

Effects of nedocromil sodium on allergen-induced rhinitis in humans

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Sixteen patients with allergic rhinitis were recruited into a double-blind crossover protocol studying the immediate effect of nedocromil sodium (NS) on the pattern of nasal symptoms and secretions after allergen challenge. After pretreatment with placebo or NS, allergen challenge resulted in pruritus, rhinorrhea, nasal congestion, and/or sneezing within 10 minutes in 12 of 16 subjects. Prostaglandin D₂ (PGD₂), a marker of mast cell degranulation, increased proportionately with symptom scores, remaining above the 95% confidence interval for 120 minutes after both pretreatments. No difference in PGD₂ between the NS-treatment and placebo-treatment days was observed. Protein markers extravasated through the vasculature (albumin and IgG) or secreted by mucosal glands (lactoferrin) were assayed. Total protein, albumin, IgG, and lactoferrin all remained >95% confidence interval for 100 minutes after allergen challenge in the placebo-pretreated group and 120 minutes in the NS-pretreated group. Although there appeared to be a trend for lower secretion of PGD₂, albumin, and IgG in the NS-treated group, the overall differences did not achieve statistical significance. This protocol revealed that two topical 130 µl doses of a 1% solution of NS failed to significantly reduce allergen-induced symptoms, PGD₂ generation, or secretion of albumin, IgG, or lactoferrin when NS was compared with placebo. The anti-inflammatory and mast cell-stabilizing effects of NS may require more prolonged pretreatment before provocation to be effective. (J ALLERGY CLIN IMMUNOL 1992;89:599-610.)

Key words: Nedocromil sodium, nasal secretions, allergen challenge

NS, the disodium salt of a pyranoquinolone dicarboxylic acid, has been found to exhibit protective antiallergic and anti-inflammatory activity at mucosal surfaces. Its action is believed to be similar to the action of sodium cromoglycate in that it stabilizes mucosal mast cells, preventing the release of inflammatory mediators involved in IgE-mediated immediate hypersensitivity reactions. NS has been studied extensively with in vitro and in vivo models of parasite-infected monkeys,¹ human lung,² and al-

Abbreviations used

NS: Nedocromil sodium
LPR: Late-phase reaction
CI: Confidence interval
AR: Allergic rhinitis
PGD₂: Prostaglandin D₂

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lergic sheep.³ The development program for the 1% nasal spray has included therapeutic trials conducted throughout Europe and North America.⁴

In this study, the immediate effect of NS on the pattern of nasal secretions after allergen challenge was examined in patients with AR. Mast cell degranulation and the physiologic responses involved in AR were determined by assaying nasal lavage fluids for inflammatory mediators, PGD₂, and histamine, as well as protein markers secreted by seromucous glands (lactoferrin) or by the vasculature during periods of increased permeability (albumin and IgG). The capacity of NS to affect both mediator release and protein secretion was ascertained.

MATERIAL AND METHODS

Subjects

Sixteen adult male subjects, between the ages of 24 and 41 years with AR, were studied after informed consent was obtained and after the protocol was approved by the Clinical Review Committee of the National Institute of Allergy and Infectious Diseases. Eligibility for the study required a history of rhinitis during the appropriate allergy season and skin test (epicutaneous) positivity to the appropriate allergen (a wheal 4 mm by 4 mm or larger than the wheal of control) within 2 years of the study. At the time of the study, November to January 1989 to 1990, all subjects were asymptomatic. No subject was studied within 3 weeks of recovery from an upper respiratory tract infection. No patients were receiving immunotherapy. Subjects with systemic diseases (renal, hepatic, cardiovascular, or respiratory) or with active asthma were excluded. Female subjects of child-bearing potential, patients with nasal polyps, a history of systemic anaphylaxis, or sensitivity to edetate sodium or benzalkonium chloride were also excluded. All medications were stopped before entering the study. Intracocular cromolyn sodium, intranasal corticosteroids, and oral antihistamines were stopped 1 month before study day 1 in three different subjects. Two other subjects stopped intranasal corticosteroids and oral antihistamines within 3 weeks of the study. Three subjects had used aspirin or acetaminophen 1 to 3 weeks before the study. After completing the screening allergen challenge or the challenge on study days 1 or 15, eight subjects required topical beclomethasone for 24 hours after challenge to prevent LPRs. Six subjects required oral terfenadine. In addition, chlorpheniramine, diphenhydramine, oxymetazoline, phenylpropanolamine/guaifenesin, and acetaminophen were taken by a total of seven subjects after challenge to relieve nasal symptoms. All subjects stopped topical corticosteroids within 2 weeks and antihistamines and analgesics within 1 week of both the first and second study day.

Material

Nasal-challenge material included normal saline, glycerinated extracts of mixed ragweed pollen, mixed grass pollen, house dust mix (each 1:20 wt/vol), and dust mites (*Dermatophagoides pteronyssinus*) (1:100 wt/vol) (Walter Reed Army Medical Center, Washington, D.C.), NS 1% in edetate sodium, and benzalkonium chloride and vehicle controls, which were prepared by Fisons Corp., Rochester, N.Y. The NS and placebo were in identical, unlabeled spray bottles supplied by the manufacturer. Allergen-challenge solutions were prepared by diluting the extracts tenfold with normal saline, producing 1:200 dilutions of ragweed, grass, and dust mix and a 1:1000 dilution of dust mite. Based on skin test results and the subject's allergy history, the allergen to which the subject was most sensitive was chosen for nasal challenge.

