Nedocromil Sodium Inhibits Bradykinin- and Antigen-induced Plasma Extravasation in Guinea Pig Airways

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ABSTRACT

This study was designed to investigate the inhibitory effect of Nedocromil sodium (NS) on bradykinin- and antigen-induced plasma extravasation in guinea pig trachea. Plasma extravasation was assessed by photometric measurement of extravasated Evans blue dye after formamide extraction. Pretreatment with NS (0.1, 1, and 10 mg/kg *i.v.*) significantly and dose-dependently reduced bradykinin (inhalation, 0.1 mM, 2 min)-induced plasma extravasation in the presence of neutral endopeptidase inhibitor, phosphoramidon (2.5 mg/kg *i.v.*), in the trachea. Pretreatment with NS (10 mg/kg *i.v.*) or disodium cromoglycate (DSCG; 10 mg/kg *i.v.*) significantly reduced antigen-induced (inhalation of ovalbumin; 5%, 2 min) plasma extravasation in the sensitized guinea pig trachea. Inhibition of the antigen-induced plasma extravasation in the trachea by NS (10 mg/kg *i.v.*) was significantly greater than the inhibition by DSCG (10 mg/kg *i.v.*) and similar to the inhibition by pretreatment with a combination of NS and FK224. Pretreatment with the combination of NS and antihistaminic drugs inhibited the antigen-induced plasma extravasation in the trachea more than the antihistaminic drugs alone. However, the inhibition by pretreatment with the combination of NS and antihistaminic drugs did not differ from the inhibition by NS alone. These findings suggest that NS inhibits antigen-induced plasma extravasation, histamine release by mast cells and tachykinin release from sensory nerves activated from bradykinin stimulation in sensitized guinea pig airway.

Asthma is a chronic inflammatory disease characterized by eosinophil and lymphocyte infiltration and epithelial shedding (10) in the airway. There is increasing evidence that the sensory nerves of the airway play a role in the asthmatic response. Nerve endings are exposed by the epithelial shedding associated with asthma, and they may become sensitized and activated by inflammatory mediators and release neuropeptides that

then spread and amplify the inflammatory process throughout the airway (3, 15).

Disodium cromoglycate (DSCG) was the first prophylactic non-steroidal drug licensed for the treatment of chronic asthma. It is a derivative of chromone-2-carboxylic acid and has a flexible hydrophilic structure with a bicyclic ring system. Nedocromil sodium (NS) is a new pyranoquinoline dicarboxylic acid derivative developed to manage reversible airflow obstruction. NS, but not DSCG, reduces antigen-induced plasma extravasation in guinea pig nasal mucosa (8). The combination of NS and the NK₁ receptor antagonist CP-99,994 did not augment the inhibition caused by NS alone (8). NS also reduces the

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early-phase reaction of the allergic response as effectively as DSCG in a guinea pig model of ocular anaphylaxis (6), and NS has been found to be at least one order of magnitude more potent than DSCG in suppressing histamine release from mast cells (9). Since NS alone had only a small effect on leakage directly induced by histamine and platelet activating factor (PAF), its effect is probably due to inhibition of mediator release from inflammatory cells (presumably mast cells) (2). NS may have effects on sensory nerve endings, which may be an important site of action in the reduction of both neurogenic inflammation in airways and the symptoms of asthma, including cough(2), although the mechanism of action is not clear.

The specific aim of the present study was three-fold: first, to determine whether NS inhibits bradykinin-induced plasma extravasation in guinea pig trachea and to compare it with DSCG; second, to determine whether NS inhibits antigen-induced plasma extravasation in sensitized guinea pig airway and to compare it with DSCG and the combination of NS and NK₁ and NK₂ receptor antagonist FK224, and third, to determine whether NS inhibits antigen-induced plasma extravasation in response to antigen in sensitized guinea pig airway and compare it with antihistaminic drugs and the combination of NS and antihistaminic drugs.

