Nedocromil Sodium Inhibits Hypertonic Saline-induced Plasma Extravasation in Guinea Pig Airways

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ABSTRACT

We investigated the potential of nedocromil sodium (NS) to reduce neurogenic plasma extravasation induced by hypertonic saline in guinea pig airways. Plasma extravasation was assessed by photometric measurement of extravasated Evans blue after formamide extraction. Hypertonic saline was delivered to anesthetized, artificially ventilated guinea pigs through a tracheal cannula. Inhalation of hypertonic saline for 2 min increased Evans blue dye extravasation in the trachea in a concentration-dependent (0.9, 5, 10, 15 and 20 %) manner. The increase in plasma extravasation induced by hypertonic saline (15 %) was significantly reduced by pretreatment with NS (10 mg/kg, *i. v.*). The inhibition of plasma extravasation by NS (10 mg/kg, *i. v.*) alone was not different from the inhibition induced by the combination of NS and the bradykinin B₂ receptor antagonist, HOE140 (0.1 μ mol/kg, *i. v.*) or HOE140 (0.1 μ mol/kg, *i. v.*) alone. However, pretreatment with the combination of NS (10 mg/kg, *i. v.*) and the tachykinin NK₁ and NK₂ receptor antagonist, FK224 (3.2 mg/kg, *i. v.*) abolished the increase in plasma extravasation induced by hypertonic saline (15 %). The increase in plasma extravasation induced by inhalation of bradykinin (0.1 mM) for 2 min was abolished by pretreatment with NS (10 mg/kg, *i. v.*). These findings suggest that NS reduced the hypertonic saline-induced plasma extravasation by inhibiting bradykinin-dependent neurogenic inflammation in guinea pig airways.

The inhalation of aerosols of hypertonic saline causes airways to narrow in the majority of patients with symptoms of asthma (20). The stimulus causing the airways to narrow is thought to be an increase in the osmolarity of the periciliary fluid, thus providing a favorable environment for mast cell release of histamine and other mediators. In most asthmatic subjects the airway response to hypertonic saline can be reduced, and in some cases inhibited, by medication with anti-histamines given either orally or by aerosol (19). In addition to mast cell release of mediators, the possibility exists that increases in osmolarity

cause narrowing of the airways by the release of tachykinins from sensory nerves. Although there is no evidence from humans to support this, recent studies in rats have demonstrated increases in vascular permeability directly related to changes in osmolarity that are prevented by treatment with capsaicin (21). Furthermore, recent studies of human lung tissue *in vitro* support a role for mast cell release of mediators and release of tachykinins in the presence of hyperosmolarity (8).

Nedocromil sodium (NS) is a disodium salt of a pyranoquinoline dicarboxylic acid (17). It inhibits the *in vitro* release of inflammatory mediators from sensitized human lung mast cells (11,12) and a range of inflammatory cells, including eosinophils, neutrophils, monocytes, macro-

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phages, and platelets (17). It is also effective in producing a dose-dependent inhibition of histamine release from mast cells obtained from lung fragments and bronchoalveolar lavage fluid in both normal and asthmatic subjects (11,12). When NS is inhaled by asthmatic subjects, it inhibits airway narrowing caused by antigens (22), cold air (5), distilled water aerosol (18), hypertonic saline (10), exercise (9), and substance P (4). These challenges are thought to provoke narrowing of the airways by the release of chemical mediators. In addition, NS is effective against bronchoconstriction induced by challenge with bradykinins (6) and tachykinins (4). Some of these challenges are thought to narrow the airways via a parasympathetic reflex, but neurogenic inflammation by the release of tachykinins from sensory nerves may also be important (1,2). As an increase in airway osmolarity is likely to involve both release of mediators from mast cells and neuropeptides from sensory nerves, it would be expected that NS would attenuate the airway inflammation to a hypertonic challenge.

