Prostaglandin D₂ Modulates Neuronal Excitation of the Trigeminal Ganglion to Augment Allergic Rhinitis in Guinea Pigs

Yoji Nagira, Kumiko Goto, Hiroyuki Tanaka, Miwa Aoki, Shingo Furue, Naoki Inagaki, Yasuhiro Tomita, and Michitaka Shichijo

ABSTRACT

Prostaglandin D₂ (PGD₂) is involved in the pathogenesis of allergic rhinitis. However, the sensory nervous system–mediated contributions of PGD₂ to the symptoms of allergic rhinitis remain unclear. We investigated the involvement of PGD₂ in these symptoms and in neuronal excitation by in vivo and ex vivo experiments. In an ovalbumin–induced model of allergic rhinitis in guinea pigs, the number of sneezing, nasal rubbing, and nasal secretion events were assessed after the nasal cavity instillation of PGD₂, histamine, or a combination of PGD₂ and histamine. In situ hybridization for PGD₂ receptor 1 (DP₁) mRNA transcripts and immunohistochemical analysis of histamine H₁ receptor protein expression in guinea pig trigeminal ganglion (TRG) were performed. The effects of DP₁ receptor antagonist on the excitability of TRG neurons to electrical and histamine stimuli were assessed using whole-cell patch-clamp recordings.

Histamine induced more sneezing, nasal rubbing, and nasal secretion events than PGD₂. PGD₂ augmented histamine-induced responses, whereas pretreatment with a DP₁ receptor–selective antagonist completely suppressed PGD₂–induced augmentation. DP₁ receptor mRNA transcripts and H₁ receptor protein expression could be detected in TRG neurons. Moreover, a DP₁ receptor agonist caused significant increases in the number of histamine-induced action potentials and depolarization, and reduced the current threshold in small-diameter neurons. Our findings show that PGD₂–DP₁ receptor signaling augments the symptoms of allergic rhinitis via the sensory nervous system by modulating nasal neuronal activation to various stimuli, such as histamine. These findings suggest that DP₁ receptor antagonist has therapeutic potential for the treatment of allergic rhinitis.

Introduction

Allergic rhinitis is an IgE-mediated inflammatory disease that is characterized by nasal obstruction, sneezing, pruritus, and rhinorrhea. Upon the crosslinking of antigen to high-affinity IgE, mast cells can discharge secretory granules, including histamine and tryptase, along with the release of newly synthesized leukotriene and prostaglandin molecules. These chemical mediators can induce the major symptoms of allergic rhinitis (Naclerio et al., 1983; Bousquet et al., 1996). Among these symptoms, sneezing, pruritus, and rhinorrhea (at least in part) may be induced via the sensory nervous system. Histamine is considered a major mediator of those symptoms of allergic rhinitis induced by neural activation, as histamine challenge in allergic rhinitis patients can induce sneezing, nasal itching, and rhinorrhea (Maidonna et al., 1987), and treatment with a histamine H₁ receptor antagonist can improve these symptoms (Taylor-Clark and Foreman, 2005a).

Like histamine, prostaglandin D₂ (PGD₂) is known to have important roles in allergic responses. PGD₂, a major cyclooxygenase metabolite, can act on two different receptors, DP₁ receptor (Boie et al., 1995) and chemoattractant receptor–homologous molecule expressed on T helper type 2 (Th2) cells (CRTH2) (Nagata et al., 1999). Activation of the DP₁ receptor results in vasodilation (Cheng et al., 2006) and enhances vascular permeability (Woodward et al., 1990), whereas CRTH2 activation induces the chemotaxis of inflammatory cells, such as Th2 cells, eosinophils, and basophils (Hirai et al., 2001). Thus, PGD₂ is thought to act in allergic inflammation via both of these receptors.

