

Participation of Prostaglandin E₂ Receptor in Nasal Congestion of Brown Norway Rats

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The aim of the present study was to clarify the involvement of prostaglandin E₂ (PGE₂) in nasal congestion in Brown Norway (BN) rats. For this purpose, we studied the effects of PGE₂ receptor (EP₁, EP₂, EP₃ and EP₄) agonists on nasal congestion and sneezing induced by toluene 2,4-diisocyanate (TDI). Enhanced pause (Penh) was increased 1 h (early phase) and 4 h (late phase) after TDI challenge. Sulprostone (an EP₃ receptor agonist) inhibited the increase of Penh, an index of nasal congestion, in both early and late phase responses. On the other hand, PGE₁ alcohol (an EP₄ agonist) increased Penh in the early phase response. Moreover, sulprostone inhibited sneezing, an immediate response by TDI challenge. These results indicate that EP₃ receptor is responsible for the relief of nasal congestion in both early and late phase responses, and EP₄ receptor is correlated with the development of nasal congestion in the early phase response. In addition, EP₃ receptor also participates in sneezing in allergic rhinitis induced by TDI challenge in BN rats.

Keywords nasal congestion; enhanced pause; prostaglandin E₂; toluene 2,4-diisocyanate; Brown Norway rat

It is well known that histamine, cysteinyl leukotriene (cys-LTs), thromboxanes, prostaglandins (PGs) and cytokines are responsible for allergic rhinitis.^{1,2)} There are two phases of allergic rhinitis response, named “early phase” and “late phase.” Early phase response occurs within minutes of exposure to the allergen and is characterized by sneezing, rhinorrhea and nasal congestion. Late phase response occurs 4 to 8 h after allergen exposure and is characterized mainly by nasal congestion.³⁾

Histamine H₁ receptor antagonists, such as chlorpheniramine and astemizol, are widely used to treat allergic rhinitis clinically,^{4,5)} however, these H₁ receptor antagonists are not completely effective for nasal congestion.^{6–8)} In an animal model, we have reported that although H₁ receptor antagonists inhibited the increase of enhanced pause (Penh) in early phase induced by toluene 2,4-diisocyanate (TDI) challenge,⁹⁾ a relatively high dose was needed. On the other hand, we reported that cys-LTs receptor antagonists (pranlukast, zafirlukast) and thromboxane A₂ (TXA₂) receptor antagonists (seratrodast, ramatroban) also caused an inhibitory effect on the increase of Penh in early and late phases after TDI challenge in sensitized rats.¹⁰⁾ Therefore, it seems likely that different mediator is involved in early and late phase responses in nasal congestion.

Prostaglandin E₂ (PGE₂) is arachidonic acid metabolite similar to LTs and TXA₂, and there are four different receptors designated EP₁, EP₂, EP₃ and EP₄. Kunikata *et al.*¹⁰⁾ reported that mice lacking EP₃ receptor developed potent allergic inflammation compared with wild-type mice or mice deficient in other prostaglandin E₂ receptor subtypes. On the other hand, Gomi *et al.*¹¹⁾ described that PGE₂ selectively enhanced the immunoglobulin E (IgE)-mediated production of interleukin-6 (IL-6). These actions may produce by the receptors other than EP₃ receptor.

In a previous study, we established a nasal congestion model using Brown Norway (BN) rats by intranasal immunization with toluene 2,4-diisocyanate (TDI) as an antigen.⁸⁾

In this model, the animals showed not only sneezing but also nasal congestion in the early and late phases.

In the present study, in order to clarify the role of PGE₂ receptor (EP₁, EP₂, EP₃ and EP₄) in the development of allergic rhinitis symptoms, we investigated the effects of each PGE₂ receptor agonists with a nasal congestion model in BN rats.

