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Inducible nitric oxide synthase evoked nitric oxide counteracts capsaicin-induced airway smooth muscle contraction, but exacerbates plasma extravasation

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Abstract

The contribution of nitric oxide (NO) to capsaicin-evoked airway responses was investigated in rats. The measurement of plasma NO level, airway dynamics, airway smooth muscle electromyogram, and plasma extravasation by India ink and Evans blue leakage technique was adapted. Capsaicin-evoked hypotension, bronchoconstriction, trachea plasma extravasation as well as increases in plasma NO level in a dose-dependent manner. L-732138 (NK_1 receptor antagonist) or SR-48968 (NK_2 receptor antagonist) pretreatment reduced capsaicin-enhanced hypotension, bronchoconstriction, plasma extravasation, and plasma NO level. N^G-nitro-L-Arginine methyl ester (L-NAME, 10 mg/kg, i.v.), a non-selective NO synthase (NOS) inhibitor, or aminoguanidine (10 mg/kg, i.v.), a selective inducible NOS (iNOS) inhibitor, reduced capsaicin-induced increases in plasma NO level and protected against capsaicin-induced plasma extravasation, whereas L-arginine (150 mg/kg, i.v.), a NO precursor, enhanced capsaicin-evoked plasma NO level and plasma extravasation. L-Arginine pretreatment ameliorated capsaicin-induced bronchoconstriction, whereas L-NAME and aminoguanidine exaggerated capsaicin-induced bronchoconstriction. In summary, NK_1 and NK_2 receptors and iNOS play a role in NO formation and on capsaicin-induced bronchoconstriction and plasma extravasation. NO generated by iNOS counteracts tachykinin-mediated bronchoconstriction, but exacerbates tachykinin-mediated plasma extravasation.

Keywords: Bronchoconstriction; Plasma extravasation; Nitric oxide; Tachykinins; Inducible nitric oxide synthase

Although autonomic nervous system mainly innervates the airway [2,28], non-adrenergic and non-cholinergic (NANC) nervous system is important in the regulation of various airway responses via tachykinins and nitric oxide (NO) [1,21,25,28,30]. Tachykinins are neuropeptides that are released from lung C-fiber nerve endings when stimulated and that are known to induce neurogenic airway responses including bronchoconstriction and airway plasma exudation [15,20,25,26]. NO synthesis is mediated by three different types of nitric oxide synthases (NOS): neuronal, endothelial, and inducible [27]. The first two synthases are expressed constitutively (cNOS), whereas inducible NOS (iNOS) must be induced [27]. Endogenous NO generated by different NOS has been reported to have different effects on the airways including bronchodilation and airway plasma exudation [1,3,4,7]. Likewise, exogenous NO has also been demonstrated to have conflict results in regulation of airway responses [14,27,31]. Tachykinins can induce the synthesis and release of NO via neurokinin type 1 (NK_1) and type 2 (NK_2) receptor activation [19,26]. Inhibition or activation of NK receptors affected NOS activity, NO amounts, and airway responses [7,14–16,29]. However, the role of NO in the C-fiber-mediated neurogenic airway responses is not completely understood and whether NO generated by cNOS and iNOS may have different involvements in the C-fiber-mediated neurogenic airway responses is not clear.

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Capsaicin acts on vanilloid type 1 receptor to stimulate lung C-fiber nerve endings and cause a release of tachykinins [2]. The released tachykinins subsequently may activate NK_1 receptor mediated vascular permeability and plasma protein extravasation in the airway [6,13] and activate NK_2 receptor provoked bronchoconstriction [5,6]. In the present study, we used capsaicin to stimulate these nerve endings, to cause the release of tachykinins, and to produce the resultant neurogenic airway responses. We used a non-selective and selective iNOS inhibitors to investigate the role of NO generated from different NOS in the modulation of capsaicin-induced airway responses. We also investigated the effects of increased production of NO, which arises from exogenous administration of L-arginine, on capsaicin-induced airway responses.

On the day of experiment, L-arginine ($C_6H_{14}N_4O_2$), L-N^G-nitro-arginine methyl ester (L-NAME) (Sigma Chemical Co., USA), aminoguanidine hydrochloride (Cayman Chemical, Ann Arbor, MI, USA) were dissolved in normal saline. Capsaicin (8-methyl-*N*-vanillyl-nonenamide; Sigma) was dissolved in 1% ethanol and 1% Tween 80 solution at the concentration of 300 nmol/ml. The non-peptide NK_1 receptor antagonist, L-732138 (*N*-acetyl-L-tryptophan-3,5-bistrifluoromethyl benzyl ester, Sigma), and non-peptide NK_2 receptor antagonist, SR-48968 {(*S*)-*N*-methyl-*N*-[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl] benzamide} (Sanofi Recherche, France) were dissolved in 10% dimethyl sulphoxide in 0.9% saline at the concentration of 1 μmol.