Local antigen challenge induces increase in PGD₂ levels in the nasal lavage fluid of patients with allergic rhinitis (Wagenmann et al., 1996) and in the bronchial lavage fluid of asthma patients (Murray et al., 1986). Furthermore, we previously reported that a potent and selective DP₁ receptor antagonist, asapirprant (S-555739), inhibited both nasal congestion and secretion in a guinea pig model of allergic rhinitis, and ameliorated airway hyper-responsiveness in rats and...
sheep (Takahashi et al., 2015). We also showed that PGD₂ directly induced the dilatation of the sinusoid vessels within nasal mucosa, and that a DP₁ receptor agonist, BW245C, induced nasal congestion in ovalbumin (OVA)-sensitized guinea pigs (Takahashi et al., 2012). Additionally, PGD₂ could enhance histamine-induced nasal blockage via the DP₁ receptor in guinea pigs (Yasui et al., 2008). Thus, we revealed previously that PGD₂-DP₁ receptor signaling plays an important role in nasal congestion. However, we had not yet investigated the involvement of the DP₁ receptor in other nasal symptoms, such as sneezing and nasal itching. Several studies have suggested that PGD₂ might be involved in rhinitis symptoms via the sensory nervous system (Rahman et al., 2007b), but the mechanism whereby PGD₂ contributes to these symptoms remained unclear.

In the present study, to better characterize the role of PGD₂ in nasal symptoms induced via sensory nerve stimulation, we examined the effects of PGD₂ on sneezing, nasal itching, and rhinorrhea using a guinea pig model of allergic rhinitis. Additionally, we examined whether PGD₂ could interact with guinea pig nasal trigeminal ganglion (TRG) neurons that innervate the nasal mucosa. Our data suggest that PGD₂ could augment the nasal symptoms of rhinitis via the sensory nervous system by modulating nasal neuronal activation in response to various stimuli in guinea pigs.

Materials and Methods

Chemicals and Reagents. Asapiprant ([2-oxazol-2-yl]-5-(4-[[(propan-2-yl)oxy]phenylsulfonyl]piperazine-1-yl)phenoxy) acetic acid) was synthesized by Shionogi & Co., Ltd. (Osaka, Japan). The pharmacological properties of asapiprant were described in our previous report (Takahashi et al., 2015). In brief, the following observations were made: The inhibition-constant value of asapiprant to the human DP₁ receptor was 0.44 nM; asapiprant inhibited the cAMP elevation induced by PGD₂ in platelet-rich plasma in human (IC₅₀: 16 nM) and guinea pigs (IC₅₀: 61 nM); its potency to the other prostanoid receptors, including CRTH₂, is 0 or 300 times less than its potency to the DP₁ receptor. These results suggested that asapiprant has highly potent and selective activity at the DP₁ receptor. Ovalbumin, atropine, cyclophosphamide monohydrate, and urethane were obtained from Sigma-Aldrich/SigmaMillipore (St. Louis, MO). PGD₂ and BW245C, a DP₁ receptor agonist, were purchased from Cayman Chemicals (Ann Arbor, MI). We purchased histamine dihydrochloride (histamine) from Nacalai Tesque (Kyoto, Japan). H-9, a protein kinase A (PKA) inhibitor, was purchased from Cell Signaling Technologies (Danvers, MA). For in vivo study, PGD₂, BW245C, and histamine were dissolved in ethanol and diluted in saline to a final concentration of 10%. In the case of simultaneous administration, histamine was mixed with PGD₂ or BW245C dissolved in 10% ethanol.

Animals. Male Hartley guinea pigs were purchased from Japan SLC Inc. (Hamamatsu, Japan). Animals were housed in an air-conditioned room at 20–26°C with a relative humidity of 30–70% and were provided standard laboratory chow and water ad libitum. The animal study protocol was approved by the Shionogi Animal Use and Care Committee. Animals were randomized by weight prior to the start of the experiments.

Sensitization and Challenge. Sensitization and challenge procedures were performed as described previously (Takahashi et al., 2012). Briefly, 6-week-old guinea pigs pretreated with cyclophosphamide monohydrate (30 mg/kg, i.p.) 2 days earlier were sensitized by the intraperitoneal injection of 1 mg OVA with 10 mg alum. Then, 7 days after sensitization, guinea pigs were challenged with 20 μl of 1% OVA into both nostrils. Beginning 7 days later, nasal challenge with 10 μl of 2% OVA per nostril was performed twice at 7-day intervals.

Counting of Sneezing and Nasal Rubbing. Various chemical mediators were instilled into both nostrils 1 day after the final OVA challenge. The numbers of sneezing and nasal rubbing events were counted for 20 minutes following nasal challenge. Sneezing was characterized by an explosive expiration after deep inspiration (Mizutani et al., 2003), and nasal rubbing was characterized by nasal scratching with forelimbs. As a control, 10% ethanol was instilled.

