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Involvement of K^+ channels in the augmented nasal venous responsiveness to nitric oxide in rat model of allergic rhinitis

Hiroyasu Sakai *, Jun Enzaka, Michiko Sakai-Oshita, Futa Uto, Yoshihiko Chiba, Miwa Misawa

Department of Pharmacology, School of Pharmacy, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

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ABSTRACT

Objective: One of the factors of nasal obstruction observed in allergic rhinitis is thought to be a dilatation of microveins in nasal mucosa, although the exact mechanism(s) is not fully understood. In nasal mucosae of repeatedly antigen challenged rats, NO-induced venodilatation itself is augmented. In the present study, the roles of K^+ channels in sodium nitroprusside (NO donor; SNP)-induced venodilatation of nasal mucosae in antigen-challenged rats were investigated.

Methods: Actively sensitized rats were repeatedly challenged with aerosolized antigen. Twenty-four hours after the final antigen challenge, nasal septum mucosa was exposed surgically and observed directly *in vivo* under a stereoscopic microscope. The 20 μl reagents were administered onto the exposed septal mucosal surface, and the venous diameters of nasal mucosa were observed.

Results: The SNP-induced venodilatation of septal mucosa was markedly and significantly increased in the antigen-challenged rats. The SNP-induced venodilatation was significantly inhibited by pretreatment with either tetraethylammonium [TEA; a large-conductance Ca^{2+} activated-K^+ (K_{Ca}) and voltage dependent K^+ (Kv) channel inhibitor] or glibenclamide [an ATP sensitive K^+ (K_{ATP}) channel inhibitor].

Conclusions: These findings suggest that NO-induced venodilatation is augmented in nasal mucosae of challenged rats, and K^+ channels play an important role in the augmented venous responsiveness to NO in nasal mucosae of repeatedly antigen challenged rats.

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Introduction

Allergic rhinitis is a common inflammatory disease with an increasing worldwide prevalence. Its major nasal symptoms, nasal blockage, sneezing, rhinorrhea and pruritus are thought to be induced by several chemical mediators released from mast cells and other inflammatory cells after provocation of relevant allergen (Bousquet et al., 1996; Naclerio and Baroody, 1994; Naclerio et al., 1983). In addition to the specific antigen-induced nasal symptoms, the state of nasal hyperresponsiveness to non-specific stimuli other than the relevant antigen is one of the characteristic features of patients with allergic rhinitis. Nitric oxide (NO) is released following the antigen–antibody reaction (Miadonna et al., 1999; Shaw et al., 1985), and has an ability to induce nasal obstruction in a guinea pig model of allergic rhinitis (Mizutani et al., 2001). One of the functions of NO is the relaxation of vascular smooth muscle cells, regulating blood flow through vessels (Bredt and Snyder, 1994; Moncada et al., 1991). Thus, NO also plays a crucial role in nasal obstruction in allergic rhinitis.

It is now well established that the endothelium plays a critical role in the control of peripheral vascular resistance and blood pressure via the release of NO. The NO is thought to be responsible for endothelium-dependent vascular smooth muscle relaxation in many vessels. Nitrovasodilators, including sodium nitroprusside (SNP), release NO and increase cGMP levels via activation of soluble guanylate cyclase (sGC). As a consequence, cGMP-dependent protein kinase (PKG) activity is enhanced causing modulation (phosphorylation) of various intracellular proteins and vascular relaxation (Waldman and Murad, 1987). Electrophysiological and pharmacomechanical studies in a variety of vascular tissues have pointed out to a pivotal role of plasmalemmal K^+ channels in PKG-induced relaxation. NO also involves hyperpolarization due to the activation of smooth muscle K^+ channels (Brayden, 2002; Chen and Cheung, 1997; Plane and Garland, 1996; Tare et al., 1990). NO can activate one or more of at least three different types of smooth muscle K^+ channels, including large conductance Ca^{2+}-activated potassium channels (BK_{Ca}), ATP-sensitive potassium channels (K_{ATP}), and voltage-gated potassium (Kv) channels in vessels (Waldron and Cole, 1999).