Challenge methods

Twenty-two subjects had a screening allergen challenge to confirm reactivity to the allergen suggested by history

(Table I). Each subject was challenged with the appropriate allergen, as described below. After 20 minutes, symptoms (itching, rhinorrhea, and nasal congestion) and signs (sneezing) were recorded. Sneezing, nasal congestion, rhinorrhea, and itching were each rated on a scale of 0 to 2. A maximum of 8 could be reached. A response to allergen was considered positive if the symptom score was 4 or higher. If a symptom score of 4 had not been attained, the subject was excluded from the study.

At least 2 weeks after the screening challenge, the subjects were studied. Each subject was assigned a number corresponding to the drug/placebo combination to be administered. In this double-blind, crossover study, each patient received the 1% NS solution on one day and received placebo on the other day.

Eligibility was reconfirmed on study day 1. The nasal-challenge technique has been described previously.⁵⁻⁷ A nasal catheter was inserted 4 cm into the right nasal cavity below the inferior turbinate and attached to a wall suction unit. Four nasal prewashes with saline (4 ml each) were administered before beginning the challenge protocol to reduce baseline secretions. Five minutes later, the right nasal cavity was pretreated with 130 μ l of NS or placebo. After 15 minutes, 75 μ l of saline was instilled into the nasal cavity. Low suction (120 mm Hg) was applied for 10 minutes; then the cavity was lavaged at high suction (200 mm Hg) with 4 ml of saline. This baseline saline challenge served as the control for all later collections.

Five minutes after the lavage, the right nostril was again pretreated with 130 μ l of the drug or placebo. After 15 minutes, the nasal cavity was challenged with 75 μ l of the allergen. After a 3-minute incubation, the allergen was removed with a 4 ml saline lavage (to allow measurement of secreted proteins without having to account for the protein contributed by the allergen itself). Nasal lavages (4 ml each) were subsequently collected at 10 (7 minutes after removing the allergen), 20, 30, 40, 50, 60, 80, 100, and 120 minutes after allergen challenge. Between each collection, suction at 120 mm Hg was maintained. Symptoms (itching, rhinorrhea, and nasal congestion) and signs (sneezing) were recorded before each lavage. A symptom score was determined for each time period. Nasal-lavage samples were kept on ice until the end of the challenge and were then aliquoted and stored at -70° C for subsequent analysis (see below). Excessive mucus was aliquoted into separate tubes.

Exactly 2 weeks later, each subject returned for study day 15. The procedure outlined above was repeated with either NS or placebo.

Epinephrine, 1:1000, was available at all times, and vital signs were monitored throughout the procedure. No changes in vital signs occurred in this study, and no patient required treatment for an adverse reaction.

Assays

Total protein. Protein in each sample was measured by the method according to Lowry et al.⁸ with bovine serum albumin as the standard. The assay range is between 31 and 1000 μ g/ml. It has been demonstrated in preliminary studies⁷ that removal of the allergen by the 4 ml saline wash

TABLE I. Patients' data

Patient No.	Sex	Age (yr)	Height (in)	Weight (lb)	Allergen	Skin wheal diameter (mm)†
1	M	32	71.0	155	Ragweed	8
2*	M	36	73.0	160	Dust	3
3*	M	29	70.0	225	Grasses	5
4*	M	35	67.0	150	Ragweed	11
5*	M	34	66.0	140	Ragweed	13
6*	M	27	73.5	178	Grasses	11
7*	M	24	68.0	130	Grasses	11
8	M	31	71.0	220	Grasses	8
9*	M	26	72.5	200	Ragweed	7
10*	M	30	72.0	180	Ragweed	4
11	M	34	70.0	183	Dust	4
12*	M	33	68.0	170	Mites	5
13	M	33	73.0	181	Mites	4
14*	M	32	70.5	165	Ragweed	8
15	M	29	71.0	165	Grasses	8
16	M	39	73.0	185	Mites	8
17*	M	33	74.0	170	Grasses	10
18*	M	30	72.0	165	Ragweed	8
19*	M	34	69.0	180	Grasses	4
20*	M	36	61.5	100	Ragweed	11
21*	M	41	70.5	170	Dust	4
22*	M	33	71.0	174	Mites	6
Mean		32.32	70.3	170.27		7.32
SEM		0.85	0.6	5.78		0.63

*Subjects used in study.

†Wheal was determined as the largest diameter. All saline control sites were negative.

effectively reduces this source of exogenous protein, thus allowing measurement of secreted proteins.

The NS that remained in the nasal cavities after administration was included in the first two lavages of the study (the baseline saline wash and the first wash after allergen challenge). Although NS had no effect on the ELISAs, it did affect the Lowry protein assay, a spectrophotometric assay, causing falsely elevated protein levels. With the extinction coefficient of NS at 346 nm, the concentration of NS contained in each sample could be determined, and the results could be corrected for the contribution by NS.

Albumin. Human albumin was measured by a competitive ELISA as previously described.⁵ The assay range is between 0.75 and 100 μ g/ml.

Lactoferrin. Human lactoferrin was measured by a non-competitive ELISA as previously reported.^{9*} The assay range is between 1.5 and 200 ng/ml.

IgG. Human IgG was measured by a noncompetitive ELISA as previously described.¹⁰ The assay range is between 31 and 100 ng/ml.