MATERIALS AND METHODS

Animals Six-week-old male BN rats were obtained from Kyudo Co., Ltd. (Saga, Japan). The animals were housed in an air-conditioned room, maintained at 24±2 °C, with relative humidity of 55±10%. The rats were given standard laboratory rodent food (Oriental Yeast, Tokyo, Japan) and water *ad libitum*. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Materials The following reagents were obtained from the sources shown in parentheses: TDI (Wako, Tokyo, Japan), ethyl acetate (Wako), 17-phenyl trinor PGE₂ (9-oxo-11 α , 15S-dihydroxy-17-phenyl-18,19,20-trinor-prosta-5Z,13E-dien-1-oic acid; Cayman Chemical Co., Ann Arbor, MI, U.S.A.), butaprost (9-oxo-11 α ,16S-dihydroxy-17-cyclobutyl-prost-13E-en-1-oic acid, methyl ester; Cayman Chemical Co.), sulprostone (*N*-(methylsulfonyl)-9-oxo-11 α ,15R-dihydroxy-16-pheoxy-17,18,19,20-tetranor-prosta-5Z,13E-dien-1-amide; Cayman Chemical Co.) and PGE₁ alcohol (1,11 α ,15S-trihydroxy-prost-13E-en-9-one; Cayman Chemical Co.). TDI was dissolved in ethyl acetate to a concentration of 10% for sensitization and to a concentration of 5% for challenge. 17-Phenyl trinor PGE₂, butaprost, sulprostone and PGE₁ alcohol were dissolved in saline and administered intravenously 10 min before antigen application.

Sensitization and Challenge Procedures Sensitization and challenge were performed according to the method described by Tsumuro *et al.*⁹⁾ In brief, rats were sensitized by intranasal application of 10% TDI solution in ethyl acetate

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once a day for 5 consecutive days. Two days later, the rats were sensitized with TDI again for 5 d. One week after completion of the bilateral sensitization, 5% TDI solution in ethyl acetate was applied to the nasal cavities to induce nasal allergy-like symptoms.

Evaluation of TDI-Induced Nasal Congestion and Sneezing Before the experiment, the rats were placed into the animal chamber for 10 min for acclimatization. Nasal congestion was measured in freely moving rats for 6 h after nasal application of 5% TDI solution (5 μ l/site) using whole body plethysmography-free moving application (Buxco, Biosystem XA for Windows SFT3410 version 2.7 r4). The Penh was used as an index of nasal congestion. Changes in Penh were expressed as an average over 1 h. The increase in Penh 1 and 4 h after TDI challenge expressed “early phase” and “late phase,” respectively. Sneezing was measured in the same animal for 30 min after antigen application in the same method described by Kishi *et al.*⁸⁾

Effects of PGE₂ Receptor Agonists on Nasal Congestion and Sneezing In this study, 17-phenyl trinor PGE₂ (an EP₁ agonist), butaprost (an EP₂ agonist), sulprostone (an EP₃ agonist) and PGE₁ alcohol (an EP₄ agonist) were administered intravenously 10 min before challenge with 5% TDI at doses of 0.1, 0.3 and 1 mg/kg. In the control group, saline was given instead of PGE₂ receptor agonists. Eight rats were used for each group. Drug tests were repeated 3 times in the same animals, and PGE₂ receptor agonists were administered at an interval of 7 d.

Statistical Analysis All experimental data are shown as the means $\pm$ S.E.M. Statistical analysis was performed using Dunnett's test with a control group and drug-treated groups. A probability value of less than 0.05 was considered significant.

RESULTS

Changes in Penh in TDI Challenge As shown in Fig. 1, in TDI-sensitized rats, Penh increased significantly after TDI challenge for successive 6 h. A time course study showed peaks at 1 and 4 h after the TDI challenge; however, no observable effects were noted in non-sensitized rats.

Effects of PGE₂ Receptor Agonists on TDI-Induced Increase in Penh The effects of PGE₂ receptor agonists on Penh in early phase are shown in Fig. 2. 17-Phenyl trinor

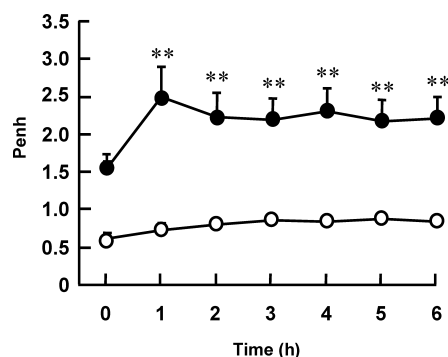


Fig. 1. Comparison of Penh for 6 h between Non-sensitized and Sensitized Rats

○: non-sensitized rats, ●: sensitized rats. Time 0 means the value before sensitization measured as average over 10 min. Each value is the means $\pm$ S.E.M. of 8 experiments. ** Significantly different from non-sensitized rats with $p < 0.01$.

