Involvement of Thromboxane A$_2$ and Histamine in Experimental Allergic Rhinitis of Guinea Pigs

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ABSTRACT
To identify the chemical mediators involved in the pathogenesis of allergic rhinitis, we studied the effects of the thromboxane (TX) A$_2$ receptor antagonist seratrodast, the peptide leukotriene receptor antagonist pranlukast and the antihistamine azelastine using a guinea pig model of allergic rhinitis. In guinea pigs actively sensitized by aerosol inhalation of antigen, antigen challenge into the nasal cavity increased both the nasal vascular permeability and the intranasal pressure; it also induced swelling of the nasal mucosa, which was evaluated by magnetic resonance imaging. Both seratrodast and azelastine significantly inhibited these antigen-induced responses when the drugs were administered p.o. 1 hr before antigen challenge. Also, the TX synthetase inhibitor ozagrel reduced the antigen-induced increase in nasal vascular permeability. On the other hand, pranlukast had little effect on the antigen-induced increases in nasal vascular permeability and intranasal pressure. Perfusions and inhalations of U-46619, a stable TXA$_2$ mimetic, or of histamine into the nasal cavity caused concentration-dependent increases in nasal vascular permeability and intranasal pressure in normal guinea pigs. Leukotriene C$_4$ also induced these responses, but the maximal responses to leukotriene C$_4$ were less than the maximal responses to U-46619 or histamine. On the other hand, these responses were not induced by prostaglandin D$_2$ or prostaglandin F$_2$-$\alpha$. Moreover, the U-46619- and histamine-induced increases in vascular permeability and intranasal pressure were significantly inhibited by seratrodast and azelastine, respectively. In addition, levels of TXB$_2$, a stable breakdown product of TXA$_2$, and histamine in nasal lavage fluid increased after antigen challenge in actively sensitized guinea pigs. These results suggest that TXA$_2$ and histamine play important roles in the pathogenesis of experimental allergic rhinitis in guinea pigs.

Nasal blockage is one of the typical symptoms of allergic rhinitis. It is caused by edema and congestion induced by chemical mediators that are released by several inflammatory cell types after antigen challenge and are capable of increasing vascular permeability and dilating the capacitance vessels of the nasal mucosa (Holmberg et al., 1989; Norman, 1985; Raphael et al., 1991; Sherwood et al., 1993). Histamine provocation into the nasal cavity increases the vascular permeability of the nasal mucosa (Svensson et al., 1992) and increases nasal airway resistance (Austin and Foreman, 1994). In addition, high levels of histamine have been identified in nasal secretions of patients with allergic rhinitis after antigen challenge (Naclerio et al., 1983). These results suggest that histamine is a chemical mediator responsible for the induction and pathogenesis of nasal blockage. However, other chemical mediators may be implicated in the pathogenesis of nasal blockage, because antihistamine drugs have little effect on nasal blockage in allergic rhinitis (Horak et al., 1993 and 1994).

Recently, in addition to histamine, arachidonic acid metabolites including TXA$_2$ and peptide leukotrienes, which have various biological activities, were detected in nasal lavage fluid from patients with allergic rhinitis after antigen provocation (Creticos et al., 1984; Brown et al., 1987). TXA$_2$ and peptide leukotrienes have been considered to be important mediators in allergic diseases because of their potential ability to contract airway smooth muscle (Dahlén et al., 1980; Svensson et al., 1977) and to increase vascular permeability (Hay et al., 1995; Lötvall et al., 1992). On the other hand, the roles of TXA$_2$ and peptide leukotrienes in allergic rhinitis are not yet clear.

In this study, to determine whether histamine and arachidonic acid metabolites contribute to the pathogenesis of allergic rhinitis, we investigated 1) changes in the vascular permeability of the nasal mucosa and intranasal pressure, by intranasal challenge with histamine or arachidonic acid metabolites in normal guinea pigs and 2) the effects of azelastine, an antihistamine drug, seratrodast, a TXA$_2$ receptor antagonist, and pranlukast, a peptide leukotriene receptor antagonist, on a) antigen-induced increases in vascular permeability and intranasal pressure and b) an antigen-induced decrease in the area of the airway lumen in actively sensi-
tized guinea pigs. In addition, we measured the levels of histamine and TXB₂ in nasal lavage fluid after antigen challenge.

