Protease-activated receptor 2 mediated mucus secretion in airway submucosal gland

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Directed by Professor Jae Young Choi

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ABSTRACT

Protease-activated preceptor 2 mediated mucus secretion in airway submucosal gland

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Protease-activated receptor 2 (PAR2) is a G protein-coupled receptor, and its activation leads to various biological responses. PAR2 is expressed in airway epithelia and smooth muscle and plays an important role in airway inflammation. In this study, we showed that the activation of PAR2 induces mucus secretion from the human airway gland. We also dissected the mechanism of PAR2-induced mucus secretion in the porcine airway gland.

Human tracheal tissues were obtained following tracheotomies, and pig tracheal tissues were obtained following robot surgery training. The mucosa with underlying glands were dissected from the cartilage of the tracheal tissues, pinned mucosal side up at the gas/bath solution interface of a physiological chamber, and covered with oil so that secretions from individual

glands could be visualized as spherical bubbles in the oil. Secretion rates were determined by optical monitoring of bubble diameter. The Ca^{2+}-sensitive dye Fura2-AM was used to determine intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by means of spectroflurometry in dissected airway gland.

Stimulation of human tracheal mucosa with PAR2-activating peptide (AP) elevated intracellular Ca^{2+} and induced glandular secretion equal to about 20% of the carbachol response in the human airway. Porcine gland tissue was more sensitive to PAR2-AP, which is dependent on Ca^{2+} and anion secretion. Endogenous PAR2 activators, trypsin, and neutrophil elastase, also induced mucus secretion from airway gland. PAR2-induced mucus secretion was preserved in ΔF508 CFTR mutant mice.

PAR2-AP is a strong agonist for mucus secretion from the airway gland that is Ca^{2+}-dependent and CFTR-independent. Because PAR2 is involved in airway host defense mechanisms by stimulating airway mucus secretion from the submucosal gland, future research should investigate the potential of this receptor as a target for therapeutic intervention in the airway defense system.

Key words : *innate immunity, fluid, cftr, PGE_2, Ca^{2+}*

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Ⅰ. INTRODUCTION

Protease-activated receptors (PAR)s are G protein-coupled receptors that are activated by proteolytic cleavage of the N-terminal extracellular domain, leading to intracellular Ca^{2+} elevation[1, 2]. PARs have a variety of biologic roles, and are involved in inflammatory diseases including inflammatory bowel disease and rheumatoid arthritis[3]. PARs are expressed in airway epithelia[3, 4] and play an important role in inflammation and adaptive immunity[5]. Endogenous PAR activators such as mast cell tryptase and neutrophil elastase induce airway inflammation and immune responses[6]. Microorganism-derived proteases such as house dust mite allergens are also capable of activating PARs and stimulating release of pro-inflammatory cytokines from airway epithelial cells[7, 8]. More interestingly, bacterial protease disables PARs and

inhibits PAR-triggered signaling in airway epithelial cells[9]. Thus, PARs are an integral component of the airway defense system and may reveal the exact pathway by which proteases affect innate immune responses. However, the role of PARs in the innate immune system in human airway under physiological and pathophysiological conditions is still unclear.

Airway submucosal glands produce most of the airway mucus which is essential for mucociliary clearance. The submucosal gland also secretes various antimicrobial components such as lysozyme to protect the airway from bacteria[10]. Adequate mucus secretion from airway submucosal glands is essential for maintaining the airway defense system. Defective mucus secretion may result in failure of host defense against pathogens that in turn could be the underlying pathogenesis of airway infection in patients with cystic fibrosis[11]. Overproduction of mucus secretion from airway glands also leads to airway diseases such as chronic obstructive lung disease and asthma[12]. Secretion from airway glands is mainly controlled by central parasymphathetic input[13]. In addition to the autonomic nervous system, airways have abundant intrinsic neurons and pathogen-sensing receptors, and their activation induces mucus secretion from airway glands via a neuronal mediator such as substance P or VIP[11, 14]. There is accumulating evidence supporting an important role for these local reflexes in the airway innate

immune response.

Among various subtypes, PAR2 plays major roles in ion transport and fluid secretion from airway epithelial cell cultures. PAR2 activates the Ca^{2+}-activated Cl^- channel (CaCC) in human bronchial epithelial cell lines and mouse trachea[15]. PAR2 also induces a transepithelial current through the CFTR by cytosolic Ca^{2+} mobilization in Calu-3 cells[16, 17]. Miotto et al. reported that PAR2 is also expressed in the airway gland[18]. These findings suggest that PAR2 can regulate anion and fluid secretion in the airway submucosal gland. However, the role of PAR2 in mucus secretion in the airway is controversial. PAR2-activating peptide (AP) is unable to induce mucin production in NCI-H292 cells[19] and proved to be only a weak enhancer of mucin secretion in human bronchial epithelial cells[20]. Until now there has been no evidence that a PAR is involved in mucus secretion from the airway submucosal gland. Therefore, demonstrating the role of PAR2 in mucus secretion from the airway submucosal gland provides a better understanding of the host defense system in the airway.