Male Sprague–Dawley rats (260–280 g) were purchased from National Laboratory Animal Center and were housed at the Experimental Animal Center, National Sun Yat-Sen University, at a constant temperature and with a consistent light cycle (light from 00:700 to 18:00 h). On the day of experiment, the rats were anesthetized with subcutaneous urethane (1.2 g/kg). The body temperature was kept at 36.5–37.0 °C by an infrared light and was monitored with a rectal thermometer. The study was conducted according to the *Guiding Principles in the Care and Use of Animals* of the American Physiological Society and was approved by the Animal Care and Use Committee of the National Sun Yat-Sen University. All efforts were made to minimize both animal suffering and the number of animals used throughout the experiment.

The trachea was cannulated caudal to the larynx (PE-200) and the animal breathed spontaneously through a Pneumotachometer (TSD 137C, Biopac Systems) connected to a flow transducer (TSD 160A, amplifier DA 100C, Biopac Systems) for monitoring airflow with a zero-flow method. Intratracheal pressure was measured by a pressure transducer (Model DP 103-24) connected to a manometer (Model CD-15-A-1-B-1, validyne). A fluid-filled PE-50 cannula was introduced into the esophagus to measure the esophageal pressure as an approximation of pleural pressure. The transpulmonary pressure (defined as the pressure difference between the intratracheal and the esophageal pressures) was measured with a manometer. Total pulmonary resistance (R_L) was calculated as previously described [12]. PE-50 catheters were placed in the left femoral artery for measurement of arterial blood pressure and in the left femoral vein for administration of test drugs. Arterial blood pressure was recorded on a polygraph (DA 100C, Biopac Systems, Inc., Goleta, CA, USA) with a transducer (TCI 100, Biopac Systems). Plasma NO concentration was examined with the NO chemiluminescent probe containing luminol 0.018 (Sigma), H_2O_2 10, desferrioxamine 0.15 (Sigma) and K_2CO_3 2 mmol/l (Sigma) and detected by a Chemiluminescence Analyzing System (CLD-110, Tohoku Electronic Inc. Co., Sendai, Japan) [9,22]. Because capsaicin activates cholinergic reflexes [5], atropine (1 mol/kg) was administrated 15 min before drug challenge in all the animals. Ten minutes after vehicle, L-NAME (10 mg/kg), aminoguanidine (10 mg/kg), L-arginine (150 mg/kg), L-732138 (1 μmol/kg), or SR-48968 (1 μmol/kg) pretreatment ($n = 6$ each), the rats were received intravenous injection of capsaicin (0–100 nmol/kg).

For electromyogram recordings, epoxy-coated stainless steel wire (50 μm; M.T. Giken Co., Ltd, Tokyo, Japan) was placed into the inner layer of airway smooth muscle under the dissecting microscope (Nikon, Japan). The EMG electrodes were embedded into the smooth muscle ∼1–2 mm. EMG signals were connected to an EMG amplifier (EMG 100C, Biopac Systems) and recorded on the recording system [8].

For evaluation of plasma extravasation, 10 min after drug pretreatment, the rats were received intravenous injection of India ink (1 mg/kg, over 5 s, Chroma-Gesellschaft, Kongen, Germany) ($n = 4$) [24] or Evans blue dye (50 mg/kg, $n = 5$) [4] followed by capsaicin stimulation (100 nmol/kg). Five minutes after capsaicin, the animal was exsanguinated by infusion of 50 ml of 0.9% (w/v) saline, at 37 °C, into the left cardiac ventricle. The trachea of India-ink was analyzed with a microscope (Leica DMRD) and of Evans blue was determined with a spectrophotometer (Beckman Coulter DU 6408, USA) at the absorbance maximum of 620 nm wavelength after extraction in a known volume of formamide at 60 °C for 24 h. Plasma extravasation was expressed as the content of Evans blue dye in μg/g of tissue.

All the signals collected are stored in the IBM computer (ThinkPad R40) and analyzed with software (AcqKnowledge 3.7.3 Biopac System). The rate of rise of EMG activity was calculated by dividing peak integrated EMG activity by the time to peak, and expressed the result as a percentage of the maximal activity.

Values are mean ± standard error of mean. Differences in parameters among groups were analyzed with analysis of variance. Post hoc analyses were performed by means of the Newman–Keuls test. For all tests, differences were considered significant if $P < 0.05$.

In our experiments, the baseline level of arterial blood pressure was 102–109 mmHg. Atropine treatment did not significantly increase the arterial blood pressure in all the animals. Intravenous capsaicin (from 25 to 100 nmol/kg) evoked hypotension, apnea, or bradypnea (decreased respiratory frequency) in a dose-dependent manner (Fig. 1). The cardiovas-