Albumin percent, lactoferrin percent, and IgG percent. The albumin percent, lactoferrin percent, and IgG percent were all calculated by dividing the respective proteins by the total protein and multiplying by 100%.

*Pharmacia technique, file No. 110. LKB, Bromma, Sweden.

Histamine. Histamine was measured by a competitive RIA as described (AMAC Inc., Westbrook, Me.).¹¹ The range of this assay is between 0.017 and 16.7 ng/ml.

PGD₂. PGD₂ was also measured by a competitive RIA, as previously described (Amersham Corp., Arlington Heights, Ill.).¹² The assay range is between 30 and 2000 pg/ml.

All results are expressed as the quantity of product secreted per 10-minute collection period, with the exception of the first postallergen-challenge collection that occurred during 7 minutes.

Statistics. The mean and standard errors for each study day were calculated. To compare the postallergen-challenge results to baseline, upper 95% confidence limits for the mean were established. Wilcoxon's signed-rank tests were used to compare the effect of NS with placebo.

RESULTS

Of the 22 subjects screened for the protocol, 16 had positive provocations and were considered eligible for the protocol. Of the six subjects who were not included in the study, one subject was found to have nasal polyps and was therefore excluded. One skin test-positive subject failed to experience any symptoms or signs of rhinitis despite multiple allergen

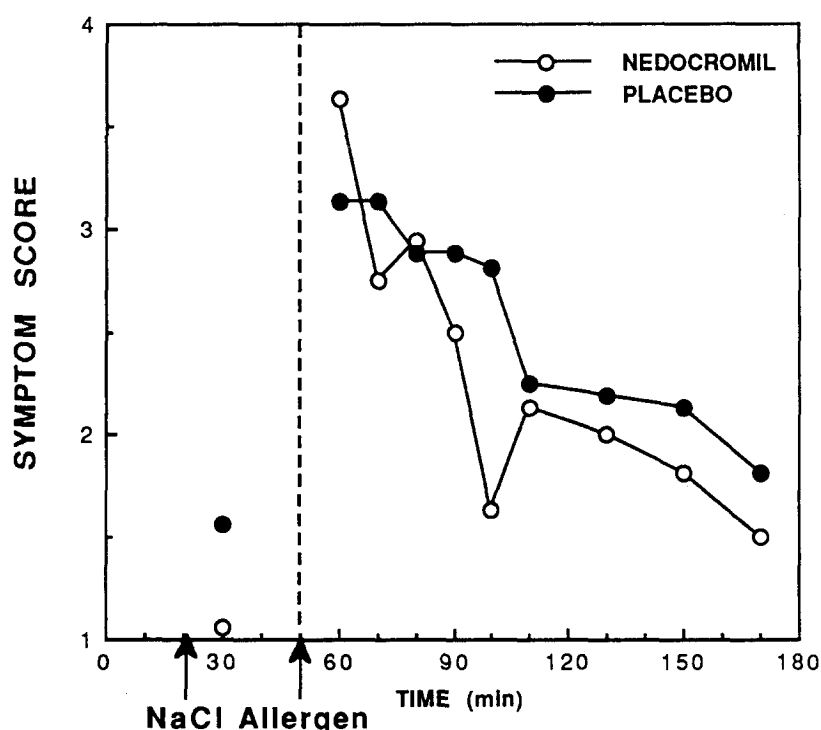


FIG. 1. Time course of mean symptom scores after saline and allergen challenges. In both NS- and placebo-treated subjects, symptoms were significantly increased over baseline after allergen challenge, remaining above the 95% CI for 50 minutes in placebo-treated group and 40 minutes in NS-treated group. Total symptoms were significantly higher in placebo-treated group at 100 minutes (50 minutes after allergen challenge) as compared with that in NS-treated group (Wilcoxon's signed-rank test, $p = 0.007$).

challenges. Two subjects experienced severe congestion concurrent with symptoms consistent with acute sinusitis 6 to 24 hours after allergen challenge and refused to continue the study. One subject was unable to stop his allergy medications. One subject had recurrent upper respiratory tract infections. Therefore, 16 patients were admitted into the study.

Control saline challenges were used as baselines for judging the response to the drug or placebo. As has been previously reported with the provocation system used, saline challenges produce neither nasal symptoms nor signs,^{5,6} and levels of lavage proteins and mediators do not change.

When subjects were pretreated with the placebo, thirteen subjects had a positive response (sneezing, nasal congestion, rhinorrhea, or itching, each rated 0 to 2 for a total of 4 or more) to the allergen (Fig. 1). The reaction occurred within 10 minutes in 12 of these patients. One subject attained an increase in symptoms 50 minutes after allergen challenge. Three subjects had no increase in symptoms above baseline. On average, there was a significant increase in all symptoms after allergen challenge. The mean symptom score increased from a baseline of 1.56 with saline challenge

to 3.13 after allergen challenge. The symptomatic increase remained >95% CI for 50 minutes after allergen challenge.

In contrast, only 11 subjects pretreated with NS experienced a 4 or higher response to allergen. The other five subjects attained a symptom score of <4, which was considered to be a negative response. On average, there was a significant increase in all symptoms after allergen challenge. The mean symptom score rose significantly from 1.06 with saline challenge to 3.63 after allergen and remained above the 95% CI for 40 minutes after challenge. At 50 minutes after allergen challenge, the mean symptom score was noted to be significantly higher in the placebo-treated group as compared to that of the NS-treated group with Wilcoxon's signed-rank test ($p = 0.007$). No differences were noted when each symptom was compared separately. The increase in symptoms above baseline levels are summarized in Table II.