Materials and Methods

Drugs and chemicals. Seratrodast and LTB₄ were synthesized by Takeda Chemical Industries, Ltd. (Osaka, Japan). Ozagrel hydrochloride and pranlukast were extracted from tablets of VEFA (Oto Pharmaceutical Co., Ltd., Osaka, Japan) and capsules of NONON (Oto Pharmaceutical Co., Ltd., Osaka, Japan), respectively. Azelastine hydrochloride was extracted from tablets of AZEPITIN (Eisai Co., Ltd., Tokyo, Japan). Indomethacin and OA (Grade III) were purchased from Sigma Chemical Co. (St. Louis, MO). U-46619 was from Cayman Chemical Co. (Ann Arbor, MI). Histamine dihydrochloride, PGD₂, PGF₂α, methanol, ethanol and EDTA were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Pontamine sky blue was from Tokyo Kasei, Ltd. (Tokyo, Japan). Azelastine hydrochloride was extracted from tablets of AZEPTIN (Eisai Co., Ltd., Tokyo, Japan). Indomethacin and OA (Grade III) were purchased from Sigma Chemical Co. (St. Louis, MO). U-46619 was from Cayman Chemical Co. (Ann Arbor, MI). Histamine dihydrochloride, PGD₂, PGF₂α, methanol, ethanol and EDTA were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals and sensitization procedure. Male Hartley guinea pigs, weighing 300 to 350 g (Seiwa Experimental Animals Ltd., Fukuoka, Japan), were sensitized by exposure to an OA aerosol (1% by volume) for 10 min on two occasions, separated by 7 days. The animals were positioned in a box (28 × 30 × 20 cm) made of polyvinyl chloride and were anesthetized with ketamine (50 mg/kg i.m.; Sankyo Co., Ltd.) and xylazine (5 mg/kg i.m.; Bayer). As described above, cannulas were inserted into the trachea and the nasal cavity. The nasal cavity was perfused with saline, and perfusate dropping from the nostrils was collected (Period 1). Vehicle, arachidonic acid metabolites, histamine or OA aerosols, generated by an ultrasonic nebulizer (model 5000D; DeVilbiss, Somerset, PA) placed between the nasopharynx and the artificial respirator, were inhaled for 3 min into the nasal cavity. The increase in intranasal pressure was expressed as the pressure minus the prechallenge base-line pressure. The effects of histamine and arachidonic acid metabolites on intranasal pressure were determined by calculating the AUC for the increase in intranasal pressure from 0 to 15 min after inhalation ended. Evaluation of the effects of the drugs on the induced increase in intranasal pressure was also based on the AUC for the increase in intranasal pressure.

Measurement of intranasal pressure. The intranasal pressure was continuously measured by the method described by Mizuno et al. (1991), with slight modifications. Guinean pigs were anesthetized with pentobarbital sodium (30 mg/kg i.p.) and were placed in a supine position. Animals breathed spontaneously through a cannula inserted into the trachea. A polyethylene cannula was inserted into the nasopharynx from the side of the larynx, and the other end of the cannula was connected to an artificial respirator (model 683; Harvard Bioscience, South Natick, MA) set at a flow volume of 4 ml and a frequency of 70 strokes/min. The two duct pores, which are situated in the upper oral cavity wall and lead to the nasal cavity, were closed with Aron alpha A. Intranasal pressure was measured by a transducer (model DP-45-22; Validyne Corp., Northridge, CA) connected to a lateral port at the proximal end of the endonasopharynx cannula. Vehicle, arachidonic acid metabolites, histamine or OA aerosols, generated by an ultrasonic nebulizer (model 5000D; DeVilbiss, Somerset, PA) placed between the nasopharynx and the artificial respirator, were inhaled for 3 min into the nasal cavity. The increase in intranasal pressure was expressed as the pressure minus the prechallenge base-line pressure. The effects of histamine and arachidonic acid metabolites on intranasal pressure were determined by calculating the AUC for the increase in intranasal pressure from 0 to 15 min after inhalation ended. Evaluation of the effects of the drugs on the induced increase in intranasal pressure was also based on the AUC for the increase in intranasal pressure.

Imaging of nasopharynx using MRI. MRI has been used to identify pathological changes in nasal tissue (Ryan et al., 1991; Sherwood et al., 1993), because it is a very useful technique for imaging soft tissues, especially tissues with high water contents. Therefore, we used MRI to evaluate the edema of the nasopharyngeal airway after antigen challenge. Actively sensitized guinea pigs were anesthetized with ketamine (50 mg/kg i.m.; Sankyo Co., Ltd.) and xylazine (5 mg/kg i.m.; Bayer). As described above, cannulas were inserted into the trachea and the nasal cavity, and then the guinea pigs were challenged by inhalation of 0.3% OA solution for 10 min. The nasal cavities were washed by flushing with 3 ml of saline and then with 10 ml of air 10 times. Transverse images of the nasopharyngeal airway were obtained using an MRI system during the 5 to 25-min period after antigen challenge.

