

Nedocromil sodium reduces allergen-induced plasma extravasation in the guinea pig nasal mucosa by inhibition of tachykinin release

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The effect of topically applied (10 μ l) nedocromil sodium (NS) and sodium cromoglycate (CS) on the plasma extravasation induced by local application of ovalbumin (5%, 10 μ l) into the respiratory nasal mucosa of sensitized guinea pigs pretreated with the neutral endopeptidase inhibitor, phosphoramidon, was studied. Topical NS (220 nmol, 10 μ l) reduced by 57% the Evans blue dye extravasation caused by local application of ovalbumin into the nasal mucosa, whereas CS (220 nmol, 10 μ l) was without effect. The tachykinin NK₁ receptor antagonist CP-99994 (2 μ mol/kg, i.v.) reduced by 45% the plasma extravasation induced by antigen challenge. The combination of NS and CP-99994 did not increase further the inhibition caused by NS alone. Plasma extravasation evoked by instillation of bradykinin (50 nmol), which causes this response by releasing tachykinins from sensory nerves, was markedly reduced by NS, but not by CS. Plasma extravasation evoked by instillation of substance P, which acts directly on the endothelial cells, was not affected by NS. We conclude that the reduction by NS of the plasma extravasation induced by antigen challenge in the nasal mucosa of sensitized guinea pigs is due to the inhibition of tachykinin release from sensory nerve endings.

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Exposure of the nasal mucosa of sensitized guinea pigs to antigen causes various inflammatory responses including the extravasation of plasma proteins. In the presence of the neutral endopeptidase (EC 3.4.24.11) inhibitor, phosphoramidon, plasma extravasation induced by antigen in the guinea pig nasal mucosa was partly due to tachykinin release from sensory nerve endings (20). Nedocromil sodium (NS) and sodium cromoglycate (CS) are used for the treatment of allergic disorders (19), although the precise mechanism of action of these drugs has not been established. There is evidence that NS, but not CS, inhibits the noncholinergic, nonadrenergic (NANC) contraction induced by electric stimuli in the guinea pig bronchus (21). Since tachykinins released from primary sensory nerve fibers are the mediators of NANC contraction in this tissue, it was concluded that NS, but not CS, has the ability to inhibit neural transmission in sensory nerves, thus

decreasing peptide transmitter release from these nerves (21).

In the present investigation, the ability of NS and CS to inhibit plasma extravasation induced by antigen challenge in the nasal mucosa of sensitized guinea pigs, pretreated with phosphoramidon, was investigated. Since NS, but not CS, reduced antigen-induced plasma extravasation, the possibility that this effect was due to a reduction in tachykinin release from sensory nerves was also explored. To study this possibility, we compared the effect of NS on antigen-induced plasma extravasation with the effect of the tachykinin NK₁ receptor antagonist, CP-99994 (17) and with the effect of the combination of NS and CP-99994. In different tissues (3, 12, 16), including the nasal mucosa (2, 20), locally applied bradykinin increases vascular permeability by releasing tachykinins from sensory nerves (11), whereas locally applied substance P increases plasma

extravasation by acting directly on tachykinin NK₁ receptors on endothelial cells (4). To further characterize the mechanism of the inhibitory action of NS on the antigen-induced plasma extravasation, we investigated the effect of NS on the plasma extravasation induced by bradykinin and substance P.

Material and methods

Animals and sensitization procedure

Male albino Hartley guinea pigs (Rodentia, Bergamo, Italy) were used in this study. They were kept in a temperature-controlled environment with standard laboratory food and water freely available. The animals were sensitized according to a protocol described previously (1, 8, 15), which consists of injection of 70 mg ovalbumin (Grade V; Sigma Chemical Co., St Louis, MO, USA) in 1.5 ml 0.9% NaCl i.p. twice, with an interval of 1 week between injections. The animals were studied 2 weeks after the second injection. Nonsensitized animals were injected twice with 2 ml 0.9% NaCl (1, 15).

Experimental procedure

The animals (body weight, 450–550 g) were anesthetized with sodium pentobarbital (45 mg/kg, i.p.), and the jugular vein was cannulated. Deep anesthesia was monitored throughout the experiment, and additional anesthesia was administered as required. We used Evans blue dye (3% solution in 0.9% NaCl; Sigma Chemical, St Louis, MO, USA) to measure plasma extravasation. Immediately after the injection of the dye (30 mg/kg i.v. over 5 s) in the jugular vein, ovalbumin was given by local instillation (10 µl/nostril) in the nose. In a previous study (20), we found that Evans blue dye extravasation evoked by ovalbumin challenge (5%, 10 µl/nostril) increased over time, reaching a maximum 15 min after the administration of the stimulus. Therefore, experiments with ovalbumin were terminated 15 min after injection of the tracer by overdosing with pentobarbital (40 mg/kg, i.v.). After a further 30 s, the chest was opened, and a cannula inserted into the ascending aorta through the left ventricle, and the circulation was perfused for 2 min with phosphate buffer (pH 5; Sigma Chemical, St Louis, MO, USA) at a pressure of 130 mmHg. In experiments in which we studied the effect of substance P and bradykinin on plasma extravasation of nonsensitized guinea pigs in the nasal mucosa, the chest was opened 5 min after injection of the tracer.