In this study, we showed that activation of PAR-2 in the human airway gland induced mucus secretion and we also dissected the mechanism of PAR-2-induced mucus secretion in porcine airway gland.

Ⅱ. MATERIALS AND METHODS

1. Human trachea tissue

These studies were approved by the Institutional Review Board of Yonsei University. Small tracheotomy flaps (1 cm^2) were obtained from the first or second tracheal ring after tracheotomy for airway maintenance. The subjects (*n*=11) had no lung disease and their ages ranged from 35 to 72 years (mean 59.3 years). All tissues were transferred to ice cold Krebs-Ringer bicarbonate buffer (KRB) bubbled with 95% O^2 - 5% CO_2, where they were maintained until use, usually within 4 h. The KRB composition was (in mM): 115 NaCl, 2.4 K_2HPO_4, 0.4 KH_2PO_4, 25 $NaHCO_3$, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 10 glucose, and 1.0 μM indomethacin. KRB was made to 90% volume and the osmolarity was measured with a Wescor 5500 vapor pressure osmometer. Distilled water was added to adjust the osmolarity to 290±5 mOsm. The pH was verified to be 7.4 (Orion 420A pH meter) after bubbling with 95% O_2 - 5% CO_2.

2. Animals

This study was approved by the Committee on Animal Research at Yonsei

Medical Center, and all experiments with animals were performed under appropriate guidelines. Pig tracheas were harvested from 18 juvenile Yorkshire pigs of either sex weighing 40~110 kg, following robotic surgery performed for training purposes. CF model mice (n=4) containing the targeted ΔF508 CFTR mutation and WT littermates (n=4) were kindly provided by Dr. M.G. Lee (Yonsei University, South Korea). The tails were clipped at 18 d of age and genomic DNA was isolated for subsequent *cftr* genotyping using a PCR assay.

3. Optical measurement of mucus secretion rates (mucus bubble method).

To prepare tissues for optical recording of mucus secretion rates by individual glands, a piece of ventral trachea of ~ 0.5 cm^2 was pinned mucosal-side up. The mucosa with underlying glands were dissected from the cartilage and mounted in a 35 mm diameter, Sylgard (Dow Corning Corporation)-lined plastic Petri dish with the serosa in the bath (~1 ml volume) and the mucosa exposed to the air. The tissue chamber was maintained at 35-37°C with high humidity using either a Sensortek S-4 Peltier effect temperature controller and spiral glass humidifier or a thermistor-controlled warming chamber and humidifier (Medical Systems). The tissue surface was cleaned and blotted dry

with cotton swabs and further dried with a stream of gas, after which 20-30 μ-L of water-saturated mineral oil was placed on the surface. The tissue was warmed to 37°C at a rate of ~1.5° C min^{-1} and continuously superfused with warmed, humidified 95% O_2 - 5% CO_2. Pharmacological agents were diluted to final concentrations with warmed, gassed bath solution and were added to the serosal side of the tissue by complete bath replacement. Bubbles of mucus within the oil layer were visualized by oblique illumination and digital images were captured with either the macro mode of a Nikon digital camera or by mating a digital camera to one ocular of a Wild stereomicroscope. Each image contained an internal reference grid to compensate for any minor adjustments in magnification made during the experiment. Stored images were analyzed either by direct measurement or with Image J software (http://rsb.info.nih.gov/ij/). Mucous volumes were determined from the size of the spherical bubbles and are given as nanoliters/min/gland (nl/min/gland). Bubbles that were not approximately spherical were omitted from secretion rate analyses. Details of these methods are provided in a reference[21].

4. Measurement of $[Ca^{2+}]_i$

Human airway submucosal gland tissues (n=3) were dissected from

tracheotomy flaps and the tissues were further dissected using collagenase NB4 (Serva, Heidelberg, GER). The isolated submucosal glands were seeded onto glass coverslips (22×22 mm) in 35 mm dishes, and cultured for 2 d. Tissues were incubated for 1 hr in PSS containing 5 μM Fura2-AM (Teflabs, Austin, TX, USA) in the presence of Pluronic F-127 (Invitrogen) to enhance dye loading. Fura-2-loaded cells were mounted on the stage of an inverted microscope (Nikon, Tokyo, Japan) for imaging. The tissues were illuminated at 340 nm and 380 nm, and the emitted fluorescent images at 510 nm were collected with a CCD camera and analyzed using the MetaFluor system (Universal Imaging Co., Downingtown, PA, USA). The fluorescence ratio (340/380) was taken as a measure of $[Ca^{2+}]_i$, and fluorescence images were obtained at 3 sec intervals.

5. Immunohistochemistry

Tracheal mucosa harvested during tracheotomies in patients with intracranial hemorrhage were fixed with 10% formaldehyde solution for 24 h and then dehydrated and embedded in paraffin. Paraffin blocks were sectioned into 4 μm-thick slices and fixed. After deparaffinizing and rehydrating, slides were incubated in antigen retrieval solution (Tris-EDTA, pH 9.0) for 20 min at

95-100℃. To block endogenous peroxidase, slides were treated with 0.3% H_2O_2 for 15 min at room temperature. Slides were blocked in 10% normal serum with 1% BSA in TBS for 2 h at room temperature, and then incubated overnight at 4℃ with a monoclonal mouse antibody against human PAR2 (1:100, SantaCruz, CA, USA). The slides were then incubated with HRP-conjugated goat anti-mouse IgG (1:200; Jackson Immune Research, CA, USA) in antibody diluent solution (DAKO, Glostrup, DEN) for 1 hr at room temperature. Slides were developed with DAB (DAKO, Glostrup, DEN) at room temperature and counterstained with hematoxylin (Merck KGaA, Darmstadt, GER).