MATERIALS AND METHODS

Animals

Male Hartley guinea pigs (Japan SLC, Inc.), weighing 300–500 g at the time of housing, were used in this study. They were kept in a temperature controlled environment with standard laboratory food and water freely available.

Sensitization procedure

The animals were sensitized according to a protocol described previously (14) that consisted of injection of 70 mg ovalbumin (Grade V) in 1.5 mL 0.9% NaCl intraperitoneally twice with a one week interval between injections. The animals were studied two weeks after the second injection.

Experimental design

On the day of the experiment, the guinea pigs (300–500 g) were anesthetized with sodium

pentobarbital (50 mg/kg *i.p.*). A midline cervical incision was made to expose the larynx and upper trachea. The trachea was incised immediately below the larynx, and a cannula was inserted 4 mm into the trachea. The animals were then artificially ventilated at a frequency of 70 breaths/min with a tidal volume of 6 mL using a constant volume ventilator (Rodent Ventilator Model 683, Harvard Apparatus, Millis, MA, U.S.A.). Neutral endopeptidase inhibitor, phosphoramidon (2.5 mg/kg *i.v.*), dissolved in 0.9% NaCl was injected into the jugular vein over a 10 s period 5 min before the inhalation. We used Evans blue dye (3% solution in 0.9% NaCl) to measure plasma extravasation. Immediately after the injection of the dye (30 mg/kg *i.v.* over 5 s) in the jugular vein, bradykinin (0.1 mM) or ovalbumin (OVA; 5%) aerosol was delivered to animals for 2 min via the tracheal cannula, using an ultrasonic nebulizer (Pulmo-Sonic model 25, DeVilbiss Co., Somerset, PA; aerosol delivery rate 0.2 mL/min). The vehicle of bradykinin and OVA was 0.9% NaCl. Nedocromil sodium (NS; 0.1, 1 and 10 mg/kg *i.v.*), disodium cromoglycate (DSCG; 10 mg/kg *i.v.*) or vehicle was injected 5 min before the stimulus. The combination of NS (10 mg/kg *i.v.*) and FK224 (3.2 mg/kg *i.v.*) or vehicle was given 10 min before the stimulus. The H₁ and H₂ receptor antagonists pyrilamine (10 mg/kg *i.v.*) and cimetidine (10 mg/kg *i.v.*), respectively, or the combination of NS (10 mg/kg *i.v.*) and antihistaminic drugs (pyrilamine and cimetidine; 10 mg/kg *i.v.* each) were administered 30 min before the stimulus. NS, DSCG, pyrilamine, and cimetidine were dissolved in saline; FK224 was dissolved in dimethylsulfoxide (DMSO). The chest was opened 10 min after injection of the tracer, a cannula was inserted into the ascending aorta through the left ventricle, and the circulation was perfused for 3 min with a 10 mM phosphate buffer of pH 5 at a pressure of 120 mmHg. The trachea were then removed, opened longitudinally along the ventral midline, blotted on bibulous paper, and weighed. Tissue was incubated in 3 mL of formamide at 50°C for 18 h to extract the extravasated Evans blue dye. The results were compared with those obtained from animals not treated with NS or DSCG.

Measurement of plasma extravasation

Extravasation of the dye-labeled macromolecules was assessed by measuring the optical density of

the formamide extracts at a wavelength of 620 nm with a spectrophotometer (model 220A, Hitachi, Japan). The amount of Evans blue dye extravasated from the tissue, expressed in ng/mg of wet weight, was interpolated from a standard curve of Evans blue dye concentrations.

Drugs

Bradykinin and phosphoramidon were purchased from Peptide Institute Inc (Osaka, Japan). NS was kindly provided by Dr. A. Norris (Rhonne-Poulenc Rorer, PA, USA). FK224 and DSCG were supplied by Fujisawa Pharmaceutical Co. Ltd. (Tokyo, Japan). Ovalbumin, Evans blue dye, pyrilamine, and DMSO were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Cimetidien was purchased from SmithKline Beecham (Tokyo, Japan).