Bradykinin may cause inflammation by a direct action on bradykinin receptors or effector cells, or via indirect mechanisms. For instance, part of the inflammatory response produced by locally applied bradykinin in certain tissues, such as airways, is due to stimulation of the release of neuropeptides, including tachykinins, from peripheral endings of primary sensory nerves (7). In the present study, we examined the question of whether NS inhibits plasma extravasation in response to hypertonic saline in guinea pig airways. Furthermore, we investigated whether NS inhibits bradykinin-dependent neurogenic inflammation induced by hypertonic saline using a recently developed tachykinin NK₁ and NK₂ receptor antagonist, FK224, bradykinin B₂ receptor antagonist, HOE140, the combination of NS and HOE140 and the combination of NS and FK224.

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.

Exposure to hypertonic saline

On the day of the experiment, the animals (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 (3, 23) using a constant volume ventilator (Rodent Ventilator Model 683, Harvard Apparatus, Millis, MA, U.S.A.). Hypertonic saline (5 %, 10 %, 15 % and 20 %), bradykinin (0.1 mM) or vehicle alone was delivered to animals for 2 min via the tracheal cannula, using an ultrasonic nebuliser, (Pulmo-Sonic model 25, De Vilbiss Co., Somerset, PA; aerosol delivery rate 0.2 mL/min).

Experimental procedure

Vascular extravasation was quantified by injection of Evans blue dye (3 % solution in 0.9 % NaCl; 30 mg/kg, *i. v.* over 5 s) at the beginning of each test. At the end of the experiment, the chest was opened, the aorta was cannulated through the left ventricle, and the circulation was perfused for 3 min with a phosphate buffer of pH 5 at a pressure of 120 mmHg. The trachea was dissected and opened along the ventral midline, from the first cartilaginous ring to the carina, blotted on absorbent paper, and weighed. Tissues were incubated in 3 mL formamide at 50°C for 18 h to extract the extravasated Evans blue dye (13). In experiments in which hypertonic saline (15 %) was used, vascular perfusion was performed following 10 min of exposure. To obtain a baseline value for Evans blue dye extravasation, saline was delivered for periods identical to those for hypertonic saline. NS (10 mg/kg, *i. v.*) (1), disodium cromoglycate (DSCG; 10 mg/kg, *i. v.*) (6) or tachykinin NK₁ and NK₂ receptor antagonist FK224, (3.2 mg/kg, *i. v.*) (14,15) was injected 5 min before the inhalation of hypertonic saline (15 %) or bradykinin (0.1 mM) (3, 25). Hypertonic saline (15 %), bradykinin (0.1 mM) or vehicle was delivered to animals for 2 min via the tracheal cannula, using an ultrasonic nebuliser (Pulmo-Sonic model 25, De Vilbiss Co., Somerset, PA; aerosol delivery rate 0.2 mL/min). Bradykinin B₂ receptor antagonist HOE140 (0.1 µmol/kg, *i. v.*) (3, 24) or vehicle was injected 10 min

before the inhalation of hypertonic saline. Phosphoramidon (2.5 mg/kg, *i. v.*) (3, 26) was injected 5 min before the inhalation of bradykinin (0.1 mM). NS was dissolved in saline, and HOE140 and bradykinin were delivered in distilled water. FK224 was dissolved in dimethylsulfoxide.

Measurement of plasma extravasation

Extravasation of the dye-labelled 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 as ng/mg wet weight, was interpolated from a standard curve of Evans blue dye concentrations.

Drugs

Hypertonic saline (5 %, 10 %, 15 % and 20 %) was made from NaCl (Wako Pure Chemical Industries Ltd., Osaka, Japan) solution in 0.9 % NaCl. Evans blue dye and DMSO were obtained from Sigma Chemical Co. (St. Louis, MO). Bradykinin and HOE140 were purchased from Peptide Institute Inc. (Osaka, Japan). NS was kindly provided by Dr. A. Norris (Roohne-Poulenc Rorer). FK224 and DSCG were supplied from Fujisawa Pharmaceutical Co. Ltd. (Tokyo, Japan).