Mast cell degranulation in response to topical allergen challenge was assessed by measuring histamine (Fig. 2) and PGD₂ (Fig. 3). The mean baseline histamine levels were comparable after pretreatment with either placebo or NS (3.13 and 5.41 ng/ml, respec-

TABLE II. Patients' symptom data after pretreatment with placebo or with NS

Patient No.	Sneezing	Rhinorrhea	Congestion	Itching	Net symptoms
Placebo					
2	-1	0	0	0	-1
3	0	0	1	0	1
4	1	2	1	2	6
5	1	2	1	0	4
6	0	2	0	2	4
7	2	2	2	0	6
9	2	1	1	1	5
10	0	0	1	1	2
12	2	2	1	0	5
14	-1	0	0	0	-1
17	2	2	0	2	6
18	0	2	1	2	5
19	0	0	0	0	0
20	0	1	0	0	1
21	0	1	1	0	2
22	1	0	0	2	3
Mean	0.56	1.06	0.63	0.75	3.00
SEM	0.26	0.23	0.15	0.23	0.62
NC					
2	0	1	0	0	1
3	0	1	1	1	3
4	0	1	0	2	3
5	0	2	1	1	4
6	2	2	0	2	6
7	2	0	1	0	3
9	0	0	1	0	1
10	0	-1	1	1	1
12	1	1	1	1	4
14	2	0	1	2	5
17	1	1	1	2	5
18	1	2	0	2	5
19	2	2	1	1	6
20	1	1	0	0	2
21	0	0	1	0	1
22	2	2	2	1	7
Mean	0.88	0.94	0.75	1.00	3.56
SEM	0.22	0.23	0.14	0.20	0.50

Maximal increase over baseline levels of symptoms after allergen challenge.

tively). Ten minutes after allergen challenge, both groups experienced a drop in lavage histamine to 0.35 and 0.59 ng/ml, respectively, followed by a maximum level of 4.05 and 6.24 ng/ml 30 minutes after challenge. In both cases, a gradual decline to below baseline levels (1.01 and 1.72 ng/ml) occurred thereafter. However, there was no significant difference in histamine levels before and after allergen challenge, nor was there any significant difference between NS- and placebo-treated subjects.

PGD₂, another mast cell mediator, increased proportionately with symptom scores. Baseline levels af-

ter saline challenge (27.54 pg/ml for placebo- and 12.68 pg/ml for NS-treated subjects) were followed by maximum levels of 282.19 and 200.84 pg/ml within 10 minutes of allergen stimulation. These maximum values persisted for 10 to 20 minutes and were followed by sharp declines, returning to near baseline levels of 35.26 and 41.98 pg/ml after 2 hours. PGD₂ levels remained above the 95% CI for 120 minutes after allergen challenge with both placebo and NS pretreatment. There was no significant difference between NS- and placebo-treated subjects.

Nasal lavages were also assayed for the following

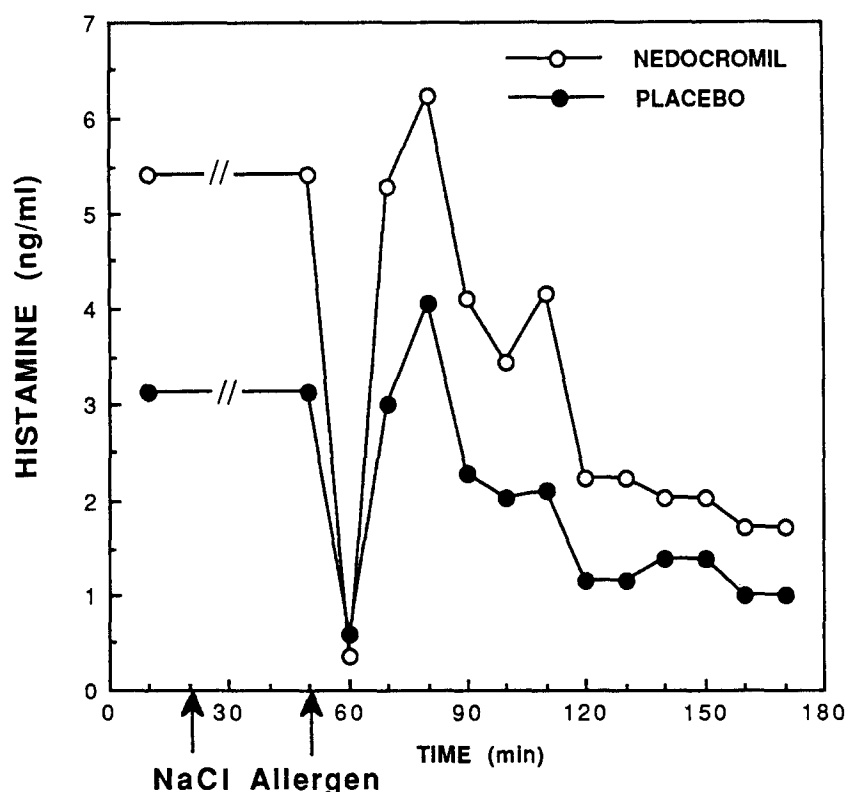


FIG. 2. Time course of mean histamine levels after saline and allergen challenges. No significant difference between pre- and post-challenge levels or between NS- and placebo-treated subjects.