6. Reagents

Fura-2 acetoxymethyl ester (fura-2-AM) was purchased from Teflabs (Austin, TX, USA). PAR2 activating peptides (PAR2-AP and SLIGRL-NH2) were purchased from the Korea Basic Science Institute (Seoul, Korea). Peptide structure was confirmed by liquid chromatography/mass spectroscopy (HP 1100 series HPLC System). Trypsin, indomethacin, thrombin, bumetanide, and 1,2-bis(2-aminophenoxy) ethane-N,N,N ,N-tetraacetic acid-acetoxymethyl ester (BAPTA-AM) were purchased from Sigma (St. Louis,

MO, USA). Human neutrophil elastase was purchased from Calbiochem (San Diego, CA, USA). Collagenase NB 4 was purchased from Serva (Heidelberg, GER).

Statistical Analysis

Data shown are means ± SEM, and the Student's t-test for unpaired data was used to compare the means of different treatment groups unless otherwise indicated. The difference between two means was considered to be significant when $P<0.05$. Curves were fit with Origin software (OriginLab Corporation, Northampton, MA) using a sigmoid function.

Ⅲ. RESULTS

1. PAR2-AP induced mucus secretion in human airway gland

Serosal application of PAR2-AP (100 μM) to human tracheal mucosa markedly increased glandular secretion, resulting in bubble formation that was comparable to the response achieved with carbachol (10 μM), a potent cholinergic agonist. Along with the bubbles from submucosal glands, PAR2 stimulation induced tiny bubbles which appeared to originate from the surface of the epithelial cells (Fig. 1A). A plot of mucus volume versus time for six individual glands from a single subject is shown in Figure 1B. PAR2-AP produced a short-latency transient peak followed by sustained secretion of more than 20 min. The secretion rate varied among the glands but all the carbachol-responding glands were also activated by PAR2-AP. Summary data from 11 human subjects are shown in Figure 1C. The mean secretion rate for the first 20 min of PAR2-AP treatment (100 μM) was 1.42±0.2, which is about 20% of the response achieved with 10 μM carbachol (6.65±1.8).

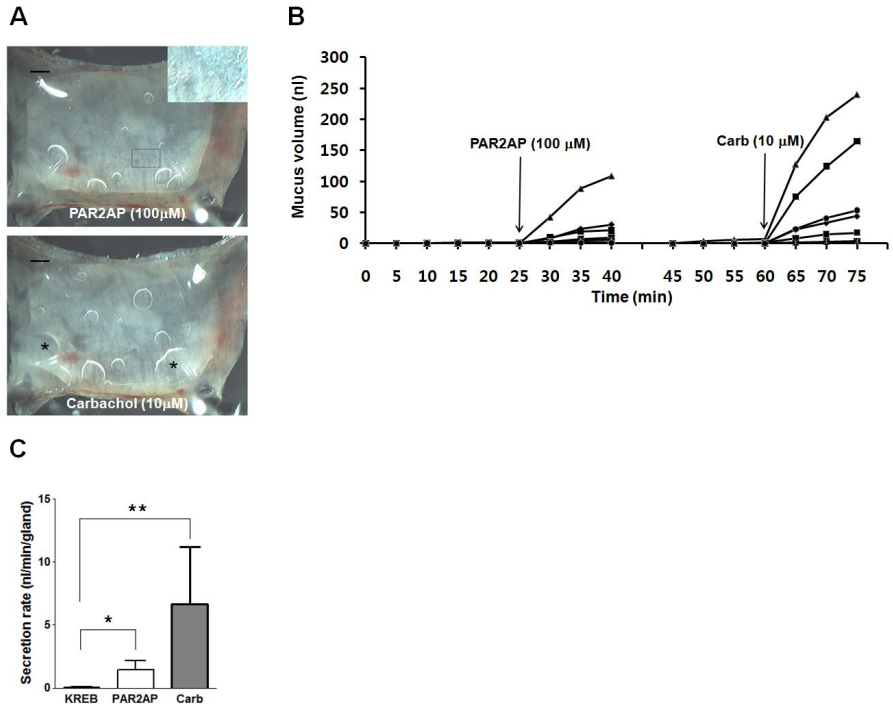

Figure 1. Serosal PAR2-AP stimulates mucus secretion from human airway submucosal gland. (A) Images of mucus bubbles formed under oil at the orifices of single submucosal glands 15 min after PAR2-AP (100 μM) or carbachol (10 μM) stimulation. PAR2-AP also induces small bubbles on the mucosal surface which seem to have originated from the epithelial surface. Some bubbles merged together. (*) Scale bar: 0.5 mm. (B) Plots of secreted mucus volume over time for 6 individual glands are shown. Each line represents a single gland. (C) Summary data of 52 glands from 11 subjects showing average secretion rates (± S.E.M) for 20-min periods following application of PAR2-AP (100 μM) or carbachol (10 μM). * and **, indicate significant differences from the response measured with Kreb's solution alone, $P<0.05$ and 0.005, respectively.