Imaging of the nasopharynx was performed with a nuclear magnetic resonance spectrometer (CSI-II OMEGA system; Bruker, Germany) operating at 4.7 Tesla magnet/33 cm according to the method described by Sherwood et al. (1993), with slight modifications. A multislice longitudinal relaxation time (T₁)-weighted spin-echo imaging sequence was used to acquire proton density images. The recycle time (T₁r) and echo time (Tₑ) were 800 and 25 msec, respectively. T₁-weighted sagittal images were first obtained to determine the region in which transverse slices would be taken. Contiguous T₁-weighted transverse slices 4 mm thick were then obtained. The cross-sectional area of the nasopharyngeal airway lumen in the transverse views was measured using an image analysis system (IBAS2000; Zeiss, Germany). The cross-sectional area of the nasopharyngeal airway was expressed as the total value of three contiguous slices.

Measurement of TXB₂ and histamine in nasal lavage fluid. As described above, actively sensitized guinea pigs were challenged by perfusing the nasal cavity with 0.3% OA solution for 10 min. Then the nasal cavity was perfused with saline, and perfusate dropping from the nostrils was collected for 10-min periods into ice-cooled
polyethylene tubes containing 0.1 ml of 250 μM indomethacin and 2% (w/v) EDTA. Perfusate samples were centrifuged at 1700 × g for 10 min at 4°C. The supernatant was stored at −80°C until assay. The levels of TXB2 and histamine were measured using an enzyme immunoassay kit (Amersham International plc., Buckinghamshire, U.K.) and a radioimmunoassay kit (Immunotech S.A., Marseille, France), respectively.

**Statistical analysis.** All values are presented as the mean ± S.E. Statistical comparisons were performed with a one-way analysis of variance and Dunnett’s test or bilateral unpaired Student’s t tests, when appropriate. Differences were considered significant when P < .05. All statistical calculations were made using a SAS (Statistical Analysis System).

**Results**

**Changes in vascular permeability of the nasal mucosa.** Perfusion of U-46619 (0.1 ~ 100 μg/ml) through the nasal cavity caused a concentration-dependent increase in dye exudation. This response peaked during Period 3 at 100 μg/ml U-46619, during Period 4 at 1 and 10 μg/ml U-46619 and during Period 5 at 0.1 μg/ml U-46619 (fig. 1A). In the groups perfused with 10 and 100 μg/ml U-46619, the concentrations of dye in the Period 3 perfusate were 4.8 ± 0.8 (P < .05) and 8.5 ± 1.4 μg/ml (P < .01), respectively, and were significantly higher than in the vehicle (2% ethanol)-perfused group (0.9 ± 0.2 μg/ml). Perfusion of histamine (3 ~ 100 μg/ml) also caused a concentration-dependent increase in exudation of dye into the nasal cavity, peaking during Period 3 (fig. 1B). The concentrations of dye in the Period 3 perfusate in the groups perfused with 30 and 100 μg/ml histamine were 9.8 ± 1.8 (P < .05) and 12.8 ± 4.2 μg/ml (P < .01), respectively, and were significantly higher than in the saline-perfused group (1.0 ± 0.2 μg/ml). Perfusion of LTC4 (1 ~ 10 μg/ml) caused a concentration-dependent increase in dye exudation that showed a maximal response at 3 μg/ml LTC4 (table 1). On the other hand, perfusion of PGD2 or PGF2α into the nasal cavity did not induce greater dye exudation than occurred in the vehicle group perfused with 1% ethanol (table 1).

**Changes in intranasal pressure.** Aerosol inhalation of U-46619 (10 ~ 100 μg/ml) into the nasal cavity caused a concentration-dependent increase in intranasal pressure. The response to 30 μg/ml U-46619 continued to rise until 15 min after inhalation ended, but the response to 100 μg/ml U-46619 peaked from 5 to 10 min after inhalation ended (fig. 2A). The AUCs for the increase in intranasal pressure from 0 to 15 min after inhalation of 30 and 100 μg/ml U-46619 were 75.0 ± 25.8 (P < .05) and 102.9 ± 28.5 cm H2O · min (P < .01), respectively, and were significantly greater than in the vehicle group, which inhaled 2% ethanol (18.0 ± 5.8 cm H2O · min). Inhalation of histamine also caused an increase in intranasal pressure, which was already increased at the end of inhalation and plateaued 10 to 15 min after inhalation ended (fig. 2B). The AUCs for the increase in intranasal pressure from 0 to 15 min after inhalation of 100 and 1000 μg/ml histamine were 110.4 ± 21.9 (P < .05) and 124.8 ± 24.4 cm H2O · min (P < .01), respectively, and were significantly greater than in the vehicle group, which inhaled saline (24.9 ± 6.6 cm H2O · min). Inhalation of LTC4 (0.3 ~ 3 μg/ml) caused a concentration-dependent increase in intranasal pressure that showed a maximal response at 1 μg/ml LTC4 (table 2). After aerosol inhalation of PGD2 or PGF2α, on the other hand, intranasal pressure fell in comparison with the vehicle group, which inhaled 3% ethanol (table 2).

