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Brief Communications

Elevated guanylate cyclase and cyclic-guanosine monophosphate-dependent protein kinase levels in nasal mucosae of antigen-challenged rats

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ABSTRACT

Objective: In patients with severe allergic rhinitis, the most serious symptom is rhinostenosis, which is considered to be induced by a dilatation of plexus cavernosum. The vascular relaxing responses to chemical mediators are mainly mediated by the production of nitric oxide (NO). However, the exact mechanism(s) in nasal venoresponsiveness of allergic rhinitis is not fully understood. In the present study, we investigated the roles of soluble guanylate cyclase (sGC) and cyclic-guanosine monophosphate (c-GMP)-dependent protein kinase G (PKG) in venodilatation of nasal mucosae of antigen-challenged rats.
Methods: Actively sensitized rats were repeatedly challenged with aerosolized antigen (2,4-dinitrophenylated *Ascaris suum*). Twenty-four hours after the final antigen challenge, nasal septum mucosa was exposed surgically and observed directly in vivo under a stereoscopic microscope. The sodium nitroprusside (SNP) and 8-Br-cGMP (a PKG activator) were administered into arterial injection, and the venous diameters of nasal mucosa were observed.
Results: The intra-arterial injections of SNP and 8-Br-cGMP-induced venodilatation were significantly augmented in the nasal mucosae of repeatedly antigen-challenged rats. Furthermore, protein expressions of sGC and PKG were significantly increased in nasal mucosae of the antigen-challenged rats.
Conclusion: The present findings suggest the idea that the promoted cGMP/PKG pathway may be involved in the enhanced NO-induced venodilatation in nasal mucosae of antigen-challenged rats.

Introduction

Allergic rhinitis is a common inflammatory disease with an increasing worldwide prevalence. The nasal blockage, sneezing, rhinorrhea and pruritus are thought to be induced by several chemical mediators and inflammatory cytokines released from various 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 plays important and crucial roles in nasal obstruction in allergic rhinitis. 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 cyclic-guanosine monophosphate-dependent protein (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 resulting vascular relaxation (Waldman and Murad, 1987). In the present study, to determine the roles of sGC and PKG in the augmentation of responsiveness to NO observed in nasal mucosae of repeatedly antigen challenged rats, we investigated the effects of 8-Br-cGMP on the venoresponsiveness in nasal septal mucosa.

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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 of 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 area of nasal mucosal veins

Venous area of nasal septal mucosae in anesthetized rats was measured by the method of previous paper (Chiba et al., 2006, 2007; Sakai et al., 2010, 2011a). 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 area and then 30 μg sodium nitroprusside (SNP)/0.1 mL/animal or 300 μg8-Br-cGMP/0.1 mL/animal was administered into the external carotid artery (i.a.). After the application of the agent, the nasal mucosa was photographed continuously at 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 min to examine the time-course changes in the area of veins. In case of dose–response experiment, after the application of the 1 –100μg SNP/animal or 10–1000 μg 8-Br-cGMP/animal, the nasal mucosa was photographed continuously at 3 min to examine the dose–response changes in the diameter of veins. The area 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 area. The basal areas of vasculature in nasal mucosae were not statistically different between control and antigen-challenged rats (control: 45,962 ± 8894 pixels, antigen-challenged: 40,889 ± 9036 pixels).

Immunoblotting

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). The tissue was then homogenized in ice-cold T-PER™ Tissue Protein Extraction Reagent (Pierce). The tissue homogenate was centrifuged (1 000×*g*, 4 °C for 1 min), and supernatants were stored at −85 °C until use. To

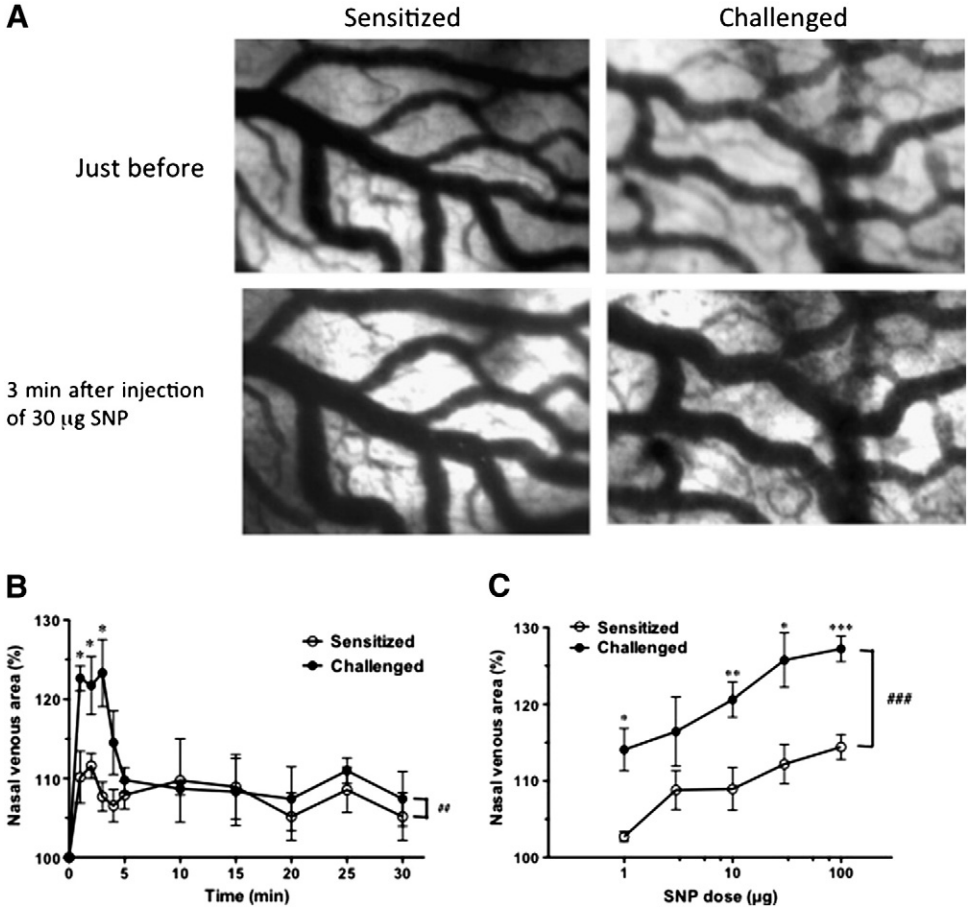

Fig. 1. Sodium nitroprusside (SNP)-induced venodilatation in nasal septum of sensitized control and challenged rats. A. Typical stereomicroscopic photos of veins of nasal septum just before (*upper* panels) and 3 min (*lower* panels) after intra-arterial (*i.a.*) injection of SNP (30 μg) into the external carotid artery in sensitized control (Sensitized; *left* panels) and challenged (Challenged; *right* panels) rats. B. Time course changes in the SNP (30 μg)-induced venodilatation in nasal septum of sensitized control and challenged rats. C. Dose–response curves of the SNP-induced venodilatation in sensitized control and challenged rats. Each point represents the mean with S.E. from 4 experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ or $^{***}p < 0.001$ and ##p < 0.01 or ###p < 0.001 (ANOVA) vs. sensitized.