Statistical analysis

All data are expressed as means $\pm$ S.E.M. Statistical comparisons were performed using a one-way analysis of variance and Dunnett's test or bilateral unpaired Students *t*-test, when appropriate. In all cases, a *P* value of less than 0.05 was considered significant.

RESULTS

Effect of NS and DSCG on bradykinin-induced plasma extravasation

Extravasation of Evans blue dye in the guinea pig trachea after exposure to aerosolized vehicle of bradykinin in the presence of phosphoramidon (2.5 mg/kg, *i.v.*) was 33.3 ± 5.8 ng/mg ($n=5$; Fig. 1). Injection of the vehicle for bradykinin (0.9% NaCl) did not affect baseline plasma extravasation (data not shown). Inhalation of 0.1 mM bradykinin for 2 min in the presence of phosphoramidon (2.5 mg/kg, *i.v.*) significantly increased the extravasation of Evans blue dye in the trachea (97.9 ± 7.9 ng/mg, $n=5$; Fig. 1). Pretreatment with DSCG (10 mg/kg *i.v.*) 5 min before aerosol challenge significantly reduced the plasma extravasation in the trachea (60.8 ± 5.8 ng/mg, $n=5$; Fig. 1). Pretreatment with NS (0.1, 1, and 10 mg/kg *i.v.*) 5 min before aerosol challenge significantly and dose-dependently reduced the bradykinin-induced plasma extravasation in the trachea (60.5 ± 5.5 , 52.9 ± 3.1 , and 38.9 ± 3.4 ng/mg, $P<0.05$, $n=5$ each; Fig. 1). The inhi-

Fig. 1 Effect of nedocromil sodium (0.1, 1 and 10 mg/kg *i.v.*) or disodium cromoglycate (10 mg/kg *i.v.*) on Evans blue extravasation in guinea pig trachea induced by inhalation of bradykinin (0.1 mM, 2 min) in presence of phosphoramidon (2.5 mg/kg *i.v.*, 5 min before exposure). Plasma extravasation was evaluated by measuring the amount of Evans blue dye extravasated in the trachea after 10 min. Values are means $\pm$ S.E.M; $n=5$ per group. * $P<0.05$ versus control (Inhalation of bradykinin in presence of phosphoramidon) in trachea.

bition of bradykinin-induced plasma extravasation caused by NS (10 mg/kg *i.v.*) was similar to the baseline value, but the inhibition caused by DSCG (10 mg/kg *i.v.*) was less prominent as compared with the NS administration. The NS and DSCG vehicle had no effect on the bradykinin-induced plasma extravasation (data not shown).

Effect of NS, DSCG, and the combination of NS and FK224 on ovalbumin-induced plasma extravasation

The extravasation of Evans blue dye in the trachea guinea pig trachea after exposure to the aerosolized vehicle of ovalbumin in the presence of phosphoramidon (2.5 mg/kg, *i.v.*) was 23.1 ± 4.4 ng/mg ($n=5$; Fig. 2). Inhalation of 5% ovalbumin for 2 min in the presence of phosphoramidon (2.5 mg/kg *i.v.*) significantly increased the extravasation of the Evans blue dye in the trachea (119.5 ± 10.0 ng/mg, $n=5$; Fig. 2). Pretreatment with DSCG (10 mg/kg *i.v.*) 5 min before aerosol challenge significantly reduced the antigen-induced plasma extravasation in the trachea (90.0 ± 6.17 ng/mg, $n=5$; Fig. 2). Pretreat-

Fig. 2 Effect of nedocromil sodium (NS; 10 mg/kg *i.v.*), disodium cromoglycate (DSCG; 10 mg/kg *i.v.*) or the combination of NS (10 mg/kg *i.v.*) and NK₁ and NK₂ receptor antagonist FK224 (3.2 mg/kg *i.v.*) on Evans blue extravasation in guinea pig trachea induced by inhalation of ovalbumin (OVA; 5% for 2 min) in presence of phosphoramidon (2.5 mg/kg *i.v.*, 5 min before exposure). Plasma extravasation was evaluated by measuring the amount of Evans blue dye extravasated in the trachea after 10 min. Values are means $\pm$ S.E.M; *n* = 5 per group. **P* < 0.05, ***P* < 0.01 versus control (Inhalation of OVA in presence of phosphoramidon) in trachea.