Measurement of Nasal Secretion. At 24 hours after the final antigen challenge, guinea pigs were anesthetized with urethane (1.4 g/kg, i.p.) and nasal secretion was provoked by the intranasal injection of 20 μl of various chemical mediators into the unilateral anterior naris. Five minutes after nasal provocation, a piece of preweighed paper (Kimwipe; Nippon Paper Creca, Tokyo, Japan) was inserted into the contralateral nostril of guinea pigs fixed in an abdominal position. After 20 minutes the paper was removed and weighed. Nasal secretions were determined as an increase in weight from the prechallenge weight. As a control, 10% ethanol was instilled unilaterally into naris of guinea pigs. To clarify the neuronal reflex, anesthetized guinea pigs were exposed for 3 minutes to an aerosolized solution of atropine (0.1%; diluted in saline) with an ultrasonic nebulizer, and 5 minutes after inhalation, concomitant challenge of 2% PGD₂ and 1% histamine into unilateral naris was performed. As a vehicle control, aerosolized saline was exposed.

Drug Administration. Asapiprant was suspended in 0.5% methylcellulose and administered orally to guinea pigs 1 hour prior to challenge with chemical mediators. As a vehicle control, 0.5% methylcellulose was administered orally to guinea pigs.

Isolation of TRG Neuron Cells and Fixation. Under deep anesthesia with pentobarbital sodium (40 mg/kg, i.p.), guinea pigs were decapitated, the source of the ophthalmic branch of the TRG nerve was isolated, and connective tissues were removed in ice-cold saline. Isolated TRG ganglia were fixed in 4% paraformaldehyde in PBS at 4°C overnight, dehydrated in an ascending ethanol series, and then embedded in paraffin.

Probe Preparation. Digoxigenin-labeled RNA probes were prepared using a DIG RNA Labeling Kit (Roche, Basel, Schweiz) and were synthesized from pCR-Blunt II-TOPO vectors (ThermoFisher Scientific; Waltham, MA) that contained dual SP6 and T7 promoters and the following sequence, which is a part of guinea pig DP₁ cDNA: DP₁ (GenBank accession number XM_013154746). DP₁/pCR II was digested with XhoI (sense linearization) or HindIII (antisense linearization) and then was treated with SP6 (GenBank accession number MG_020928) or T7 polymerase to produce sense and antisense probes, respectively.

In Situ Hybridization. Blocks were cut into 4-μm-thick sections that were mounted on slides and then deparaffinized in xylene, rehydrated via a descending series of ethanol, and rinsed with distilled water and phosphate-buffered saline (PBS). Sections were treated with 0.2 N HCl for 8 minutes, preincubated in protease K buffer (0.1 M Tris-HCl and 0.05 M EDTA) at 37°C for 5 minutes, treated with proteinase K (1.0 μg/ml) in protease K buffer at 37°C for 10 minutes, and acetylated with 0.1 M triethanolamine-HCl (pH 8.0) and 0.25% acetic anhydride at room temperature for 15 minutes. Following prehybridization, sections were incubated for 16 hours at 50°C in hybridization buffer [4× SSC, 50% formamide, 1× Denhardt’s solution, 10 mM Tris-HCl (pH 8.0), 0.1% SDS, 250 μg/ml tRNA, 5 mM EDTA, and 10% dextran sulfate] containing a DIG-labeled antisense or sense probe. After hybridization, sections were washed with 2× SSC at 55°C for 20 minutes three times, 1× SSC at 55°C for 20 minutes, 0.1× SSC at 55°C for 20 minutes, and finally with PBS. Detection of DIG-labeled antisense was performed with anti-digoxigenin-AP (1:50)

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primary antibody and 1-Step NBT/BCIP (ThermoFisher Scientific; Waltham, MA).

**Immunohistochemistry.** Tissue blocks were cut into 4-μm-thick sections, mounted on slides, dewaxed in xylene, rinsed with distilled water, and transferred to a microwave-resistant plastic staining jar that contained, as retrieval solution, 10 mM citrate buffer (pH 6.0). Fully covered slides were heated twice for 5 minutes in a microwave oven and then allowed to cool to 20°C within 60 minutes. Slides were immersed into 3% H2O2 for 10 minutes at room temperature to quench endogenous peroxidase activity, and then were transferred to a blocking solution containing 10% normal goat serum in 0.1% Triton-PBS for 30 minutes at room temperature. After a washing, slides were incubated overnight at 4°C in corresponding buffer that contained a polyclonal antibody against the histamine H1 receptor (dilution 1:50; Santa Cruz Biotechnology, Dallas, Texas). After a washing in PBS, samples were incubated with biotinylated affinity-purified anti-rabbit IgG for 1 hour. Slides were then incubated with VECTASTAIN ABC reagent (Vector Laboratories, Burlingame, CA) for 30 minutes. After a washing, immune complexes were detected by staining with 3,3′-diaminobenzidine. Slides were counterstained with hematoxylin after immunostaining. After three washes with PBS, TRG sections were mounted in Mount-Quick and covered with a coverslip.