**Effect of seratrodast and azelastine on U-46619- and histamine-induced responses.** Seratrodast (0.3, 1 and 3 mg/kg p.o.) given 1 hr before 3 μg/ml U-46619 perfusion dose-dependently inhibited the U-46619-induced increase in dye exudation by 41, 67 (P < .01) and 71% (P < .01), respectively (table 3). Azelastine (1 mg/kg p.o.) significantly inhibited the histamine (100 μg/ml)-induced increase in dye exudation by 41, 67 (P < .01) and 71% (P < .01), respectively.
TABLE 2
Changes in intranasal pressure induced by inhalation of various stimulants into the nasal cavity of normal guinea pigs

<table>
<thead>
<tr>
<th>Stimulants</th>
<th>Concentration (μg/ml)</th>
<th>No. of Animals</th>
<th>Increase in Intranasal Pressure AUC (0–15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>—</td>
<td>5</td>
<td>32.7 ± 7.9</td>
</tr>
<tr>
<td>LTC4</td>
<td>0.3</td>
<td>5</td>
<td>45.7 ± 7.0</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>5</td>
<td>71.0 ± 9.5**</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td></td>
<td>64.9 ± 8.8</td>
</tr>
<tr>
<td>3% Ethanol</td>
<td>—</td>
<td>3</td>
<td>16.3 ± 4.8</td>
</tr>
<tr>
<td>PGD2</td>
<td>300</td>
<td>5</td>
<td>2.6 ± 4.6</td>
</tr>
<tr>
<td>PGF2α</td>
<td>300</td>
<td>5</td>
<td>-2.6 ± 3.2</td>
</tr>
</tbody>
</table>

**P < .01 compared with the vehicle (saline)-inhalation group (Dunnett’s test).

Exudation of dye induced by antigen challenge in actively sensitized guinea pigs. In guinea pigs actively sensitized by antigen aerosol inhalation, perfusion of OA (0.1 – 1%) into the nasal cavity caused a concentration-dependent increase in dye exudation that increased slightly in Period 2, peaked in Period 3, and decreased gradually thereafter (fig. 3). In the groups challenged with 0.1, 0.3 and 1% OA, the dye concentrations in the Period 3 perfusate were 7.0 ± 1.8, 9.6 ± 1.8 and 19.8 ± 3.7 μg/ml (P < .01), respectively. The response to 1% OA was significantly different from that of the saline-perfused group (1.6 ± 0.2 μg/ml).

Seratrodast (0.3, 3 and 30 mg/kg p.o.) given 1 hr before antigen challenge significantly reduced the OA (0.3%)-induced increase in exudation of dye by 29, 51 (P < .05) and 74% (P < .01), respectively (table 5). Ozagrel (100 mg/kg p.o.) significantly suppressed this OA-induced response by 52% (P < .05), and azelastine (1 mg/kg p.o.) significantly reduced exudation by 84% (P < .05) (table 3). Seratrodast (0.01, 0.03 and 0.1 mg/kg p.o.) given 1 hr before 30 μg/ml U-46619 inhalation dose-dependently suppressed the U-46619-induced increase in intranasal pressure by 17, 55 and 79% (P < .01), respectively (table 4). Azelastine (1 mg/kg p.o.) significantly inhibited the histamine (1000 μg/ml)-induced increase in intranasal pressure by 62% (P < .05) (table 4). These drugs did not affect the time course of the U-46619- or histamine-induced responses.