ment with NS (10 mg/kg *i.v.*) 5 min before aerosol challenge more sharply inhibited the increase of antigen-induced plasma extravasation in the trachea (50.2 ± 3.48 ng/mg, *n* = 5; Fig. 2). The combination of NS (10 mg/kg *i.v.*) and FK224 (NK₁ and NK₂ receptor antagonist; 3.2 mg/kg *i.v.*) also reduced antigen-induced plasma extravasation in the trachea (42.6 ± 3.9 ng/mg, *n* = 5; Fig. 2), but the inhibition was similar to the inhibition by pretreatment with NS alone. The NS, DSCG, and FK224 vehicle did not affect OVA-induced plasma extravasation (data not shown).

Effect of NS, Antihistaminics, and the combination of NS and Antihistaminics on ovalbumin-induced plasma extravasation

The extravasation of Evans blue dye in the guinea pig trachea after exposure to aerosolized vehicle of OVA in the presence of phosphoramidon (2.5 mg/kg *i.v.*) was 22.0 ± 3.7 ng/mg (*n* = 5; Fig. 3). Inhalation of 5% OVA for 2 min in the presence of phosphoramidon (2.5 mg/kg *i.v.*) significantly increased the extravasation of the Evans blue dye in the trachea (121.9 ± 10.5 ng/mg, *n* = 5; Fig. 3).

Fig. 3 Effect of nedocromil sodium (NS; 10 mg/kg *i.v.*), H₁ and H₂ receptor antagonists pyrilamine (10 mg/kg *i.v.*) and cimetidine (10 mg/kg *i.v.*) or the combination of NS (10 mg/kg *i.v.*) and anti-histaminic drug (pyrilamine and cimetidine; 10 mg/kg *i.v.* each) on Evans blue extravasation in guinea pig trachea induced by inhalation of ovalbumin (5% for 2 min) in presence of phosphoramidon (2.5 mg/kg *i.v.*, 5 min before exposure). Plasma extravasation was evaluated by measuring the amount of Evans blue dye extravasated in the trachea after 10 min. Values are means $\pm$ S.E.M; *n* = 5 per group. **P* < 0.05, ***P* < 0.01 versus control (Inhalation of OVA in presence of phosphoramidon) in trachea.

Pretreatment with NS (10 mg/kg *i.v.*) 5 min before OVA aerosol challenge inhibited the plasma extravasation in the trachea (49.4 ± 4.9 ng/mg, *n* = 5; Fig. 3). Pretreatment with the combination of pyrilamine (10 mg/kg *i.v.*) and cimetidine (10 mg/kg *i.v.*) 30 min before OVA aerosol challenge also significantly reduced the plasma extravasation, but the inhibition was less prominent as compared with the NS administration. Lastly, pretreatment with the combination of NS (10 mg/kg *i.v.*) and antihistaminic drugs (pyrilamine and cimetidine; 10 mg/kg *i.v.* each) 30 min before OVA aerosol challenge inhibited the extravasation in the trachea (48.6 ± 2.9 ng/mg, *n* = 5; Fig. 3). The inhibition by pretreatment with the combination of NS (10 mg/kg *i.v.*) and antihistaminic drugs (pyrilamine and cimetidine; 10 mg/kg *i.v.* each) did not differ from the inhibition of antigen-induced plasma extravasation in the trachea by NS alone. The NS, pyrilamine, and cimetidine vehicle had no effect on OVA-induced plasma extravasation (data not shown).