**Isolation of TRG Neurons.** Juvenile guinea pigs were decapitated under deep anesthesia with pentobarbital sodium (40 mg/kg, i.p.), the source of the ophthalmic branch of the TRG nerve was isolated, and connective tissues were removed in ice-cold Locke’s solution (136 mM NaCl, 5.6 mM KCl, 1.2 mM MgCl2, 2.2 mM CaCl2, 1.2 mM NaH2PO4, 14.3 mM NaHCO3, and 10 mM d-glucose). The location of the ophthalmic branch projecting into the nasal mucous tissue was confirmed by Di labeling, according to the method of Taylor-Clark et al. (2005b). Isolated TRG ganglia were incubated in Locke’s solution containing 1 mg/ml collagenase for 40 minutes at 37°C and subsequently treated with 0.05% trypsin-EDTA for 5 minutes. Trituration was gently applied to dissociate neurons from TRG ganglia in culture medium (α-minimum essential medium with 10% fetal bovine serum, 20 mM HEPES, 1% penicillin-streptomycin solution, and 4 mM l-glutamine). After centrifugation (8800; KUBOTA, Tokyo, Japan) at 210 g for 5 minutes at 25°C, supernatant was removed and pellets containing TRG cells and surrounding tissues were resuspended in 10 ml of culture medium. After removing surrounding tissues using a cell strainer (70 μm; BD Bioscience, San Jose, CA) and centrifugation at 210 g for 5 minutes at 25°C, TRG cells were resuspended in 2 ml of culture medium. Finally, TRG cells were mounted on poly-l-lysine/ laminin-coated glass cover slips and incubated at 37°C with 5% CO2 (MCO-17A1; SANYO, Tokyo, Japan) for 15–25 hours.

**Histamine-Induced Action Potentials and Depolarization.** Whole-cell current-clamp recordings were made from visually identified TRG neurons using an upright microscope (Olympus, Tokyo, Japan). The external solution for recordings contained 136 mM NaCl, 5.6 mM KCl, 1.2 mM MgCl2, 2.2 mM CaCl2, 1.2 mM NaH2PO4, 14.3 mM NaHCO3, and 10 mM d-glucose. Patch electrodes were fabricated from borosilicate glass capillaries using an electrode puller (Sutter Instrument Co., Novato, CA). The tip resistance of patch electrode was 3.4–6.9 MΩ when filled with internal solution (140 mM KCl, 10 mM HEPES, 11 mM EGTA, 2 mM MgCl2, 1 mM CaCl2, and 10 mM d-glucose, adjusted to pH 7.3 with KOH). With a whole-cell configuration at a holding potential of ~60 mV, histamine-induced action potentials and depolarization were measured in the current clamp mode (EPC-10; HEKA Electronik, Lambrecht/Pfalz, Germany). Histamine-induced action potentials and depolarization were digitized at 1 kHz and filtered at a cut-off frequency of 2.9 kHz for computer analysis using Chart 7 software (ADInstruments, Colorado Springs, CO). In these studies, the number of action potentials elicited for 30 seconds and depolarized membrane potentials were calculated using Chart 7 software. After obtaining a stable baseline recording, the effects of BW245C on histamine-induced responses were evaluated according to the following protocols. To evaluate the effects of BW245C on histamine-induced responses, 10 μM histamine was applied with or without pretreatment with BW245C (0.1 μM). To evaluate the effects of asapiprant or H-89 on the augmentation of histamine-induced action potentials by BW245C, 1 μM asapiprant or 0.1 μM H-89 were added before examination of histamine-induced responses with BW245C. During these experiments, cells were continuously superfused with Locke’s solution.