proteins: total protein (Fig. 4), lactoferrin (Fig. 5), albumin (Fig. 6), and IgG (Fig. 7). After saline challenge, the subjects had a baseline total protein of 60 $\mu\text{g}/\text{ml}$ after the placebo pretreatment and 34 $\mu\text{g}/\text{ml}$ after NS pretreatment. In both cases, the maximal increase in total protein occurred within 20 minutes of the allergen challenge. The mean total protein rose significantly to $>330 \mu\text{g}/\text{ml}$ in both cases after allergen challenge with subsequent drops to 80 to 100 $\mu\text{g}/\text{ml}$ after 2 hours. Total protein remained above the 95% CI for 100 minutes after allergen challenge in the placebo-treated group and 120 minutes in the NS-treated group. From 70 to 80 minutes after allergen challenge, total protein was significantly lower in subjects pretreated with NS by Wilcoxon's signed-rank test ($p = 0.03$).

Albumin, secreted during periods of vascular permeability, increased dramatically from a mean baseline of 6.24 $\mu\text{g}/\text{ml}$ in the placebo-treated group and 7.90 $\mu\text{g}/\text{ml}$ in the NS-treated group to 65.17 $\mu\text{g}/\text{ml}$ (placebo) and 42.06 $\mu\text{g}/\text{ml}$ (NS) within 20 minutes of allergen challenge (Fig. 5). Albumin secretion remained above the 95% CI for 100 minutes after allergen challenge in the placebo-treated group and 120 minutes in the NS-treated group. Ten minutes after

allergen challenge, subjects pretreated with placebo were noted to have significantly increased levels of albumin compared to that of subjects pretreated with NS with Wilcoxon's signed-rank test ($p = 0.02$). When albumin was compared to total protein, the mean baseline albumin percent of the placebo-treated group was lower than that in the NS-treated group (15.22% versus 33.15%). Although allergen caused a slight rise in albumin percent in the placebo-treated group from 15.22% to 23.38%, it caused a large drop in the NS-treated group from 33.15% to 9.09% in the first 10 minutes, followed by a return to 33.48% 70 minutes after challenge. No significant changes were noted.

IgG, also a vascular protein, mimicked albumin during the challenge protocol. Beginning with baseline levels of 1.91 and 3.45 $\mu\text{g}/\text{ml}$ in the NS- and placebo-treated group, respectively, IgG rose to a maximum of 28.43 $\mu\text{g}/\text{ml}$ after placebo and 20.49 $\mu\text{g}/\text{ml}$ after NS at 20 minutes after allergen challenge (Fig. 6). IgG remained $>95\%$ CI for 100 minutes after allergen challenge in the placebo-treated group and 120 minutes in the NS-treated group. However, no significant differences were noted between NS- and placebo-treated subjects. Similar to albumin percent,

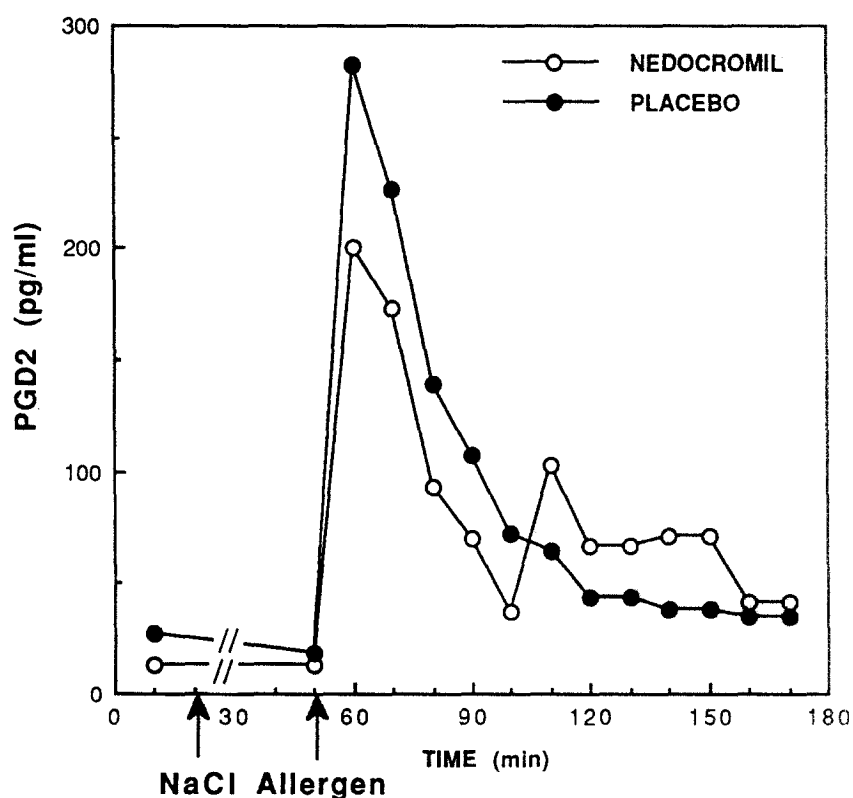


FIG. 3. Time course of mean PGD₂ levels after saline and allergen challenges. PGD₂ levels increased significantly after allergen challenge, remaining above the 95% CI for 120 minutes in both NS- and placebo-treated subjects. No significant differences were noted between NS- and placebo-treated subjects.