TABLE 4
Effects of seratrodast and azelastine on the U-46619- and histamine-induced increase in intranasal pressure in normal guinea pigs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>No. of Animals</th>
<th>Increase in Intranasal Pressure AUC (0–15 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>8</td>
<td>78.7 ± 11.7</td>
</tr>
<tr>
<td>Seratrodast</td>
<td>0.01</td>
<td>8</td>
<td>65.0 ± 15.5</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>8</td>
<td>35.7 ± 9.1</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>8</td>
<td>16.7 ± 3.3**</td>
</tr>
<tr>
<td>Azelastine</td>
<td>1</td>
<td>5</td>
<td>44.5 ± 14.8*</td>
</tr>
</tbody>
</table>

**P < .01 compared with the control group (Dunnett’s test or unpaired t test).

TABLE 3
Effects of seratrodast and azelastine on the U-46619- and histamine-induced increase in nasal vascular permeability in normal guinea pigs

Normal guinea pigs were challenged by perfusion of U-46619 (3 μg/ml) or histamine (100 μg/ml) through the nasal cavity. The drug or 5% gum arabic (control) was administered p.o. 1 hr before U-46619 or histamine perfusion. The increases in dye concentration in the perfusate during Period 3 are shown. Each value represents the mean ± S.E. for 5 to 8 animals.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>No. of Animals</th>
<th>Increase in Exudate (μg/ml)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-46619-induced response</td>
<td>—</td>
<td>6</td>
<td>4.9 ± 0.8</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>6</td>
<td>2.9 ± 0.8</td>
<td>41**</td>
</tr>
<tr>
<td>Seratrodast</td>
<td>0.3</td>
<td>6</td>
<td>1.6 ± 0.9**</td>
<td>67</td>
</tr>
<tr>
<td>Azelastine</td>
<td>1</td>
<td>4</td>
<td>1.4 ± 0.3**</td>
<td>71</td>
</tr>
</tbody>
</table>

*P < .05, **P < .01 compared with the control group (Dunnett’s test or unpaired t test).
it by 69% (P < .01) (table 5). On the other hand, pranlukast (3 and 30 mg/kg p.o.) given 1 hr before antigen challenge did not reduce the OA-induced increase in dye exudation (table 5).

**Increase in intranasal pressure induced by antigen challenge in actively sensitized guinea pigs.** In guinea pigs actively sensitized by antigen aerosol inhalation, aerosol inhalation of OA (0.1 ~ 3%) into the nasal cavity caused a concentration-dependent increase in intranasal pressure that plateaued at 5 to 10 min after antigen challenge (fig. 4). In the groups challenged with 0.1, 0.3, 1 and 3% OA, the increases in intranasal pressure 10 min after antigen challenge were 2.2 ± 1.3, 5.6 ± 1.1, 10.7 ± 2.2 (P < .01) and 10.5 ± 2.1 cm H2O (P < .05), respectively; two of these values were significantly higher than in the group that inhaled saline (1.9 ± 0.7 cm H2O). Seratrodast (0.3, 3 and 30 mg/kg p.o.) given 1 hr before antigen challenge dose-dependently reduced the OA (3%)-induced increase in intranasal pressure by 11, 27 and 48% (P < .05), respectively (fig. 5, table 6). Azelastine (1 mg/kg p.o.) reduced this OA-induced increase in intranasal pressure, but the value of the AUC did not reach statistical significance (fig. 5, table 6). Pranlukast (3 and 30 mg/kg) given 1 hr before antigen challenge did not inhibit the OA-induced increase in intranasal pressure (table 6).

**Decrease in area of the nasopharyngeal airway induced by antigen challenge in actively sensitized guinea pigs.** The nasopharyngeal airway was observed by MRI after antigen challenge in actively sensitized guinea pigs. As shown in figure 6, the mucosa surrounding the nasopharyngeal airway was observed as an area with a high signal intensity. When compared with the saline-challenged group, the OA-challenged group showed swelling of the mucosa, and the cross-sectional area of the airway lumen was significantly reduced by 32 (P < .05) or 33% (P < .01) (fig. 6, A and B; table 7). Seratrodast (30 mg/kg p.o.) given 1 hr before antigen challenge significantly suppressed the decrease in the area of the airway lumen by 48% (P < .05) (fig. 6C; table 7). Azelastine (1 mg/kg p.o.) significantly inhibited the decrease in the area of the airway lumen by 67% (P < .05) (table 7).

**Levels of TXB2 and histamine in the perfusate.** Concentrations of TXB2 and histamine in nasal lavage fluid were measured after antigen challenge in actively sensitized guinea pigs. As shown in figure 7, the concentration of TXB2 in nasal cavity perfusate was increased by intranasal antigen challenge, peaked at Period 2 to Period 3 and gradually decreased thereafter. The concentrations of TXB2 in the perfusates at Periods 2 and 3 were 270.2 ± 62.2 (P < .01) and 269.6 ± 57.6 pg/ml (P < .01), respectively, and were significantly higher than in the saline-challenged group (43.8 ± 12.3 pg/ml at Period 2 and 40.7 ± 12.4 pg/ml at Period 3). The concentration of histamine was markedly increased during Period 2 by antigen challenge and declined rapidly thereafter (fig. 7). The concentration of histamine in the Period 2 perfusate was 22.5 ± 5.2 ng/ml (P < .01) and was significantly higher than in the saline-challenged group (0.3 ± 0.1 ng/ml).
Discussion