**Current-Induced Action Potential.** In a whole-cell configuration at a holding potential of ~60 mV, current-induced action potentials were measured in the current clamp mode with Pulse software (EPC-10; both from HEKA). The action potential threshold was determined in the current clamp mode using stepwise current pulses (10–300 pA). The threshold current required to elicit an action potential discharge was used for current-clamp electrical excitability studies, in which each neuron was subjected to current pulses (200-millisecond duration, 0.2 Hz). Current-induced action potentials were digitized at 10 kHz and filtered at a cut-off frequency of 2.9 kHz for computer analysis with Pulse software (HEKA). After obtaining a stable baseline recording, the effects of BW245C (0.1 μM) on current-induced action potential thresholds were evaluated. To evaluate the inhibitory effect of asapiprant on the augmentation of current-induced action potential thresholds by BW245C, 1 μM asapiprant was pretreated before measurement of the current-induced action potential threshold with BW245C. During these experiments, cells were continuously superfused with Locke’s solution.

**Statistical Analysis.** All results are expressed as means ± S.E.M. Statistical significance was assessed using Windows SAS program. P-values < 0.05 were considered statistically significant differences. Statistical analysis was performed by Welch’s t test for two-group analysis or Dunnett’s test for multiple group comparisons.

**Results**

**Involvement of PGD2 and Histamine in Sneezing and Nasal Rubbing.** To investigate the effect of PGD2 and histamine on sneezing and nasal rubbing in an OVA-induced model, both nostrils of guinea pigs were exposed to PGD2 or histamine 24 hours after a final OVA challenge. Histamine significantly increased the number of sneezing and nasal rubbing events (P < 0.001 and 0.001, respectively), whereas PGD2 alone did not induce these responses (Fig. 1, A and C). To examine the effects of PGD2 or histamine-induced increases in sneezing and nasal rubbing, a combination of PGD2 and histamine was instilled into both nostrils. Concomitant stimulation by 2% PGD2 and 1% histamine induced a significant increase in the number of sneezing and nasal rubbing (26.9 ± 3.2, and 18.0 ± 1.9, respectively, N = 9) compared with histamine alone (sneezing: 12.1 ± 1.1, rubbing: 9.75 ± 1.1, N = 8). To test the involvement of the DP1 receptor in PGD2-induced augmentation of nasal symptoms, a DP1 receptor–specific antagonist asapiprant was orally administered 1 hour prior to challenge with 2% PGD2 and 1% histamine. The PGD2-induced augmentation of sneezing and rubbing events could be completely suppressed by 30 mg/kg asapiprant (13.1 ± 1.5 and 10.6 ± 1.1, respectively, N = 9). Moreover, a 2% solution of the DP1 receptor agonist BW245C did not affect sneezing or nasal rubbing events, but coadministration of 2% BW245C and 1% histamine significantly increased the number of sneezing and nasal rubbing events (21.1 ± 2.0, and 15.8 ± 0.9, respectively, N = 9) compared with administration of histamine alone (sneezing: 11.0 ± 1.4, rubbing: 10.1 ± 1.1, N = 9) (Fig. 1, B and D).

**Involvement of PGD2 and Histamine in Nasal Secretion Induced by the Sensory events Nervous System.** In part, nasal secretion can be induced by nerve reflexes; secretory...
response in the contralateral nostril that is induced by allergen and various stimuli to unilateral nostrils can be mediated by nerve reflexes since contralateral secretory response is inhibited by vidian neuroectomy or nerve blockade with local anesthetic drug (Konno and Togawa, 1979; Sanico et al., 1999a). To examine the effects of PGD2 and histamine on nasal secretion induced by the nervous system, PGD2 or histamine was instilled unilaterally into naris of guinea pigs in an OVA-induced allergy model, and nasal secretions in the contralateral naris were measured. We found that PGD2 did not induce an increase in nasal secretions on the contralateral side (13.3 ± 2.6 mg, N = 7) compared with 1% histamine alone (5.2 ± 1.2 mg, N = 7) (Fig. 2A and C). Pretreatment of muscarinic receptor antagonist, atropine inhalation significantly inhibited contralateral secretion induced by concomitant stimulation with 2% PGD2 and 1% histamine (P < 0.05, N = 6), suggesting that contralateral hypersecretions are induced by parasympathetic reflex (Fig. 2B). Additionally, oral administration of 30 mg/kg asapiprant completely inhibited PGD2-induced exacerbation of nasal secretions (P < 0.05, N = 6) (Fig. 2C). As with 2% PGD2, 2% BW245C did not induce a change in nasal secretion, but a significant increase of nasal secretion on the contralateral nasal cavity was observed after the simultaneous instillation of 2% BW245C and 1% histamine (P < 0.05, N = 9) (Fig. 2D).