IgG percent of total protein was initially elevated in the NS-treated group (8.48% versus 5.13% in the placebo-treated group). On allergen stimulation, IgG percent increased to 7.92% in the placebo-treated group with a gradual decrease during the following 2 hours. In the NS-treated group, IgG percent dropped to 5.08% in the first 20 minutes, followed by an increase to 6.06%, and then a decrease to 4.96% after 2 hours. No significant changes were noted.

Lactoferrin, a nasal mucosal glandular marker, increased in parallel with total protein after allergen challenge (Fig. 7). With a mean baseline lactoferrin level of 1.5 to 2.0 $\mu\text{g/ml}$ after either placebo or NS, allergen caused a marked increase that peaked at 7 to 8 $\mu\text{g/ml}$ after 20 minutes after both treatments. Lactoferrin remained above the 95% CI for 100 minutes after allergen challenge in the placebo-treated group and 120 minutes in the NS-treated group. Levels dropped to 3.23 and 2.65 $\mu\text{g/ml}$ after 2 hours for placebo- and NS-treated subjects, respectively. No significant differences were noted between NS- and placebo-treated subjects. The mean baseline levels of lactoferrin percent in the subjects pretreated with NS were elevated at 6.42%, compared to 3.13% found in

subjects treated with placebo. After allergen challenge, there was a decrease to 3% in the NS-treated group and an increase to 5% in the placebo-treated group within 10 minutes of the allergen challenge with subsequent stabilization at 4% for both groups after 2 hours. None of these results were significantly different as compared with the baseline.

DISCUSSION

Allergen challenge of 16 of 22 subjects resulted in a significant increase in subjective symptoms, sneezing, PGD₂ formation, and the secretion of albumin, IgG, lactoferrin, and total protein. These responses were noted within minutes of allergen challenge and lasted more than 1 hour. It should be noted that the allergen challenge used was roughly equivalent to the amount of pollen to which subjects might be exposed in 30 to 60 minutes during the height of the allergy season. Other studies routinely use three to 30 times this amount of pollen, and therefore, the results of this study may be somewhat different from results of other studies on this basis alone. Moreover, the nasal mucosa is washed to remove the allergen after only 3 minutes to allow accurate measurement of total pro-

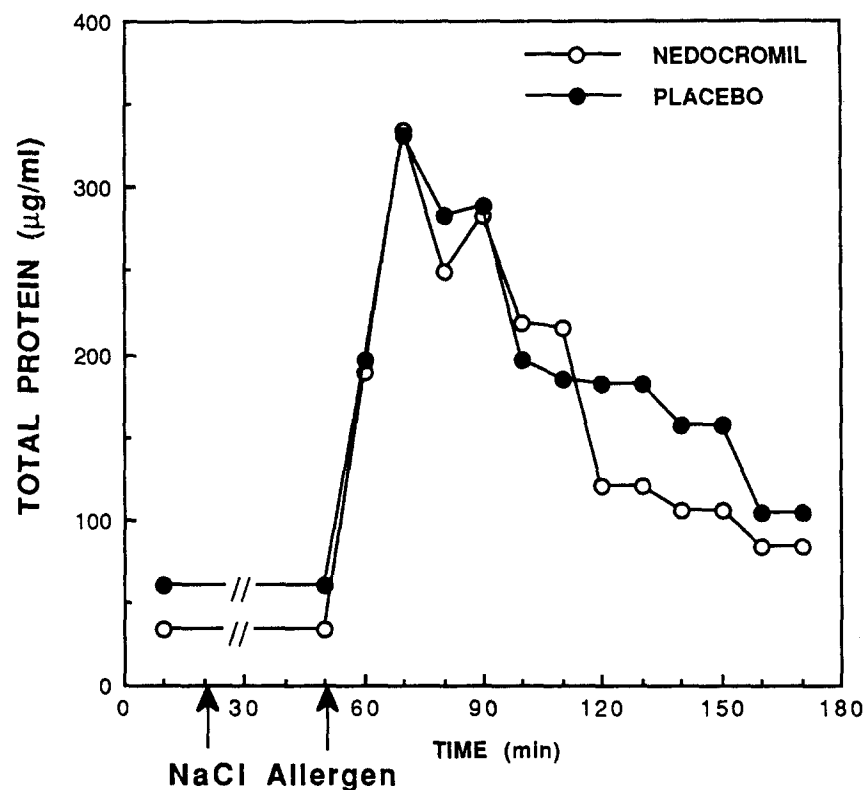


FIG. 4. Time course of mean total protein levels after saline and allergen challenges. Total protein remained above the 95% CI for 100 minutes in placebo-treated subjects and 120 minutes in NS-treated subjects. Total protein levels were significantly higher in placebo-treated group at 70 to 80 minutes after allergen challenge as compared with that in NS-treated group (Wilcoxon's signed-rank test, $p = 0.03$).

tein secreted in response to challenge. Again, most other studies allow a much longer challenge period and, in addition, often rechallenge subjects with larger doses of allergen after only 10 or 20 minutes.

Although all study designs have their own advantages, this procedure allows for the quantitative collection of secretions and mediators over time and demonstrates that even a single, relatively low-dose allergen challenge has profound and prolonged responses. Although PGD₂ rose dramatically after allergen challenge, much as noted in an earlier allergen study,⁷ histamine levels did not increase. Perhaps the histamine RIA used was too specific.¹¹ Many earlier studies used a fluorometric assay for histamine that also recognized other compounds.¹³ In the allergen studies performed at National Institutes of Health, no increase in histamine after allergen challenge has been detected.⁷ However, in both studies, PGD₂ levels increased markedly above baseline within 10 minutes of allergen challenge. Symptoms of sneezing, itching, nasal congestion, and rhinorrhea were significantly elevated after allergen challenge. Our continuing studies with higher doses and multiple doses of allergen

have also failed to elicit increased levels of histamine despite marked increases in symptom scores and PGD₂ release.

