in the airway.

The increased secretion of glandular-kallikrein in bronchial washings caused by LTC_4 was almost completely suppressed by either indomethacin, a thromboxane synthetase inhibitor (OKY-046) or a thromboxane antagonist (S-1452), and it resembled our previous report showing that bronchoconstriction induced by peptide leukotrienes in guinea-pigs was inhibited by indomethacin or a thromboxane synthetase inhibitor (Ueno *et al.*, 1982). Furthermore, this increased secretion by LTC_4 was also inhibited by atropine or scopolamine in the present experiment, suggesting the increased secretion of glandular kallikrein may be mediated by the release of acetylcholine from cholinergic nerves.

The cellular source of thromboxane A₂ formation is still

unclear, but the adventitial layer of the vessels may be a possible site, since constriction of the arterioles in the hamster cheek pouch by LTC_4 can be almost completely inhibited by a thromboxane synthetase inhibitor (Ueno *et al.*, 1983) and the bronchoconstriction induced by inhalation of LTD_4 is not inhibited by indomethacin, although that induced by intravenous LTD_4 is suppressed by the cyclo-oxygenase inhibitor (Hamel *et al.*, 1982). Intravenously administered STA_2 increased the secretion of kallikrein in bronchial washings. However, only small doses of STA_2 could be used, because death by thromboembolism occurs with large doses of this agonist. The observation that atropine inhibited the increased secretion of glandular kallikrein caused by STA_2 and the observation that indomethacin did not suppress that caused by pilocarpine (Figure 4) indicates that thromboxane A_2 formed in response to LTC_4 may stimulate the cholinergic nerve endings.

There are few reports in which thromboxane A_2 is described as stimulating cholinergic nerve endings. Thromboxane A_2 does, however, stimulate sensory nerves of dogs with the same potency as prostaglandin E_2 , when injected into the canine splenic artery (Hori *et al.*, 1986).

Secreted glandular kallikrein may cause the release of kinin from LMW kininogen present in the surrounding interstitial space of the bronchus. We failed, however, to detect kinin in the bronchial washings, even in the presence of inhibitors of kininase I (D,L-2-mercaptomethyl-3-guanidinoethylthiopropionic acid, *o*-phenanthroline) and of kininase II (captopril, *o*-phenanthroline), since a major kininase in the plasma of guinea-pigs is an aminopeptidase, that is resistant to these inhibitors (data to be published).

Intravenous bradykinin itself had effects similar to those of LTC_4 (Figure 5). A more recent observation suggested that kinin increases lung mucus secretions. It was reported that when canine tracheal explants were incubated with ^{14}C -labelled D-glucosamine in the medium and labelled macromolecules were isolated from the culture medium, kinin enhanced the incorporation of the labelled material into high molecular weight molecules that may be mucin-type glycoproteins (Baker *et al.*, 1977). Thus, the observation in the present study that bradykinin increased the secretion of glandular-kallikrein into the bronchial washings via thromboxane formation and acetylcholine release (Figure 6), seems to be plausible. Furthermore, the increase in release of glandular-kallikrein induced by LTC_4 and pilocarpine could be inhibited by pretreatment with bradykinin antagonist (Figure 7). These observations suggest that kinin formation stimulated by LTC_4 results in a positive feedback loop that controls the release of the glandular-kallikrein into bronchial secretions. The activation of the glandular-kallikrein-LMW kininogen system in the airways may be involved in the pathogenesis of airway diseases via the capacity of bradykinin to induce bronchoconstriction and mucus secretion.

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