2. Expression of PAR2 in human airway gland

Immunostaining for PAR2 revealed that PAR2 proteins are expressed in the basolateral side of acinar cells of the submucosal gland. Immunoreactivity was also noted in the cytoplasm of acinar serous cells, but not mucus cells (Fig. 2).

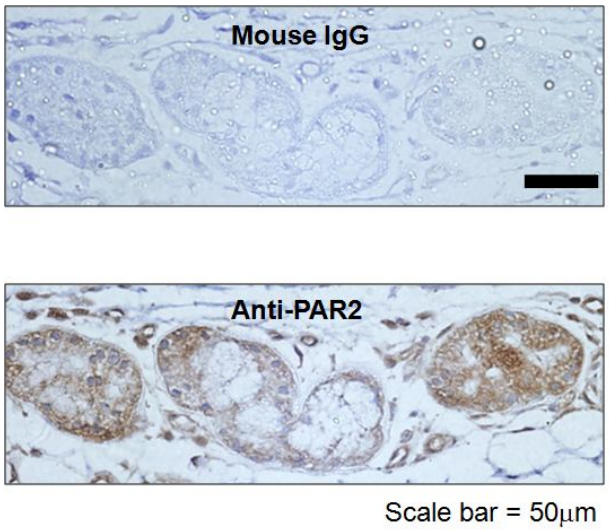

Figure 2. Expression of PAR2 in human airway gland. Immunostaining using PAR2 antibody showed that PAR2 is mainly expressed in the basolateral side of acinar cells. In serous glandular cells immunoreactivity is also noted in the cytoplasm. Negative control with mouse IgG shows no immunoreactivity. Bar indicates 50 μm.

3. PAR2-induced $[Ca^{2+}]_i$ mobilization in human airway gland

Next, we examined the changes in $[Ca^{2+}]_i$ after PAR2-AP application in dissected human airway gland cells. A slow increase in $[Ca^{2+}]_i$ was evoked by PAR2-AP(10 μM) within 100 sec, followed by a small sustained plateau. Although the response was not uniform among the cells, we observed similar responses and the peak response was approximately 40% of that seen with carbachol (10 μM) (Fig. 3A and B).

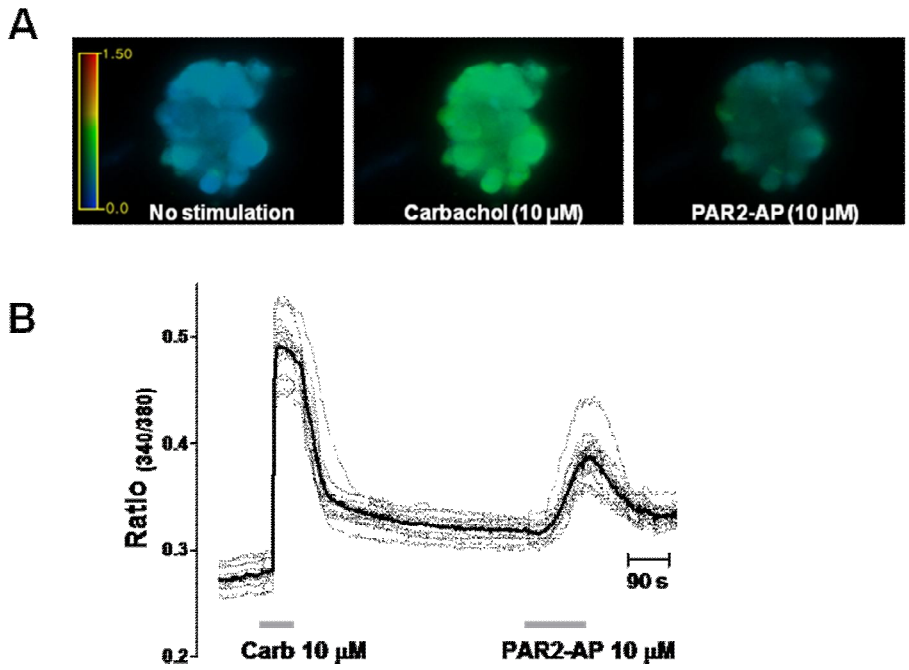

Figure 3. Evidence of PAR-2 elevating $[Ca^{2+}]_i$. (A) Fluorescence changes in response to 10 μM carbachol and 10 μM PAR2-AP. Cell diameters in images are approximately 20 microns. (B) $[Ca^{2+}]_i$ vs. time for 11 cells from images in A, measured in response to sequential pulses of 10 μM carbachol or 10 μM PAR2-AP. Fluorescence ratio, 340 nm/380 nm.