It is possible that interstitial histamine may have been released but then diluted by an outpouring of plasma exudate that is then transported into nasal secretions. This dilutional effect would not be observed in other models of nasal-antigen challenge in which oxymetazoline pretreatment is used to prevent excessive nasal congestion. By interfering with normal vascular responses, less vascular permeability would occur, and plasma exudation associated with allergen challenges would be decreased. In any case, although the present model does not demonstrate histamine release, it regularly elicits PGD₂ generation and other unequivocal signs, symptoms, and responses indicative of AR.

The increase in PGD₂ levels and symptomatology experienced by the subjects are compatible with an allergic response to the allergen. It is unlikely that the response was a nonspecific reaction to the allergen diluent, since in a previous study with the same skin test materials,⁷ the allergen diluent (glycerinated sa-

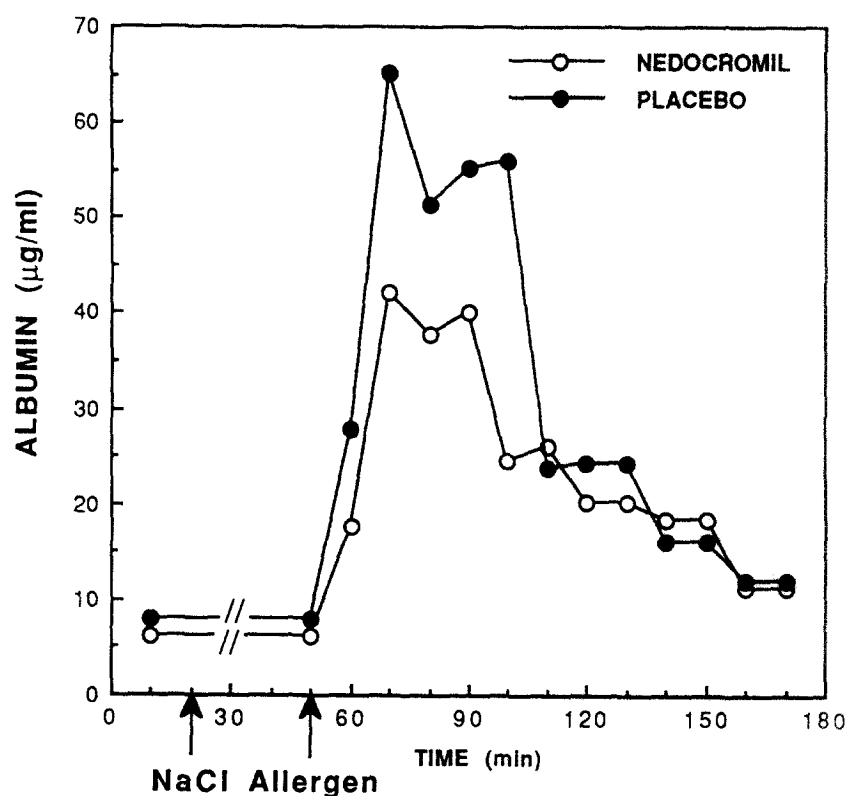


FIG. 5. Time course of mean albumin levels after saline and allergen challenges. In both NS- and placebo-treated subjects, albumin levels increased significantly over baseline values, remaining above the 95% CI for 100 minutes in placebo-treated subjects and 120 minutes in NS-treated subjects. Ten minutes after allergen challenge, significantly increased levels of albumin were noted in placebo-treated group as compared with that in NS-treated group (Wilcoxon's signed-rank test, $p = 0.02$).

line), after being diluted 1:10 with normal saline, was found to have no irritant effect on the nasal mucosa. In the present study, subjects were observed for 30 minutes after saline challenge. Neither allergic symptoms nor protein secretions were noted. We do not therefore believe that the symptoms and secretions observed after allergen challenge represent a nonspecific irritant response.

NS has been extensively tested for use as a 1% topical nasal spray in the treatment of AR. Clague et al.¹⁴ treated 10 patients with AR with NS or placebo for 1 week. The patients then had a nasal provocation with allergen in the left nostril and saline in the right nostril followed by nasal biopsies. NS was significantly more effective in reducing nasal obstruction, rhinorrhea, and sneezing. Although there was no significant increase in mast cell numbers after allergen challenge, there was significantly more mast cell degranulation noted after allergen challenge as compared to saline challenge. However, NS was no more effective than placebo in preventing histologic mast cell degranulation. Lozewicz et al.¹⁵ performed nasal biop-

sies on allergic individuals both before and during pollen season. Twenty-two patients received NS or placebo four times a day for 8 weeks. There was a significant reduction in usage of terfenadine and ocular decongestants during this period. Both groups had significantly increased mast cells and eosinophils during pollen season. In addition, mast cells were significantly decreased with NS compared to the decrease with placebo. Eosinophils were not significantly reduced.