Statistical analysis

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

RESULTS

Plasma extravasation induced by hypertonic saline

Exposure of guinea pig airways to 0.9 % NaCl produced an extravasation of Evans blue dye of 23.8 ± 3.45 ng/mg ($n=5$). Exposure to hypertonic saline produced a concentration-dependent increase in tracheal plasma extravasation (5 %, 70.6 ± 5.8 ng/mg; 10 %, 96.8 ± 6.6 ng/mg; 15 %, 115.0 ± 18.7 ng/mg; 20 %, 126.0 ± 12.9 ng/mg; $n=5$; Fig. 1).

Effect of nedocromil sodium on hypertonic saline-induced plasma extravasation

Inhalation of 15 % hypertonic saline for 2 min significantly increased the extravasation of the Evans blue dye in the trachea (126.2 ± 12.5 ng/mg; $n=5$). Pretreatment with DSCG (10 mg/kg, *i. v.*; 90.1 ± 7.32 ng/mg; $n=5$) significantly reduced the hypertonic saline-induced plasma extravasation in the trachea. Pretreatment with NS (10 mg/kg, *i. v.*; 70.7 ± 3.3 ng/mg; $n=5$) significantly reduced the plasma extravasation compared with DSCG. The inhibition of the response to hypertonic saline (15 %) produced by the combination of NS (10 mg/kg, *i. v.*) and B_2 receptor antagonist, HOE140 ($0.1 \mu\text{mol/kg}$, *i. v.*; 63.4 ± 4.1 ng/mg; $n=5$) was not significantly different from the response obtained with NS (10 mg/kg, *i. v.*) or HOE140 ($0.1 \mu\text{mol/kg}$, *i. v.*; 67.3 ± 4.2 ng/mg; $n=5$) alone. However, pretreatment with the combination of NS (10 mg/kg, *i. v.*) and FK224 (3.2 mg/kg, *i. v.*) abolished the plasma extravasation of the Evans blue dye induced by hypertonic saline (15 %) for 2 min in the trachea (33.9 ± 6.3 ng/mg; $n=5$). Pretreatment with FK224 (3.2 mg/kg, *i. v.*) alone also abolished the increase of plasma extravasation induced by hypertonic saline (data not shown) (Fig. 2).

Fig. 1 Effect of exposure to different concentration of hypertonic saline (5, 10, 15 and 20 %) on the extravasation of Evans blue dye in the trachea of pentobarbital anesthetized guinea pigs. 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 (0.9 % saline) in trachea.

Effect of nedocromil sodium on bradykinin-induced plasma extravasation

The extravasation of Evans blue dye in the trachea of the guinea pig after exposure to the aerosolised vehicle of bradykinin in the presence of phosphoramidon (2.5 mg/kg, *i. v.*) was not significantly different from the baseline (33.3 ± 5.8 ng/mg; $n=5$). 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 the Evans blue dye in the trachea (97.9 ± 4.9 ng/mg; $n=5$). The bradykinin-induced plasma extravasation was abolished completely by NS (10 mg/kg, *i. v.*) 5 min before aerosol challenge in the trachea (38.9 ± 3.4 ng/mg; $n=5$) (Fig. 3).

DISCUSSION

The major finding of the present study was that NS inhibits hypertonic saline-induced plasma extravasation, an effect caused by activation of sensory nerves induced by bradykinin, and the

subsequent release of tachykinins in the guinea pig trachea.