In this study, we showed that seratrodast (Ashida et al., 1989; Kurokawa et al., 1994), a selective TXA2 receptor antagonist, and azelastine (Katayama et al., 1981; Zechel et al., 1981), an antihistamine drug, inhibit increases in both vascular permeability of the nasal mucosa and intranasal pressure and reduce swelling of the nasal mucosa induced by antigen challenge. In addition, intranasal challenge with U-46619, a TXA2 mimetic, and histamine increased nasal vascular permeability and intranasal pressure. Levels of TXB2 and histamine in nasal lavage fluid were markedly increased after antigen challenge in actively sensitized guinea pigs. These results strongly suggest that TXA2 and histamine play pivotal roles in the increases in both vascular permeability of the nasal mucosa and intranasal pressure evoked by antigen challenge in this allergic rhinitis model. It has been suggested that histamine mediates antigen-evoked edema of the nasal mucosa in guinea pigs (Mizuno et al., 1991; Shirasaki et al., 1992, Sherwood et al., 1993). To the best of our knowledge, this is the first time that participation of TXA2 in edema of the nasal mucosa induced by an anaphylactic response has been demonstrated.

Although seratrodast is a selective TXA2 receptor antagonist, it also antagonizes the contractile responses of guinea pig tracheal strips to PGD2 and PGF2α (Ashida et al., 1989), because these prostanoids contract airway smooth muscle by acting on a TX receptor (TP receptor) on the human and guinea pig airway smooth muscle (Coleman and Sheldrick, 1989; McKenniff et al., 1991). Therefore, to elucidate further the involvement of TXA2 in the antigen-induced responses in this model, we also studied the effect of ozagrel (Hiraku et al., 1986), a TX synthetase inhibitor, on antigen-induced increases in nasal vascular permeability. Administration of ozagrel had an inhibitory effect on the antigen-induced increase in nasal vascular permeability. Administration of PGD2 and PGF2α at relatively high concentrations tended to decrease both nasal vascular permeability and intranasal pressure in normal guinea pigs (tables 1 and 2). These results strongly suggest that the inhibitory effect of seratrodast on the increase in nasal vascular permeability induced by intranasal antigen challenge resulted from an antagonistic effect against the TXA2 released by antigen challenge and that PGD2 and PGF2α were not involved in the antigen-induced responses in this model. Woodward et al. (1990) demonstrated that PGD2, but not PGF2α, increases conjunctival microvascular permeability and that this response to PGD2 could not be ascribed to the TX receptor because a selective TXA2 receptor antagonist had no effect on it. Moreover, it has been reported that PGD2 and PGF2α do not displace the specific binding of a radiolabeled TXA2 receptor antagonist to membrane preparations of pulmonary arterial endothelial cells (Sung et al., 1989). Therefore, it seems likely that there are differences in the affinities of these prostanoids for TP receptors in different regions. In addition to these observations, there are differences between tissues in the effect of TXA2 on vascular permeability. It has been reported that TXA2 mimetics, including U-46619, increase vascular permeability in the conjunctiva (Woodward et al., 1990) and in the lower airway (Lötvall et al., 1992) as well as in the nose, and that these responses are inhibited by selective TX receptor antagonists. On the other hand, Woodward et al. (1990) demonstrated that TXA2 mimetics do not increase cutaneous vascular permeability. Nothing that has been learned to date can readily account for these different activities of TXA2 mimetics and prostaglandins in different regions. Although the notion of heterogeneous subpopulations of TP receptors is currently being contested, Raychowdhury et al. (1994) have demonstrated the existence of a family of TP receptors produced by alternative splicing of the carboxyl terminus. Thus these pharmacological observations also seem to suggest the existence of heterogeneous populations of TP receptors. However, the ability of divergent carboxyl tails significantly to influence recognition of TP receptor ligands remains a topic for future detailed examination.

Administration of LTC4 into the nasal cavity also caused increases in nasal vascular permeability and intranasal pressure, as previously reported (Shirasaki et al., 1992), but the maximal responses to LTC4 were less than the maximal responses to U-46619 or histamine. Administration of pranlukast (Nakagawa et al., 1992; Yamaguchi et al., 1992), a

**Fig. 5.** Effects of seratrodast and azelastine on the antigen-induced increase in intranasal pressure in actively sensitized guinea pigs. Presently sensitized guinea pigs were challenged by inhalation of a 3% OA aerosol into the nasal cavity. Seratrodast (●) and azelastine (▲) were administered p.o. at 30 and 1 mg/kg, respectively, 1 hr before antigen challenge. Each point represents the mean for seven animals, and vertical bars represent the standard error. *P < .05, **P < .01, compared with the control group (□) (Dunnett’s test).