4. PAR2-AP induced mucus secretion in porcine airway gland

Because pig airway tissues are more readily available than human tissues, we used pig tissues to dissect the mechanism of PAR2-mediated mucus secretion. We first established dose-response relations for PAR2-AP in pig airway gland using optical methods to determine the rate of mucus secretion from single glands. The pig gland was more sensitive to PAR2-AP than human airway gland. The threshold for PAR2-AP stimulation of gland mucus secretion (defined as the concentration which produced an obvious increase in mucus secretion rates for at least two glands in the optical field) was approximately 200 nM. The EC50 was 12.98 μM, and the approximate *Vmax* achieved with 100 μM PAR2AP was 2.47 ± 0.2 nl/min/gland (n = 3, 27 glands; Fig. 4A). Mucus secretion did not decrease with repeated exposure to PAR2-AP (Fig. 4B).

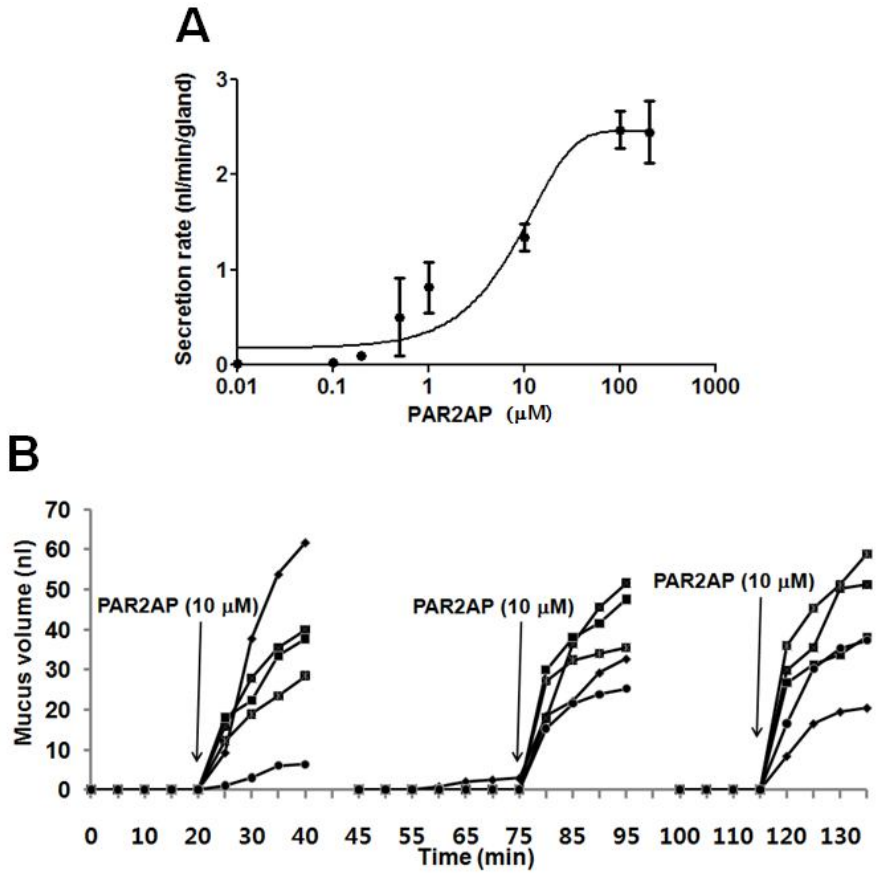

Figure 4. Mucus Secretion in pig airway gland in response to PAR2-AP. (A) Approximate dose-response relationship for submucosal gland secretion in pig glands. Each point represents the average of 10–14 glands from 3-4 different pig trachea. (B) Typical gland response to repeated PAR2-AP application. Similar secretory responses were noted with repeated PAR2-AP treatment.

5. Potential activators of protease-activated receptors in airway tissue

In order to provide supporting evidence for a physiologically relevant role of PAR2, we investigated the effects of various potential endogenous PAR activators that activate PARs by enzymatic cleavage. Trypsin (10 μM), which activates PAR2 and possibly PAR4[22] induced vigorous mucus secretion at a rate of 3.10±0.47 nl/min (41 glands, four pigs) which is a larger effect than that seen with PAR2-AP (1.61±0.06 nl/min, 41 glands, six pigs). Thrombin, an activator of PAR1, PAR3, and PAR4, did not induce mucus secretion. Human neutrophil elastase, which can also catalyze PAR2, also induced mucus secretion (0.73±0.17 nl/min, 11 glands, two pigs). After trypsin treatment, the glandular response to PAR2-AP was almost eliminated (Fig. 5).