Expression of the DP1 and Histamine H1 Receptors in Guinea Pig TRG Cells. To characterize the expression of DP1 and histamine H1 receptors in TRG neurons, we performed in situ hybridization to detect DP1 receptor mRNA transcripts, and immunohistochemistry to assess histamine H1 receptor protein expression. We detected both in TRG neurons (Fig. 3).

Effects of BW245C on Histamine-Induced Action Potentials in Guinea Pig TRG Cells. To test whether the PGD2-induced augmentation of histamine responses that we observed in in vivo experiments were the effects of PGD2 on TRG neurons, we examined the effects of BW245C on histamine-induced neuronal responses in small-diameter neurons (most probably C-fiber neurons) that innervate the nasal mucosa using whole-cell patch-clamp recordings. Typical results of histamine-induced action potential discharge are shown in Fig. 4. Perfusion with 10 μM histamine elicited depolarization and the discharge of action potentials (Fig. 4A). Treatment with 30 μM histamine induced even greater depolarization and action potential discharge (data not shown), suggesting that 10 μM was submaximal and represented an appropriate concentration for the following pharmacological investigations. Thus, we assessed the effects of BW245C on neuronal excitability in response to 10 μM histamine. We detected no differences in the percentage of small-diameter neurons that responded to histamine or in baseline membrane potential among the experimental groups (Table 1). The addition of 0.1 μM BW245C induced significant (P < 0.01) increases in the number of action potentials for 30 seconds along with depolarization (action potentials: 90.3 ± 19.5, depolarization: 15.4 ± 2.4 mV, N = 12) (Fig. 5, A–B). Pretreatment with 1 μM asapiprant significantly inhibited BW245C-induced exacerbation of action potentials and depolarization (17.1 ± 7.1, and 7.8 ± 0.9 mV, P < 0.01 and 0.001, respectively, N = 12). Furthermore, we examined the effects of PKA inhibitor H-89 on BW245C-induced augmentation of
depolarization and action potential discharge that could be induced by histamine. Significant inhibition of BW245C-induced augmentation was observed after treatment with 0.1 mM H-89 (action potentials: 12.5 ± 6.7, depolarization: 7.8 ± 0.9 mV, P < 0.001, N = 12) (Fig. 5).

**Effects of BW245C on Thresholds for Current Pulse-Induced Action Potentials in Guinea Pig TRG Cells.** We investigated whether BW245C changed the threshold for current pulse–induced action potentials. The application of 0.1 mM BW245C significantly reduced the current threshold for eliciting action potentials in small-diameter neurons (151.3 ± 21.0 pA, P < 0.05, N = 16) (Fig. 6). After washout, the threshold for current-induced action potentials was returned to its normal state, indicating that the enhancement effect of BW245C was reversible (data not shown). Pretreatment with 1 μM asapiprant significantly and completely inhibited the reduction of the current threshold for action potentials induced by BW245C (218.8 ± 19.0, P < 0.05, N = 16).

**Discussion**

In this present study, we investigated the effects of PGD₂ on sneezing, nasal rubbing, and nasal secretion in the contralateral nostril in an OVA-induced guinea pig model. Moreover, we examined the involvement of PGD₂ in the frequencies of action potentials and depolarization induced by histamine, and the threshold for action potentials caused by current pulses in guinea pig TRG cells. As a result, we report that PGD₂ augmented histamine-induced sneezing, nasal rubbing, and contralateral hypersecretion via DP₁ receptor signaling, and
PGD2 could augment histamine-induced contralateral nasal secretion via DP1 receptor signaling.

In this study, we used animals actively sensitized by repeated antigen exposure to mimic the clinical situation seen in allergic patients. Our previous study showed that nasal hyper-responsiveness in guinea pigs was obtained after sensitization and challenge with OVA, and a significant nasal obstruction was observed by stimulation with chemical mediators in sensitized animals (Yasui et al., 2008). It has been reported that nasal allergen challenge induced the recruitment of eosinophils around nerves in the nasal mucosa and augmented the neural reflex responses in allergic rhinitis patients (Thornton et al., 2013). Indeed, we confirmed that the number of inflammatory cells such as eosinophils in nasal mucous was significantly increased following final OVA challenge in sensitized guinea pigs (Takahashi et al., 2015). These results suggest that an allergen-sensitized guinea pig model would be useful in predicting the effects on nasal responses in humans and that infiltration of inflammatory cells might be necessary for PGD2-induced augmentation.