**Table 6**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>No. of Animals</th>
<th>Increase in Intranasal Pressure (AUC 0–25 min)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>7</td>
<td>287.0 ± 35.9</td>
<td>—</td>
</tr>
<tr>
<td>Seratrodast</td>
<td>0.3</td>
<td>7</td>
<td>256.2 ± 46.1</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>208.9 ± 42.0</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>7</td>
<td>150.2 ± 20.2</td>
<td>48</td>
</tr>
<tr>
<td>Azelastine</td>
<td>1</td>
<td>7</td>
<td>163.1 ± 36.2</td>
<td>43</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>10</td>
<td>222.4 ± 24.0</td>
<td>—</td>
</tr>
<tr>
<td>Pranlukast</td>
<td>3</td>
<td>10</td>
<td>186.5 ± 25.8</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>10</td>
<td>189.9 ± 16.1</td>
<td>15</td>
</tr>
</tbody>
</table>

*P < .05 compared with the control group (Dunnett’s test).
peptide leukotriene receptor antagonist, had little effect on the antigen-induced increases in nasal vascular permeability and intranasal pressure. In addition, because swelling of the nasal mucosa is caused by increased vascular permeability, we suspect that pranlukast may not inhibit the swelling of

**TABLE 7**

Effects of seratrodast and azelastine on the antigen-induced decrease in the area of the nasopharyngeal airway in actively sensitized guinea pigs

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of Animals</th>
<th>Area of Nasopharyngeal Airway (mm²)</th>
<th>Decrease in Area (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>8</td>
<td>85.1 ± 6.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>OA</td>
<td></td>
<td>56.6 ± 3.1**</td>
<td>33</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>70.2 ± 4.9†</td>
<td>18</td>
<td>48</td>
</tr>
<tr>
<td>Seratrodast</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td>90.4 ± 6.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Saline</td>
<td>7</td>
<td>61.7 ± 6.5*</td>
<td>32</td>
<td>—</td>
</tr>
<tr>
<td>OA</td>
<td></td>
<td>80.8 ± 5.2†</td>
<td>11</td>
<td>67</td>
</tr>
</tbody>
</table>

*P < .05, **P < .01 compared with the saline-challenged group (unpaired t test), †P < .05 compared with the OA-challenged control group (unpaired t test).

Fig. 6. Representative T₁-weighted transverse slice from an actively sensitized guinea pig after saline (panel A) or antigen (panels B and C) challenge. A) Nasopharyngeal airway (arrow) and nasal mucosa (arrowhead) from a saline-challenged animal. B) Swelling of the nasal mucosa (arrowheads) and a decrease in the area of the nasopharyngeal airway (arrow) are seen after antigen challenge. C) Seratrodast (30 mg/kg) was administered p.o. 1 hr before antigen challenge. Inhibition of the swelling of the nasal mucosa (arrowhead) and the inhibition of the decrease in the area of the nasopharyngeal airway (arrow) are seen.

Fig. 7. Levels of thromboxane B₂ and histamine in nasal cavity perfusates of actively sensitized guinea pigs. Actively sensitized guinea pigs were challenged by perfusion of saline (open symbol) or 0.3% OA (closed symbol) during Period 2. Each point represents the mean for 7 or 8 animals, and vertical bars represent the standard error. *P < .05, **P < .01, compared with the saline-perfused group (unpaired t test).
the nasal mucosa induced by antigen challenge in this model. Considered as a whole, these results suggest that peptide leukotrienes, including LTC₄, hardly contribute at all to these antigen-induced responses, at least in our guinea pig model.

In this study, we found that antigen challenge into the nasal cavity evoked histamine and TXB₂ release into nasal cavity perfusates that peaked during Period 2 and from Period 2 to Period 3, respectively, and that this was followed by a transient increase in dye exudation that peaked during Period 3. In addition, persistent increases in intranasal pressure and swelling of the nasal mucosa were induced by antigen challenge in actively sensitized guinea pigs. It is widely assumed that nasal blockage is caused by swelling of the nasal mucosa induced by both mucosal edema as a result of increased vascular permeability and dilation of capacitance vessels, leading to engorgement of blood in the nasal mucosa. However, Sherwood et al. (1993) reported that antigen-induced swelling of the nasal mucosa was a result of increased vascular permeability in the nasal mucosa of actively sensitized guinea pigs, because oxymetazoline, a vascular decongestant, did not inhibit the swelling. We therefore conclude that histamine and TXA₂ released by antigen challenge begin to increase nasal vascular permeability when the levels of these mediators in the nasal mucosa increase to a certain point and that the lasting increase in intranasal pressure is secondary to an increase in nasal vascular permeability with leakage of plasma that leads to prolonged edema of the nasal mucosa.