The inhalation of aerosols of hypertonic saline is thought to mediate airway narrowing, in part by the release of mast cell mediators. Evidence in rats suggests that hyperosmolarity may also increase vascular permeability by a mechanism involving sensory neuropeptides. The tachykinin receptor antagonist, CP99, 994, produces stereoselective inhibition of neurogenic plasma extravasation induced by inhalation of hypertonic saline in the rat trachea (16). NS has been shown to be capable of inhibiting chloride ion flux in mast cells, epithelial cells, and neurons (11). NS inhibits antigen-induced plasma extravasation at all airway levels in the guinea pig. Since NS alone had only a small effect on plasma extravasation induced directly by histamine and platelet activation factor, its effect is probably due to inhibition of mediator release from inflammatory cells (1). NS may have effects on sensory nerve endings, which may be an important site of action in the reduction of neurogenic inflammation in airways. NS has been found to be at least one order of magnitude more potent than DSCG in suppressing histamine release from mast cells (11,12).

In the present study, hypertonic saline-induced

Fig. 2 Effect of nedocromil sodium (NS; 10 mg/kg, *i. v.*), disodium cromoglycate (DSCG; 10 mg/kg, *i. v.*), HOE140 (0.1 μ mol/kg, *i. v.*), combination of NS (10 mg/kg, *i. v.*) and HOE140 (0.1 μ mol/kg, *i. v.*) or combination of NS (10 mg/kg, *i. v.*) and FK224 (3.2 mg/kg, *i. v.*) on Evans blue extravasation in guinea pig trachea induced by inhalation of hypertonic saline (15%, 2 min). 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 in trachea. ** $P<0.05$ versus NS (10 mg/kg, *i. v.*) in trachea.

Fig. 3 Effect of nedocromil sodium (NS; 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.

plasma extravasation was concentration-dependent. Pretreatment with NS inhibited the increase of hypertonic saline (submaximal dose; 15 %)-induced plasma extravasation. NS significantly reduced the hypertonic saline-induced plasma extravasation compared with DSCG in the guinea pig trachea. The inhibition of the response to hypertonic saline produced by NS was not significantly different from the response obtained with the combination of NS and bradykinin B₂ receptor antagonist, HOE140, or HOE140 alone. In these result, NS inhibited the increase of plasma extravasation induced by bradykinin. Furthermore, pretreatment with NS 5 min before aerosol challenge abolished the bradykinin-induced plasma extravasation in the guinea pig trachea. In these study, NS significantly inhibited hypertonic saline-induced plasma extravasation, an effect caused by activation of sensory nerves induced by bradykinin.

Ovalbumin-induced plasma extravasation was significantly reduced by the bradykinin B₂ receptor antagonist, HOE140 (3) or FR173657 (26), in the sensitized guinea pig. The combination of the tachykinin NK₁ receptor antagonist CP96,345 and HOE140 did not further increase the inhibition obtained with CP96,345 alone. These results provide evidence that the activation of sensory nerves that contribute to antigen-induced plasma extravasation is due to kinin release (3, 24). However, cigarette smoke-induced plasma extravasation is inhibited by the tachykinin NK₁ receptor antagonist, CP99,994, but not HOE140 (25). It has been reported that the involvement of kinin-dependent neurogenic inflammation differs according to the stimulus. In the present study, pretreatment with the combination of NS and tachykinin NK₁+NK₂ receptor antagonist, FK224, abolished the increase of plasma extravasation induced by hypertonic saline. FK224 alone also abolished the increase of plasma extravasation. However NS partially inhibited the plasma extravasation. In these results, the increase of hypertonic saline-induced plasma extravasation involve the release of tachykinin from sensory nerves both directly and indirectly (sensory nerve activated by bradykinin). At least, in this hypertonic saline study, NS inhibited the increase of plasma extravasation induced by tachykinin release from sensory nerves activated by bradykinin.

These findings suggest that NS significantly reduces hypertonic saline-induced plasma

extravasation in the guinea pig trachea by inhibiting bradykinin-dependent neurogenic inflammation. If these findings are substantiated in human beings, NS may prove to be a useful therapeutic agent for the treatment of inflammatory airway diseases such as asthma.

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