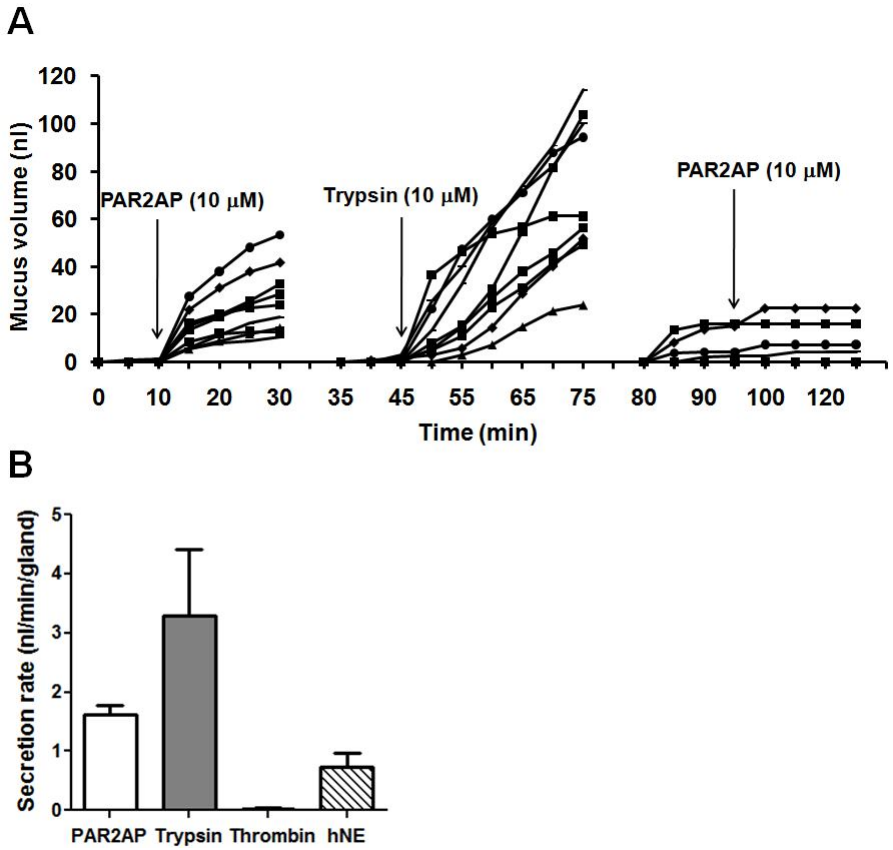

Figure 5. Effects of endogenous PAR activators in pig airway gland. (A) Trypsin (10 μM) induced vigorous mucus secretion which desensitized the PAR2-AP (10 μM) response. (B) Secretion rates from airway gland in response to endogenous PAR activators. h NE (human neutrophil elsastase). Data are means ± S.E.M from 11 to 41 glands from two to four pigs.

6. Mechanism of PAR-2-mediated mucus secretion

We compared secretory responses to PAR2-AP in the presence or absence of a Ca^{2+} chelator. BAPTA-AM (50 μM) reduced gland secretion stimulated by PAR2-AP by approximately 50% (0.71±0.08 nl/min, 21 glands, three pigs) compared with the secretion rate measured without BAPTA-AM (1.42±0.40 nl/min) (Figs. 6A and C). We used bumetanide (100 μM) to block the basolateral Na^+–K^+–$2Cl^-$ cotransporter (NKCC)1 to reduce luminal Cl^--mediated fluid transport. In a separate experiment we replaced $HCO3^-$ with HEPES and gassed with air to eliminate $HCO3^-$-mediated fluid transport. PAR2-AP-stimulated secretion (1.68±0.23 nl/min) was inhibited by both bumetanide (0.79±0.06 nl/min) and HEPES replacement (0.34±0.005 nl/min), and was almost eliminated when these treatments were used in combination (0.04±0.02 nl/min) (Figs. 6B and C). These results indicate that most PAR2-induced fluid secretion by submucosal glands is dependent on Ca^{2+} mobilization and anion movement. We also evaluated whether COX-2 or PGE2 might be involved in the process of PAR2-AP-induced mucus secretion. However, indomethacin (1 to 10 μM), a Cox-2 inhibitor, did not inhibit PAR2-AP-induced mucus secretion (data not shown).