It is well known that histamine is released from inflammatory cells in response to immunological stimuli earlier than TXA₂, because histamine and TXA₂ are preformed and newly generated mediators, respectively. In the present study, the increased level of histamine in nasal lavage fluid after antigen challenge peaked earlier than that of TXB₂ (fig. 7). In addition, histamine and U-46619 required different times to increase intranasal pressure; intranasal pressure had already increased at the end of histamine inhalation but gradually increased after U-46619 inhalation (fig. 2). Therefore, it seems that histamine and TXA₂ are involved in the antigen-induced increase in intranasal pressure at different times. This suspicion is supported by the observation that azelastine and seratrodast tended to attenuate the early (0 to 10 min after antigen challenge) and delayed (10 to 25 min after antigen challenge) increases, respectively, in intranasal pressure induced by intranasal antigen inhalation in actively sensitized guinea pigs (fig. 5). Although the source of the antigen-induced histamine release is suspected to be mast cells, the source of TXA₂ release in the present experiments is unknown. TXA₂ is known to be generated by several types of cells, including macrophages, neutrophils and mast cells (Higgs et al., 1983; Holgate et al., 1984; Schleimer et al., 1983), and any of these may be involved.

We should point out that there are two differences between our guinea pig model and human allergic rhinitis. First, whereas PGD₂ did not increase nasal vascular permeability or intranasal pressure in guinea pigs, instillation of PGD₂ into the human nasal cavity increases nasal airway resistance (Doyle et al., 1990). Second, whereas azelastine inhibited the antigen-induced responses in this guinea pig model, antihistamine drugs had little or no effect on nasal blockage in patients with allergic rhinitis (Corrado et al., 1987; Holmberg et al., 1989; Jankowski et al., 1993). Although it is difficult to determine the source of these differences between the effects in humans and in guinea pigs, it may be related to the fact that we could not evaluate congestion, which is one of the major causes of nasal blockage in human allergic rhinitis. The differences may also be attributable in part to different methods of using the antihistamine drugs, which were administered prophylactically and therapeutically. Furthermore, it has been reported that the immediate response is followed by a late-phase response that begins several hours after intranasal antigen challenge in approximately 50% of patients with allergic rhinitis (Dvoracek et al., 1984). In this study, we were concerned with only the immediate response after antigen challenge in this model. This may also have been a cause of the discrepancies. On the other hand, it has been reported that histamine increases the vascular permeability of the nasal mucosa (Svensson et al., 1992) and increases nasal airway resistance (Austin and Foreman, 1994) in humans, whereas the cyclooxygenase inhibitors indomethacin (McLean et al., 1983) and flurbiprofen (Brooks and Karl, 1988) inhibit nasal blockage in patients with allergic rhinitis. High levels of histamine (Naclerio et al., 1983) and TXB₂ (Brown et al., 1987) have been identified in the nasal lavage fluid of patients with allergic rhinitis after antigen challenge. In addition, Narita et al. (1996) have shown that BAY u3405, a TX receptor antagonist, reduces the biphasic increase in total airway resistance, probably nasal airway resistance, after intranasal antigen challenge in guinea pigs. Thus these observations, as well as the results in our guinea pig model of allergic rhinitis, suggest that TXA₂ and histamine may contribute to the development of nasal blockage in allergic rhinitis in general. It is uncertain whether the findings described in the present paper are applicable to human allergic rhinitis.

In conclusion, we report that U-46619, a TXA₂ mimetic, as well as histamine, increased both nasal vascular permeability and intranasal pressure and that seratrodast and azelastine inhibited the increases in nasal vascular permeability and intranasal pressure induced by intranasal antigen challenge in actively sensitized guinea pigs. We also demonstrated that TXB₂ and histamine levels in nasal lavage fluid increased after intranasal antigen challenge. Taken together, these results strongly suggest that TXA₂ and histamine play important roles in the development of increased nasal vascular permeability and intranasal pressure evoked by antigen challenge in this guinea pig model and that seratrodast may be a useful tool for elucidating the pathophysiological role of TXA₂ in human allergic rhinitis.

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References