In the present study, to determine the roles of K^+ channels in the augmentation of responsiveness to NO observed in nasal mucosae of repeatedly antigen challenged rats, the effects of K^+ channels inhibitors on the SNP-induced venodilatation in nasal septal mucosa were examined.

* Corresponding author. Fax: +81 3 5498 5787.
E-mail address: sakai@hoshi.ac.jp (H. Sakai).

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Materials and methods

Animals and antigen sensitization

Male Wistar rats (6 weeks of age, specific pathogen-free, 170–190 g; Charles River Japan, Inc.) were used. All experiments were approved by the Animal Care Committee at Hoshi University (Tokyo, Japan). Rats were sensitized and repeatedly challenged with 2,4-dinitrophenylated *Ascaris suum* antigen (DNP-*Asc*) by the method described in the previous paper (Chiba et al., 2008; Misawa and Chiba, 1993). In brief, the rats were sensitized with DNP-*Asc* together with *Bordetella pertussis*, and were boosted 5 days later. Eight days after the first immunization, the rats were challenged by inhaling DNP-*Asc* for 40 min under conscious state. The animals were subjected to a total of three antigen-challenges, spaced 48-h apart. The sensitized-control animals received the same immunization procedure but inhaled saline aerosol instead of antigen challenge.

Measurement of changes in diameter of nasal mucosal veins

Venous diameter of nasal septal mucosae in anesthetized rats was measured by the method of previous paper (Chiba et al., 2006, 2007). In brief, the rats were anesthetized with urethane (2 g/kg, *i.p.*) and nasal mucosa was exposed surgically. Then the nasal septal mucosa was observed under a stereoscopic microscope (SZX9; Olympus Optical Co., Ltd., Tokyo, Japan) at a magnification of 40×. A digital camera (Camedia C-4040 Zoom; Olympus Optical Co., Ltd., Tokyo, Japan) was equipped with the microscope to obtain photographs of nasal septum blood vessels. After an equilibration period, a photograph was taken for measurement of baseline venous diameter (50–80 μm) and then pre-warmed (37 °C) 20 μl of 10^{-4} M sodium nitroprusside (SNP) was applied to the exposed mucosal surface of nasal septum directly. Vehicle of SNP was used saline. After the application of the agent, the nasal mucosa was photographed continuously at 5-min interval for 60 min to examine the time-course change in the diameter of veins. In some experiments, pre-warmed (37 °C) 20 μl of 10^{-8} M tetraethylammonium (TEA) or 10^{-5} M glibenclamide was pretreated to the exposed mucosal surface 20 min before the application of SNP. The vehicles of TEA and glibenclamide used were saline and dimethyl sulfoxide (DMSO), respectively. The diameter of veins was measured using Image-Pro Plus (Media Cybernetics, San Diego, CA, USA) application with a computer and expressed as percentage of the baseline venous diameter. Three randomly selected veins were analyzed in each animal and the average was determined as $N=1$. In our preliminary study, venous diameter of septal mucosae at basal state was not changed by 20 μl of saline or DMSO for 60 min.

Reverse transcriptase chain reaction (RT-PCR)