The guinea pig was decapitated behind the orbit, and the bony nose was freed from the skin. For exclusion of the vestibulum, the rostral part of the nose was cut immediately in front of the incisors, which were then excised at their insertion in the

bone. The nasal dorsum and floor were incised by sagittal paramedian sections to open the nasal cavity. These incisions divided the nose into three parts, the septum and the right and left lateral walls (18). The septum was freed from the vomeronasal organ. Each lateral wall was dissected further to remove the naso- and maxilloturbinates. The turbinates and the mucosa removed from the septum were blotted on bibulous paper and weighed. All tissues were incubated in 1 ml of formamide (Merck, Darmstadt, Germany) at 60°C for 18 h to extract the extravasated Evans blue dye (16).

All the experiments were performed in the presence of phosphoramidon (4.5 µmol/kg i.v., injected 5 min before delivering the stimulus). All the drugs were dissolved in 0.9% NaCl. All stimuli (ovalbumin, bradykinin, and substance P) were delivered locally (10 µl/nostril) to both nostrils by instillation into the nasal mucosa by a micropipette.

Measurement of plasma extravasation

The extravasation of Evans blue dye-labeled macromolecules from the microcirculation in the tissue was quantified by measuring the optical density of the formamide extracts at a wavelength of 620 nm with a spectrophotometer (Model UV160U, Shimadzu Scientific Instruments, Inc, Columbia, MD, USA). The amount of Evans blue dye extravasated in the tissues, expressed in nanograms per milligram of wet weight, was interpolated from a standard curve of Evans blue concentrations (0.1–5 µg/ml).

Drugs

CP-99994 was a kind gift from Dr J. A. Lowe III (Pfizer Inc, Groton, CT, USA). Sodium cromoglycate and nedocromil sodium were the gift of Dr A. Norris (Fisons Pharmaceuticals, Loughborough, UK). Substance P, bradykinin, and phosphoramidon were purchased from Peninsula Laboratories, Inc, Belmont, CA, USA; capsaicin and ovalbumin were obtained from Sigma Chemical Co., St Louis, MO, USA.

Statistical analysis

All data are expressed as means ± SEM. Mean values of spectrophotometric measurements of Evans blue dye extravasation were analyzed by one-way analysis of variance. Comparisons between means in each condition were performed by Bonferroni's multiple range test. Student's *t*-test was used when two groups of data were compared. Differences of $P < 0.05$ were considered significant.

Results

Effect on ovalbumin-induced plasma extravasation

Baseline Evans blue dye extravasation in the nasal mucosa of sensitized guinea pigs pretreated with phosphoramidon (4.5 μ mol/kg, i.v.) was 8.43 ± 1.11 ng/mg ($n = 4$). Instillation of 0.9% (10 μ /nostril) saline did not significantly increase baseline Evans blue dye extravasation in the nasal mucosa of sensitized guinea pigs (7.43 ± 0.85 ng/mg, $n = 5$). Instillation of NS (220 nmol, 10 μ l) or CS (220 nmol, 10 μ l) did not increase significantly baseline plasma extravasation: Evans blue dye extravasation values were 8.86 ± 0.55 ng/mg ($n = 4$) and 8.76 ± 0.86 ng/mg ($n = 4$), respectively. A previous study (20) showed that instillation of 5% ovalbumin into the nostril of nonsensitized guinea pigs did not affect baseline Evans blue extravasation in the nasal mucosa. The marked increase in plasma extravasation induced by 5% ovalbumin in the guinea pig nasal mucosa (46.16 ± 5.63 ng/mg, $n = 5$) was not affected by local application of CS (220 nmol, 10 μ l) (Fig. 1). However, local pretreatment with NS (220 nmol, 10 μ l) significantly reduced by 57% ovalbumin-induced plasma extravasation (Fig. 1). The NK₁ antagonist CP-99994 (2 μ mol/kg, i.v.), which did not affect baseline plasma extravasation (9.41 ± 0.97 ng/mg, $n = 4$), also inhibited the increase in the Evans

blue dye extravasation induced by 5% ovalbumin by 45% (Fig. 1). The inhibition of the ovalbumin-evoked Evans blue dye extravasation caused by the combination of NS (220 nmol) and CP-99994 (2 μ mol/kg, i.v.) (51%) was not significantly different from the inhibition evoked by NS alone (Fig. 1).