As mentioned above, Doyle et al. (1990) showed that PGD2 alone did not induce sneezing; however, they also showed in the same report that PGD2 alone could induce rhinorrhea in patients with allergic rhinitis. By contrast, neither rhinorrhea nor sneezing was induced by PGD2 alone in the present study. To understand the potential reason for this discrepancy, we assessed whether the unilateral instillation of PGD2 or histamine could induce nasal secretion in the ipsilateral nostril using this model. The unilateral instillation of PGD2 or histamine induced a significant increase in nasal discharge in the ipsilateral naris compared with the contralateral naris (data not shown). In a different perspective, PGD2 has been found to increase the vascular permeability in the conjunctiva of guinea pigs and in the skin of rats (Flower et al., 1976; Woodward et al., 1990). These findings suggest that PGD2-induced rhinorrhea in patients with allergic rhinitis would result from plasma exudation induced by increased vascular permeability.

TRG neurons innervate the nasal mucous membrane, and neuronal excitation contributes to sneezing, nasal itching, and, at least in part, hypersecretion (Sarin et al., 2006). In this study, our in vivo data suggest that PGD2 may act on TRG neurons to affect histamine-induced neuronal responses, but less is known about the involvement of PGD2 in TRG neurons. Accordingly, we assessed the electrophysiological properties of guinea pig TRG C-fiber neurons to investigate the effects of histamine and PGD2. The proportion of C-fiber neurons in the TRG that responded to histamine was ∼40%, which is consistent with other findings (Taylor-Clark et al., 2005b). BW245C did not induce depolarization or action potentials at doses up to 10 μM, indicating that BW245C alone did not elicit neuronal excitation in TRG neurons (data not shown).

BW245C increased neuronal excitation to histamine and current pulses.

Sneezing can be induced by the nasal instillation of histamine, but not of PGD2, in patients with allergic rhinitis (Doyle et al., 1990). By contrast, Rahman et al. (2007a) reported that PGD2 enhanced histamine-induced sneezing and nasal rubbing in rats. Similarly, we found in the present study that PGD2 alone did not induce sneezing or nasal rubbing but did augment histamine-induced symptoms in guinea pigs. Moreover, we showed that PGD2-induced augmentation was mediated via the DP1 receptor, as BW245C also induced augmentation, whereas asapiprant inhibited this augmentation.

It is well known that rhinorrhea is mainly driven by plasma leakage from nasal blood vessels and discharge from submucosal glands via the histamine-induced neural reflex (Konno et al., 1987). Several reports have indicated that nasal secretion on the contralateral side induced by various unilateral stimuli could be induced by nervous reflexes, whereas nasal secretion on the ipsilateral side could be mediated by both plasma leakage and neural reflexes (Namimatsu et al., 1992; Baroody et al., 1993). Herein, we demonstrated that

<TABLE 1>

<table>
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<tr>
<th>Group</th>
<th>Histamine Response</th>
<th>Membrane Potentials (mV)</th>
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<tr>
<td>Histamine</td>
<td>38.5</td>
<td>−57.4 ± 1.2</td>
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<tr>
<td>Histamine+BW245C</td>
<td>44.4</td>
<td>−58.0 ± 0.7</td>
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<tr>
<td>Asapiprant</td>
<td>38.7</td>
<td>−57.2 ± 1.2</td>
</tr>
<tr>
<td>H-89</td>
<td>37.5</td>
<td>−56.4 ± 1.0</td>
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*Represents means ± S.E.M.

<FIG 4. Effect of DP1 receptor signaling on histamine-induced neuronal excitation in TRG small-sized trigeminal ganglion neurons. Data show representative traces of neuronal responses to 10 μM histamine after treatment with buffer (A), 0.1 μM BW245C alone (B), 0.1 μM BW245C followed by 1 μM asapiprant (C), or 0.1 μM H-89 (D).>
Moreover, BW245C enhanced the neuroexcitability response to histamine, which was abrogated by pretreatment with asapiprant. Moreover, DP$_1$ receptor mRNA transcript and histamine H$_1$ receptor protein expression could be detected in TRG neurons. These results suggest that PGD$_2$–DP$_1$ receptor signaling potentiates neuronal excitation induced by histamine stimulation in TRG neurons. Therefore, augmentation of histamine-induced sneezing and rhinorrhea by DP$_1$ receptor activation may be mediated by the augmentation of neuronal excitation, as observed in the TRG.