PGE₂ (an EP₁ agonist) and butaprost (an EP₂ agonist) caused no significant effects on Penh even at a dose of 1 mg/kg; however, sulprostone (an EP₃ agonist) caused a dose-related inhibition of Penh, and a significant effect was observed at a dose of 1 mg/kg. On the other hand, PGE₁ alcohol (an EP₄ agonist) caused a dose-related increase of Penh.

The effects of PGE₂ receptor agonists on Penh in late phase are shown in Fig. 3. Sulprostone showed a dose-related inhibition of Penh, and a significant effect was observed at a dose of 1 mg/kg, whereas 17-phenyl trinor PGE₂, butaprost and PGE₁ alcohol caused no significant effects on Penh even at a dose of 1 mg/kg.

Effects of PGE₂ Receptor Agonists on TDI-Induced Increase in Sneezing The effects of PGE₂ receptor agonists on sneezing after TDI challenge are shown in Table 1. 17-Phenyl trinor PGE₂, butaprost and PGE₁ alcohol caused no significant effects on sneezing, even at a dose of 1 mg/kg; however, sulprostone caused a dose-related inhibition of sneezing, and significant effects were observed at doses of 0.1, 0.3 and 1 mg/kg.

DISCUSSION

In the present study, Penh in sensitized rats was signifi-

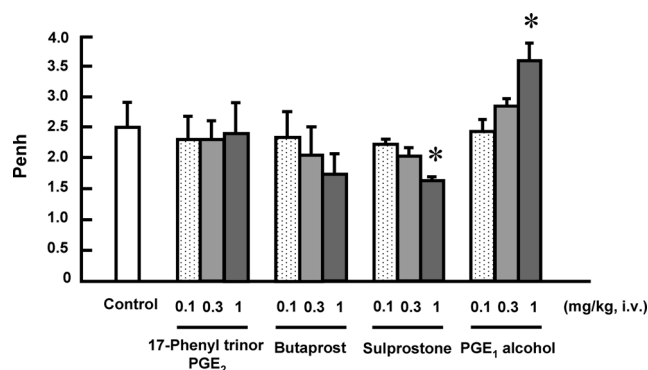


Fig. 2. Effects of PGE₂ Receptor Agonists on the Increase of Penh in the Early Phase after TDI Challenge in Sensitized Rats

17-Phenyl trinor PGE₂, butaprost, sulprostone and PGE₁ alcohol were administered intravenously 10 min before antigen application. The increase in Penh at 1 h after TDI challenge expressed as early phase. Each column and vertical bar shows means $\pm$ S.E.M. of 8 experiments. * Significantly different from control group with $p < 0.05$.

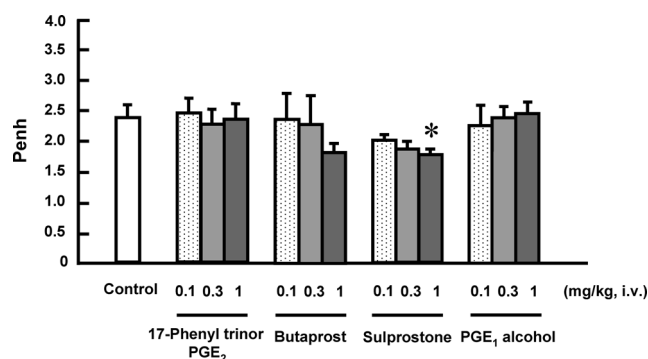


Fig. 3. Effects of PGE₂ Receptor Agonists on the Increase of Penh in the Late Phase after TDI Challenge in Sensitized Rats

17-Phenyl trinor PGE₂, butaprost, sulprostone and PGE₁ alcohol were administered intravenously 10 min before antigen application. The increase in Penh at 4 h after TDI challenge expressed as late phase. Each column and vertical bar shows means $\pm$ S.E.M. of 8 experiments. * Significantly different from control group with $p < 0.05$.