Effect on bradykinin- and substance P-induced plasma extravasation

Baseline plasma extravasation in nonsensitized guinea pigs pretreated with phosphoramidon (4.5 μ mol/kg, i.v.) was 7.43 ± 0.8 ng/mg ($n = 4$). Local application of 10 μ l of 0.9% saline did not affect baseline plasma extravasation in nonsensitized guinea pigs (8.83 ± 0.62 ng/mg, $n = 4$). Application of 50 nmol (10 μ l) of bradykinin to the nasal mucosa of nonsensitized guinea pigs pretreated with phosphoramidon (4.5 μ mol/kg i.v.) increased markedly plasma extravasation of the Evans blue dye (59.23 ± 3.58 ng/mg, $n = 5$) (Fig. 2). Local pretreatment with NS (220 nmol), but not with CS (220 nmol), reduced significantly bradykinin-evoked increase in plasma extravasation (Fig. 2). Local application of substance P (5 nmol, 10 μ l) also increased plasma extravasation (70.8 ± 4.64 ng/mg, $n = 5$). Topical pretreatment with NS (220 nmol) did not affect the substance P-induced plasma extravasation (Fig. 2), which, however, was eliminated by

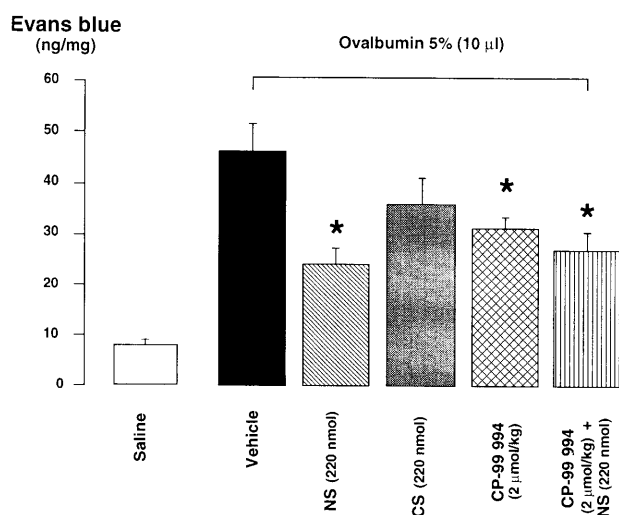


Fig. 1. Effect of nedocromil sodium (NS, local application, 10 μ l), sodium cromoglycate (CS, local application, 10 μ l), tachykinin NK₁ receptor antagonist, CP-99999 (i.v.), or combination of NS (local application) and CP-99999 (i.v.) on Evans blue dye extravasation in nasal mucosa of sensitized guinea pigs induced by local instillation of ovalbumin (10 μ l/nostril). Experiments were performed in presence of phosphoramidon (4.5 μ mol/kg, i.v.). Baseline (open column) indicates extravasation evoked by instillation of vehicle of NS (0.9% saline, 10 μ l). Vehicle (solid column) indicates extravasation evoked by ovalbumin after pretreatment with vehicles of NS or CS (0.9% saline, 10 μ l, locally) and vehicle of CP-99994 (0.9% saline, 1 ml/kg, i.v.). Values are means $\pm$ SEM of at least five experiments. * $P < 0.01$ vs vehicle.

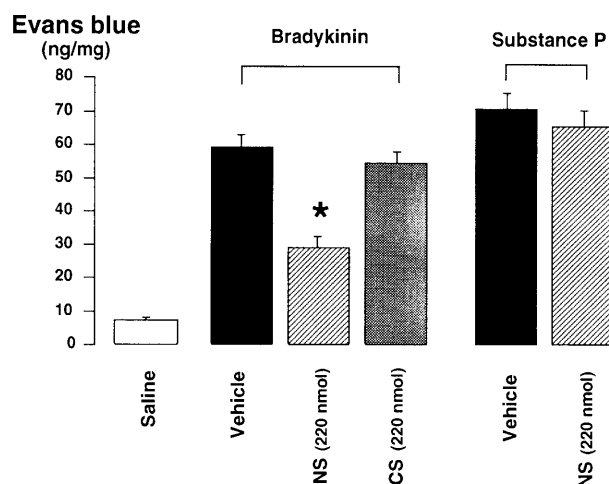


Fig. 2. Effects of nedocromil sodium (NS, local application, 10 μ l) and sodium cromoglycate (CS, local application, 10 μ l) on Evans blue dye extravasation in nasal mucosa of nonsensitized guinea pigs induced by instillation of bradykinin (50 nmol, 10 μ l) or substance P (5 nmol, 10 μ l). Experiments were performed in presence of phosphoramidon (4.5 μ mol/kg, i.v.). Baseline (open column) indicates extravasation induced by instillation of vehicle of bradykinin or substance P (0.9% saline, 10 μ l). Vehicle (solid column) indicates extravasation evoked by bradykinin or substance P after pretreatment with vehicle of NS or CS (0.9% saline, 10 μ l). Values are means $\pm$ SEM of at least five experiments. * $P < 0.01$ vs respective vehicle.

the tachykinin NK₁ receptor antagonist, CP-99994 (2 µmol/kg, i.v.) (11.71 ± 3.14 ng/mg, $n = 4$).