It has recently been reported that CRTH2 signaling can inhibit sensory nerve (Maher et al., 2002, 2013). We examined the effect of CRTH2 signaling on histamine-induced nasal symptoms and neuronal activation. Contrary to our expectations, 13,14-dihydro-15-keto (DK)-PGD$_2$, a CRTH2 agonist, did not affect histamine-induced sneezing, nasal rubbing, and neuronal activation in TRG (data not shown). It is possible that there is no or weak expression of CRTH2 in guinea pig TRG neuron, and further studies are needed to examine the CRTH2 expression.

The DP$_1$ receptor is coupled to Gs-protein subunits. Its activation can induce intracellular cAMP accumulation, which results in the activation of PKA. This signaling pathway is thought to initiate various biologic activities (Kohyama et al., 2002, 2013). Hence, we tested the effects of a PKA inhibitor, H-89, on BW245C-induced augmentation of neuronal responses to histamine. Strikingly, H-89 completely inhibited the augmentation effect, indicating that PKA signaling has a critical role in DP$_1$ receptor–induced augmentation of neuronal excitability.

We examined the effects of PGD$_2$ on action potential thresholds in a current-pulse setting and found that the threshold for elicitation of action potential was reduced after perfusion with BW245C. These findings suggest that lowering the threshold by PGD$_2$–DP$_1$ receptor signaling is a mechanism whereby augmentation of histamine-induced neuronal excitation in small-diameter TRG neurons occurs. However, the more detailed mechanisms whereby PGD$_2$ could induce neuronal hypersensitivity remain unclear. Ebersberger et al. (2011) reported that PGD$_2$ could shift conductance depending on the Nav1.9 channels, and BW245C could enhance the amplitude of tetrodotoxin-resistant (TTX-R) Na$^+$ current in rat dorsal root ganglion neurons. Additionally, they demonstrated that BW245C reduced the threshold for elicitation of action potentials that could be induced by a current pulse. Moreover, it has been reported that cAMP–PKA activation plays an important role in increasing TTX-R Na$^+$ current in rat dorsal root ganglion neurons (England et al., 1996). Therefore, it is possible that PKA activation in response to PGD$_2$–DP$_1$ receptor signaling can modulate Na$^+$ channels to elicit hypersensitivity in TRG neurons. On the other hand, H-89 inhibits several other kinases other than PKA, and it cannot be denied that another kinase could be involved in DP$_1$ receptor signaling. Further studies will be needed to confirm
the involvement of PKA with other specific PKA inhibitors, and to identify the detailed mechanisms that are involved.

Rhinitis symptoms that are produced by neural reflexes have been attributed to neural activation by various stimuli, including cold air (Philip et al., 1993), hyperosmolar saline (Sanico et al., 1999a), and chemical mediators such as histamine (Sanico et al., 1999b) and bradykinin (Riccio and Proud, 1996). In the present study, BW245C caused both the enhancement of histamine-induced neuronal excitation and a reduction in the action potential threshold induced by current pulses administered as a nonspecific irritant. This augmentation of neuroexcitability to nonspecific stimulation indicates that DP1 signaling as well as histamine may potentiate neural activity in response to various stimuli, resulting in the augmentation of symptoms in patients with allergic rhinitis.

Our findings suggest that DP1 receptor antagonist is beneficial for the treatment of rhinitis symptoms. On the other hand, cyclo-oxygenase (COX) inhibitor, which inhibits PGD2 production, is not effective in allergic rhinitis patients. It is well known that COX inhibitor elicits cysteinyl leukotriene production, and nasal provocation by leukotriene-D4 induced nasal obstruction and nasal secretion (Okuda et al., 1988) in allergic rhinitis patients, and thus it is thought that leukotriene-D4 production is the leading cause of the ineffectiveness of COX inhibitor.

In conclusion, for the first time we have demonstrated that PGD2–DP1 receptor signaling modulates neuronal excitation induced by various stimuli by reducing the action potential threshold, resulting in the augmentation of histamine-induced rhinitis symptoms in guinea pigs. These findings suggest that DP1 receptor antagonists have therapeutic potential in allergic rhinitis by acting via the nervous system. When combined with an antihistamine drug such as a histamine H1 receptor antagonist, it could more strongly ameliorate these symptoms.

Authorship Contributions

Conducted experiments: Nagira, Goto, Aoki.

Contributed new reagents or analytic tools: Nagira, Goto.

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References


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