DISCUSSION

Bradykinin may be particularly important in activating sensory nerves, and when inhaled by asthma patients, it induces marked dyspnea and coughing, similar to that seen in an asthma attack. Bradykinin is produced from plasma that exudes into the asthmatic airway lumen as a result of plasma extravasation (4). OVA-induced plasma extravasation in sensitized guinea pigs is significantly reduced by the bradykinin B_2 receptor antagonist HOE140 (5) or FR173657 (13). The combination of NK_1 receptor antagonist CP-96,345 and HOE140 does not potentiate the inhibition produced by CP-96,345 alone (5). These reports suggest that the sensory nerve activation that contributes to antigen-induced plasma extravasation is due to bradykinin release (5, 13).

In our experiment, pretreatment with NS (0.1, 1, and 10 mg/kg *i.v.*) 5 min before aerosol challenge significantly and dose-dependently reduced the bradykinin-induced plasma extravasation in the trachea. The inhibition of bradykinin-induced plasma extravasation in the guinea pig trachea by NS (10 mg/kg *i.v.*) was not significantly different from the baseline, but was significantly greater than the inhibition caused by DSCG (10 mg/kg *i.v.*). We further investigated whether NS inhibits plasma extravasation in response to antigen in sensitized guinea pig airway and compared it with DSCG and the combination of NS and the NK_1 and NK_2 receptor antagonist FK224. The inhibition by pretreatment with NS was also significantly greater than that caused by DSCG. The inhibition of antigen-induced plasma extravasation in the trachea by the combination of NS and the NK_1 and NK_2 receptor antagonist FK224 was reduced, but similar to the inhibition by pretreatment with NS alone. FK224 interacts with both NK_1 and NK_2 receptors and inhibits neurogenic inflammation completely (11, 12). These results suggest that NS completely inhibits the neurogenic inflammation activated by bradykinin during antigen-induced plasma extravasation in sensitized guinea pig trachea.

We also investigated whether NS inhibits plasma extravasation in response to antigen in sensitized guinea pig airway and compared it with antihistaminic drugs and the combination of NS and antihistaminic drugs. Some reports describe inhibition of antigen-induced plasma extravasation by NS alone or by antihistaminic drugs alone, but not by a combination of NS and

antihistaminic drugs (2, 7). In the present study, pretreatment with pyrilamine and cimetidine blocked only part of histamine significantly reduced the plasma extravasation in the trachea. Pretreatment with the combination of NS and antihistaminic drugs (pyrilamine and cimetidine) inhibited the extravasation in the trachea more than the antihistaminic drugs alone. However, the inhibition of antigen-induced plasma extravasation in the trachea by NS alone did not differ from the inhibition by pretreatment with the combination of NS and antihistaminic drugs. However, it has been reported that NS does not reduce histamine-induced plasma extravasation (2). Our findings suggest that NS inhibits antigen-induced plasma extravasation by reducing histamine release from inflammatory cells, not by blocking histamine receptors.

NS has been shown to be capable of inhibiting chloride ion flux in mast cells, epithelial cells, and neurons, and this action may explain how it prevents responses such as mast-cell degranulation, the effects of osmolarity challenges in the airways, and neuronal activation (1). NS was found to be at least one order of magnitude more potent than DSCG in suppressing histamine release from mast cells (9). Since NS alone had only a small effect on extravasation induced directly by histamine and PAF, its effect is probably due to inhibition of mediator release from inflammatory cells (presumably mast cells) (2). NS may have effects on sensory nerve endings, which may be an important site of action in the reduction of both neurogenic inflammation in the airways and the symptoms of asthma, including cough (2). We have shown that NS significantly reduces antigen-induced plasma extravasation in guinea pig airways. NS inhibits neurogenic inflammation activated by bradykinin, and its inhibitory effect is more potent than that of DSCG. These findings suggest that NS suppresses the histamine release from mast cell and the release of tachykinin from activated sensory nerves in response to bradykinin stimulation (Fig. 4).

If these findings are substantiated in humans, NS may prove to be a useful therapeutic agent compared with DSCG for the treatment of inflammatory disease such as asthma.

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Fig. 4 Schematic representation of anti-inflammatory action by nedocromil sodium (NS) in airway

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