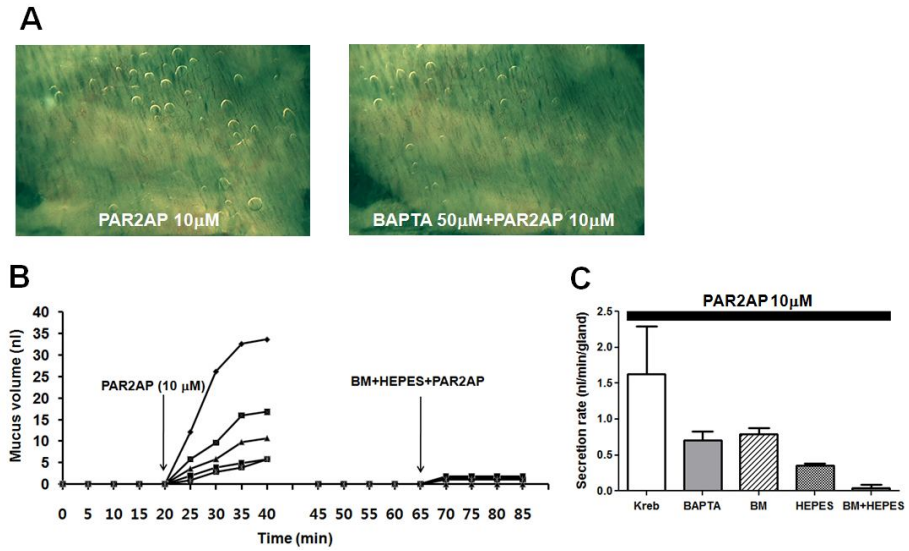

Figure 6. Ca^{2+}- and anion-dependency of PAR2-AP-induced mucus secretion in pig airway gland. (A) Images of mucus bubbles without and with BAPTA-AM (50 μM). Scale bar: 0.5 mm. (B) PAR2-AP-induced mucus secretion is also diminished in combination with bumetanide (BM) treatment (100 μM) or HEPES replacement. (C) Summary data from 21 to 33 glands from three pigs. Data are means ± S.E.M. * and ** indicate significant differences from the response seen with PAR2-AP alone, $P<0.05$ and 0.005, respectively.

7. PAR2-AP induced mucus secretion in ΔF508 CFTR mutant mice

To examine the possible role of the CFTR in PAR2-AP-induced secretion we compared responses in trachea from ΔF508 CFTR mutant mice and their

WT littermates. When the trachea were exposed to the PAR2-AP (100 μM), large amounts of secretion were observed in tracheas from both wild type (0.10±0.01 nl/min, 22 glands , four mice) and CF mice (0.15±0.01 nl/min, 15 glands three mice) There was no significant difference between wild type and mutant. (*P*>0.05) (Fig. 7).

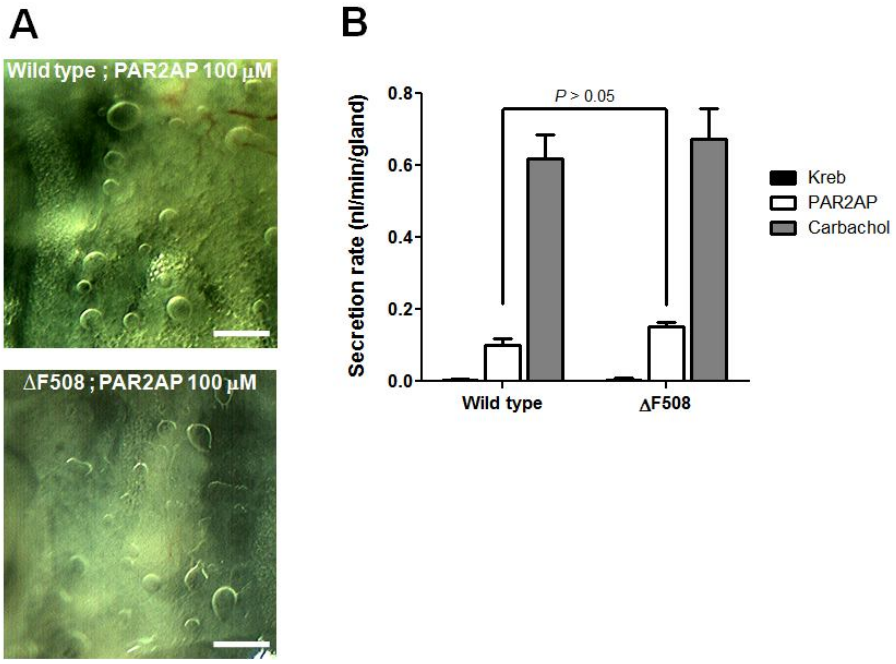

Figure 7. PAR2-AP-induced mucus secretion in mice. (A) Images of mucus bubbles in the trachea from wild type littermates or targeted ΔF508 CFTR mutant mice 10 min after PAR2-AP stimulation. Scale bar: 0.5 mm. **(**B) Secretion rates in response to PAR2-AP (100 μM) or carbachol (10 μM) in mouse tracheal gland. Data shown are means ± S.E.M from 49 glands from eight mice.

Ⅳ. DISSCUSSION

Although airway submucosal gland secretion is mainly regulated by central nervous system, parasymphatetic pathway, the role of local regulator system such as C-fiber-Substance P has being emphazised in airway epithelia, which is defective in CF airway[14]. In previous studies activation of PAR2 receptor induces transepithelial anion current in mouse trachea, which leads to a shift from absorption to secretion[15, 23]. PAR2 activation by human airway trypsin-like protease in NCI-H292 cells showed small but significant increase in mucin production while PAR2-AP did not increase mucin secretion[19]. PAR2-AP was also a weak enhancer of mucin secretion in primary human airway epithelial cell[20], which indicate PAR2 is not a significant contributor to mucus regulation. In the present investigation we directly demonstrated that PAR2 activation with a synthetic activating peptide as well as a physiological stimulator, trypsin or neutrophil elastase, potently induced airway gland mucus secretion. The secretion rate by PAR2-AP in human gland is approximately 20% of *Vmax* to carbachol[24], and was bigger that those to VIP[11] and Substance P[14]. Our data suggest that PAR2 seem to be involved in host defense mechanism against airway pathogens. PAR2 and its agonist from pathogen or immune cells could be important components of local defense

system in airway mucosa.