The isolated nasal septa tissues were quickly frozen with liquid nitrogen, and these tissues were crushed to pieces by Cryopress™ (CP-100W; Niti-on, Co. Ltd., Japan: 15 s×3). Total RNA was isolated from each frozen tissue powder by using TRI reagent (Sigma-Aldrich) and stored at −85 °C until use. cDNAs were prepared from the total RNA (1 μg) by reverse transcriptase reaction in a total volume of 40 μl reaction buffer containing 50 mM Tris–HCl, pH 8.3, 4 mM $MgCl_2$, 2 mM dNTP mixture, 1 U/μl RNase inhibitor, 0.01 mg/ml random hexamers, and 5 U/μl avian M-MuLV reverse transcriptase. The reaction mixture was incubated for 5 min at 25 °C, and then for 60 min at 42 °C to initiate the synthesis of the cDNAs. Reverse transcriptase was inactivated at 70 °C for 5 min. Then to the RT reaction mixture (10 μl) was added 1 μl of 10 μM forward primer, 1 μl of 10 μM reverse primer, 5 μl of 2× PCR Master Mix (0.05 U/ml Taq DNA polymerase, 4 mM $MgCl_2$ and 0.4 mM of each dNTP; Fermentas Life Science) in a total volume of 20 μl. RT-generated cDNAs encoding the gene sequences were amplified by PCR using specific primers (Table 1), which were designed from published sequences. The thermal cycle profile used in the present study was 1) denaturing for 3 min at 95 °C, 2) annealing primers for 15 s at 55 °C, and 3) extending the primers for 1 min at 72 °C. The PCR amplifications were performed for 30 or 35 cycles. The PCR mixtures were subjected to electrophoresis on 1% agarose gel with DNA molecular weight standard marker (100 bp DNA ladder, Takara Bio Inc.) and visualized by ethidium bromide staining.

Statistical analyses

Statistical significance of difference was determined by one- or two-way analysis of variance (ANOVA) and Bonferroni/Dunn's *post hoc*-test. A value of $P<0.05$ was considered significant.

Results and discussion

The nasal obstruction is one of the characteristics of allergic rhinitis. In patients with seasonal allergic rhinitis, nasal obstructive symptom has been induced by nasal allergen challenge (Wang et al., 1997). One possible mechanism of the nasal obstruction is thought to be a dilatation of veins in nasal mucosa, although the exact mechanism(s) is not fully understood.

We have previously demonstrated that the increase in venous diameters induced by leukotriene D_4 (LTD_4) in antigen-challenged rats was blocked by pretreatment with L-NMMA, an NO synthase inhibitor (Chiba et al., 2007). The results indicate an involvement of NO in the venodilatation induced by LTD_4 in challenged rats. NO is known as a potent vasodilatator. In nasal airways, expression of constitutive (cNOS) and inducible NO synthases (iNOS) has been demonstrated in normal subjects and animals (Chiba et al., 2006; Kawamoto et al., 1998, 1999; Oh et al., 2003). Immunohistochemical studies also demonstrated the localization of constitutive NO synthases in endothelial and epithelial cells in nasal mucosa (Kawamoto et al., 1998, 1999).

The venous constriction induced by administration of vasoconstrictor, phenylephrine (10^{-3} M), was examined. The phenylephrine-induced

Table 1
PCR primers for K^+ channels and GAPDH.

	Accession number	Primer Deoxyribonucleotide sequences	Product size (base pairs)
Kv2.1	NM_013186	Forward 5′-TTCCTCACAGAATCCAACAAGAGC-3′ Reverse 5′-GGGTAGATGTCTCCATAGCCAACAG-3′	323
Kv2.2	NM_054000	Forward 5′-ATGTAAAGAAGGGAGGAGGAAAGC-3′ Reverse 5′-GGAAGAGAAAGTGTTGACCTGGATG-3′	365
Kir6.1	NM_017099	Forward 5′-GAGTGAACTGTCGCACCAGA-3′ Reverse 5′-CGATCACCAGAACTCAGCAAAC-3′	248
Kir6.2	NM_031358	Forward 5′-TCCAACAGCCCGCTCTAC-3′ Reverse 5′-GATGGGGACAAAACGCTG-3′	168
Slo	NM_031828	Forward 5′-GGACTTAGGGGATGGTGGTT-3′ Reverse 5′-GGGATGGAGTGGACAGAGGA-3′	297
GAPDH	NM_017008	Forward 5′-CCATCACTGCCACTCAGAAGAC-3′ Reverse 5′-TACTCCTTGGAGGCCATGTAGG-3′	469

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