Discussion

The present study shows that antigen administration into the nostrils of sensitized guinea pigs, pretreated with a neutral endopeptidase inhibitor, increases vascular permeability into the respiratory nasal mucosa. Several mediators released during the anaphylactic reaction play a role in the antigen-induced leakage of plasma proteins (10). A previous report (20) indicated that, in experimental conditions similar to those adopted in this study, part of the plasma extravasation induced by antigen challenge in the guinea pig nose is mediated by tachykinins released from sensory nerve terminals (20). CP-99994, a tachykinin NK₁ receptor antagonist (17), with negligible calcium antagonist activity as compared to that of CP-96345, the parent compound used in the previous study (20), reduced antigen-induced plasma extravasation. These observations confirm that tachykinins stimulating NK₁ receptors play a role in the anaphylactic plasma exudation in the guinea pig nasal mucosa.

The major findings of the present study are, first, that NS, but not CS, reduced the antigen-induced plasma extravasation in the guinea pig nasal mucosa, and, second, that the combination of NS and CP-99994 did not increase further the inhibition caused by NS alone. These findings suggest that NS and CP-99994 act on a common final pathway to inhibit antigen-induced plasma extravasation in the guinea pig nasal mucosa. Three hypotheses may be proposed to explain the inhibitory action of NS on antigen-induced plasma extravasation in the nasal mucosa:

- 1) NS may behave as a tachykinin NK₁ receptor antagonist
- 2) NS may inhibit the action of one or more mediators released by antigen which may stimulate sensory nerves
- 3) NS may inhibit tachykinin release from sensory nerves.

Studies in the human skin and in a rabbit vascular preparation suggest that CS is a substance P antagonist (6). However, the possibility that CS and NS inhibit tachykinin NK₁ receptors has not been confirmed in the guinea pig (14). In addition, in the present study, CS, at the same dose as that of NS, was completely inactive in reducing the antigen-induced plasma extravasation mediated by tachykinin NK₁ receptor activation. Therefore, it is unlikely that NS inhibits the tachykinin NK₁ receptor. Various mediators, including histamine, leukotrienes, and kinins (5, 9), are released in the airways during the anaphylactic reaction. Although all these inflam-

matory mediators may potentially act on sensory nerves, only kinins play a major role in the stimulation of sensory nerves and the subsequent release of tachykinins in the lower (3) and upper (20) airways of the guinea pig following antigen challenge. However, there is no evidence that NS selectively reduces kinin release or antagonizes kinin receptors. The third hypothesis, that NS may inhibit the component of antigen-induced plasma extravasation mediated by tachykinins, by reducing neural transmission on sensory nerves and the subsequent release of tachykinins from sensory nerve terminals, is supported by present and previous evidence.

Verleden et al. (21) showed that NS, but not CS, reduced the NANC contraction of the guinea pig bronchus induced by electric field stimulation, but not the contraction induced by exogenously applied tachykinins. Since the response to electric field stimulation is due to tachykinins released from sensory nerve endings, it was suggested that NS reduces selectively neural transmission in these nerves (21). The present observations that NS, but not CS, inhibits plasma extravasation induced by topical application of bradykinin supports this hypothesis. Bradykinin is a stimulator of sensory nerves, and it releases peptide transmitters from these nerves (11). Bradykinin applied locally to airways tissue, including the nasal mucosa, increases plasma protein extravasation by releasing tachykinins from sensory nerves (3, 13, 20). In contrast, substance P increases plasma extravasation in the airways, presumably by acting on the tachykinin NK₁ receptors which have been described recently on endothelial cells of the postcapillary venules (4). As predicted by the hypothesis and the evidence reported above, we observed that NS markedly inhibited bradykinin-evoked plasma extravasation, but did not affect substance P-induced plasma extravasation. The finding that NS was ineffective against substance P-induced plasma extravasation also supports the view that the inhibitory effect of NS on antigen- and bradykinin-induced increases in plasma exudation in the nose was selective, and not due to changes in cardiovascular parameters or other nonspecific actions.

The present findings indicate that, at least at the dose used in this study (0.1% solution), NS exerts an anti-inflammatory action in the nasal mucosa through a mechanism that is not shared by CS. If the mechanism is present also in man, NS may have some advantage over CS in the treatment of inflammatory disorders in the nose and in other organs in which sensory nerves are involved, and may be particularly relevant to the effect of NS in rhinitis (7).

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