Table 1. Effects of PGE₂ Agonists on Sneezing Induced by TDI in Sensitized Rats

Drugs	Dose (mg/kg, i.v.)	Sneezing
Control	—	48.1±3.7
17-Phenyl trinor PGE ₂	0.1	44.2±4.5
	0.3	42.1±4.3
	1	48.4±5.1
Butaprost	0.1	40.5±3.9
	0.3	37.9±3.9
	1	34.4±3.6
Sulprostone	0.1	35.8±2.4*
	0.3	27.3±1.5**
	1	21.5±2.1**
PGE ₁ alcohol	0.1	42.6±3.0
	0.3	41.1±3.2
	1	42.1±5.3

Drugs were administered intravenously 10 min before antigen instillation. Sneezing was measured for 30 min after TDI challenge. Values are means±S.E.M. of 8 experiments. *, ** Significantly different from control with $p<0.05$ and $p<0.01$, respectively.

cantly increased at 1 and 4 h after TDI challenge. Almost the identical findings were already reported by ours.^{8,9} In other words, an increase in Penh observed after TDI challenge was reconfirmed even in the present study. It was found that 17-phenyl trinor PGE₂ and butaprost had no significant effect on an increase of Penh in early phase after TDI challenge in sensitized rats. This means that both EP₁ and EP₂ receptors are not related with nasal congestion in early phase.

On the other hand, sulprostone inhibited Penh in the early phase, whereas PGE₁ alcohol caused an increase of Penh. These findings indicate that Penh in the early phase was inhibited by EP₃ receptor and augmented by EP₄ receptor. In addition, sulprostone showed an inhibition of the Penh increase in the late phase after TDI challenge in sensitized rats. Sneezing was also inhibited by sulprostone.

Chung¹² reported that in a mouse model of allergic inflammation in which 4 different receptor subtypes (EP₁, EP₂, EP₃ and EP₄) were individually deleted, only EP₃ receptor-deficient mice showed an enhancement of inflammation, whereas EP₃ agonist was inhibitory, with PGE₂ being inactive. Kunikata *et al.*¹⁰ also described that mice lacking EP₃ receptor developed allergic inflammation that was much more pronounced than in wild-type mice or mice deficient in other PGE receptor subtypes. On the other hand, Matsuoka and Narumiya¹³ also described that EP₃ signaling negatively modulates allergic inflammation in both early and late phase reactions from the findings that EP₃ signaling suppresses mast cell degranulation and the expression of chemokines in airway epithelial cells. In addition, EP₃ receptor agonist suppressed adenylate cyclase leading to an inhibition of intracellular cAMP through inhibitory G (Gi)-protein.¹⁴ EP₃ receptor plays a role in vasoconstriction. A potent vasoconstrictor, naphazoline, showed inhibition of cAMP accumulation¹⁵ and

suppressed nasal congestion induced by TXA₂ analog and leukotriene D₄ (LTD₄).¹⁶ On the other hand, vasodilation is mediated by EP₄ receptor coupled to an increase of cAMP.¹⁷

As shown in the text, EP₄ agonist PGE₁ alcohol caused an augmentation of the increase of Penh without causing sneezing in the early phase after TDI challenge. Kabashima *et al.*¹⁸ described that EP₄ signaling has an important role in the initiation of antigen-specific immune responses in the skin. In addition, there is a report in which a selective EP₄ antagonist, CJ-023, 423, had significant inhibitory effects on paw swelling, inflammatory biomarkers, synovial inflammation and bone destruction in rats with adjuvant-induced arthritis.¹⁹ It was thought at the time, therefore, that an increase of Penh observed only in the early phase occurs by activation of EP₄ receptor.

In conclusion, the findings of this study clearly indicate that EP₃ receptor is responsible for the suppression of nasal congestion in both early and late phase responses. In addition, EP₃ receptor is involved in not only nasal congestion but also sneezing induced by TDI challenge in BN rats.

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