We further dissected the underlying mechanism for PAR2-AP-induced mucus secretion in pig airway gland. Activation of PAR2 elevated intracellular Ca^{2+} and PAR2-AP-induced mucus secretion was decreased by pretreatment with a Ca^{2+} chelator (BAPTA-AM). These results indicate that PAR-2-mediated mucus secretion is Ca^{2+} dependent. The response to PAR2-AP was not suppressed by indomethacin and PAR2-AP was still able to induce mucus secretion in ΔF508 CFTR mutant. These findings are not consistent with a previous report on research performed in Calu-3 cells[16] where Cl^- secretion induced by PAR2-AP was found to require prostaglandin release and CFTR activation. Although Calu-3 cells have properties similar to those of airway gland serous cells, their anion channel profile is quite different from that of the airway gland tissue *in vivo*. That is, CFTR is the only anion channel in the apical membranes of Calu-3 cells and the apical anion conductance is almost completely derived from CFTR channels in Calu-3 cells[25]. In contrast, both CFTR and CaCC exist in airway submucosal gland[24]. It is possible that the elevated cytosolic Ca^{2+} due to PAR2-AP opened the CaCC and induced anion secretion in airway gland *ex vivo* in our experiments.

Another discrepancy between Calu-3 cells and airway gland serous cells is

their differing patterns of response to repeated stimulation. Sato *et al.*[17] showed that PAR2-AP generates a brief response of Cl^- secretion through the PC-PLC-mediated pathway in Calu-3 cells, which was desensitized by repeated PAR2-AP treatment. However, we observed that the mucus secretion in response to PAR2-AP did not decrease with repeated treatment in airway glands. A possible reason for this difference is that the turnover rate for PAR2 is more rapid in submucosal gland tissue than in cultured cells.

Although the parasympathetic pathway is the primary controller of airway gland secretion, evidence increasingly supports a role for intrinsic control systems for airway gland secretion such as the capsaicin sensitive C-fiber system[14]. In our experiments, endogenous PAR2-AP agonists such as airway trypsin and neutrophil elastase also stimulated airway mucus secretion. This local receptor-mediated mucus secretion may be involved in host defense against pathogens in airway mucosa which is independent of parasympathetic control. If the role of PAR2 in the airway is to contribute to the control of the innate response to invading pathogens via CaCC activation in order to preserve respiratory functions, this mechanism would be preserved in the CF airway and act as a salvaged route for fluid secretion and innate immune responses. Interestingly, PAR2 expression is increased in airway epithelial cells in allergic airway disease[26] and in bronchial vessels of patients with

bronchitis[18]. Furthermore, human airway tryptase (HAT) is detected in high levels in BAL fluid from patients with chronic airway inflammatory disease[22]. Our preliminary data also showed that PAR2 expression in airway gland is increased in patients with allergic rhinitis and COPD, which could be one possible underlying mechanism of mucus hypersection in allergic or inflammatory airway disease.

Ⅴ. CONCLUSION

we showed that PAR2-AP is a strong agonist for mucus secretion from airway gland of three different species, human, pig and mouse which is Ca^{2+}-dependent and at least partially CFTR-independent. Because PAR2 is involved in the airway host defense mechanism, future research should investigate the potential of this receptor as a target for therapeutic intervention in the airway defense system.

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Abstact (in Korean)

호흡기 점막하선에서 단백분해효소 활성수용체 (PAR)-2에 의한 점액 분비

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이현재

호흡기 점막하선(Airway submucosal gland)은 호흡기 점액의 대부분을 생산해 내며, 그로부터 나오는 충분한 양의 점액분비는 외부항원이나 박테리아로부터 호흡기를 보호하는데 있어서 필수적 요소이다. 하지만 점액분비가 결핍되거나 과분비 된다면 만성 호흡기 감염, 만성폐쇄성폐질환, 천식 같은 호흡기질환을 유발한다. 따라서, 점막하선으로부터의 점액분비 기전을 이해하는 것은 호흡기 질환 치료에 있어서 중요하다고 할 수 있다. 호흡기 질환의 공통적 현상인 점액 과분비 기전을 이해하고 이를 억제할 수 있는 근본적인 치료법이 필요하다.

단백분해효소 활성수용체 (PARs)들은 사람 호흡기관을 포함하여 우리 몸에 광범위하게 분포되어 있으며 다양한 생리적 역할을 수행한다. PAR subtype 2 가 알레르기 천식이나 기관지염 같은

염증성 호흡기질환에서 중요한 역할을 수행한다는 결과가 알려져 있다. 하지만 점액분비의 시작점인 점막하선에서 PAR-2 의 역할은 알려져 있지 않다.

따라서, 본 연구에서는 사람 호흡기 점막하선에서 PAR-2 의 역할을 밝히고, PAR-2 의 활성화에 의한 점액분비 기전을 이해함으로써 다양한 호흡기 질환에서 나타나는 비특이적 점막과민반응에 대한 연구의 기본자료를 제공하고, 알레르기성, 염증성 호흡기 질환 병인의 새로운 연구가 가능하게 될 것이라 기대한다.

핵심되는 말 : 선천성 면역, 점액, 낭포성섬유증 막관통 조절인자, 프로스타글란딘 E_2, 칼슘