In separate 8-week trials, Schwartz et al.,¹⁶ Ruhno et al.,¹⁷ Schuller et al.,¹⁸ and Druce et al.¹⁹ studied 100, 38, 228, and 177 seasonal allergic patients, respectively. Patients received NS or placebo two to four times a day during peak pollen season. Concomitant medications in the studies by Schwartz et al.,¹⁶ Schuller et al.,¹⁸ and Druce et al.¹⁹ included antihistamine/decongestants and artificial tears. β_2 -Agonist bronchodilators and inhaled corticosteroids were allowed for subjects with asthma by Schuller et al.¹⁸ and Druce et al.¹⁹ In contrast, Ruhno et al.¹⁷ allowed the concomitant use of ocular cromolyn in addition to

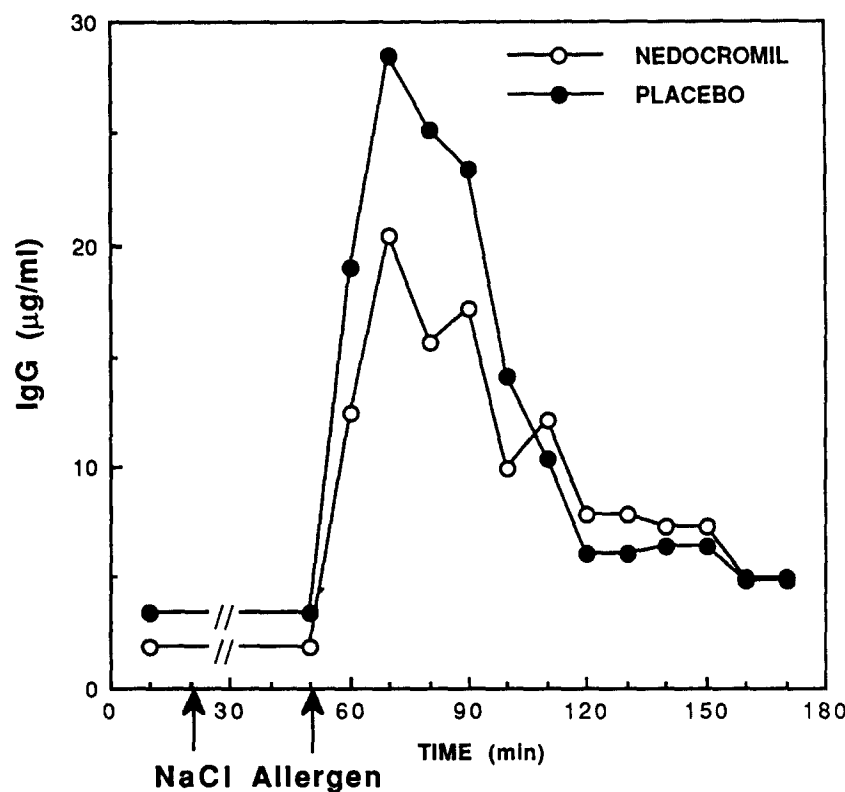


FIG. 6. Time course of mean IgG levels after saline and allergen challenges. Both NS- and placebo-treated subjects experienced a significant increase in IgG levels after allergen challenge, which remained above the 95% CI for 100 minutes in placebo-treated subjects and 120 minutes in NS-treated subjects. No significant differences were noted between two treatment groups.

artificial tears and terfenadine. Inhaled salbutamol was allowed for chest symptoms, and if symptoms were "not controlled, inhaled cromolyn or beclomethasone could be added." Although the "use of the test solution, antihistamine, salbutamol, cromolyn sodium, or other medications were recorded daily," it was not stated whether there was any significant decrease in use of salbutamol or cromolyn. During the peak pollen periods, NS was noted to cause a significant reduction in rhinorrhea, nasal congestion, sneezing, rhinitis, itchy eyes, and sleep disturbance. Ruhno et al.¹⁷ noted a significant decrease in antihistamine use. The clinical assessment was significantly in favor of NS compared to placebo in the study of Schuller et al.¹⁸

In separate 4-week trials with NS during peak pollen seasons, Juul et al.,²⁰ Lindsay-Miller,²¹ Sipila et al.,²² Bellioni et al.,²³ and Frei et al.²⁴ studied a total of 249 patients. In these studies, a significant reduction occurred in symptoms with a concurrent significant decrease in use of antihistamines. Sipila et al.²² noted that nasal peak flow rates were slightly better in the NS-treated group. Both patients and clinicians favored the treatment with NS over that with placebo.

In a study involving single-dose NS, Ruhno et al.²⁵

challenged 14 patients with ragweed at 7-day intervals in a placebo-controlled, double-blind, crossover study. No differences between NS- and placebo-treated patients were noted in lavage TAME esterase levels 10 minutes after allergen challenge. A symptom score relating the number of sneezes and nasal airway inspiratory resistance was found to be lower in patients pretreated with NS ($p = 0.015$). Another study with a single dose of NS was reported by Corrado et al.²⁶ Ten patients with AR were challenged with allergen on 2 days, 1 to 2 weeks apart. Concomitant medications included antihistamines and nasal decongestants. Each patient was pretreated with NS or placebo 20 minutes before challenge. NS was significantly more effective than placebo in inhibiting nasal obstruction ($p < 0.01$), rhinorrhea ($p < 0.01$), and sneezing ($p < 0.05$).

Unlike the previous trials,¹⁴⁻²⁴ which all involved long-term pretreatment with NS before allergen challenge (1 to 8 weeks), the current study involved two single doses of NS administered minutes before challenge. The trials by Ruhno et al.¹⁷ and Corrado et al.²⁶ with single doses of NS are therefore more comparable to the current protocol. However, Corrado et al.²⁶ al-

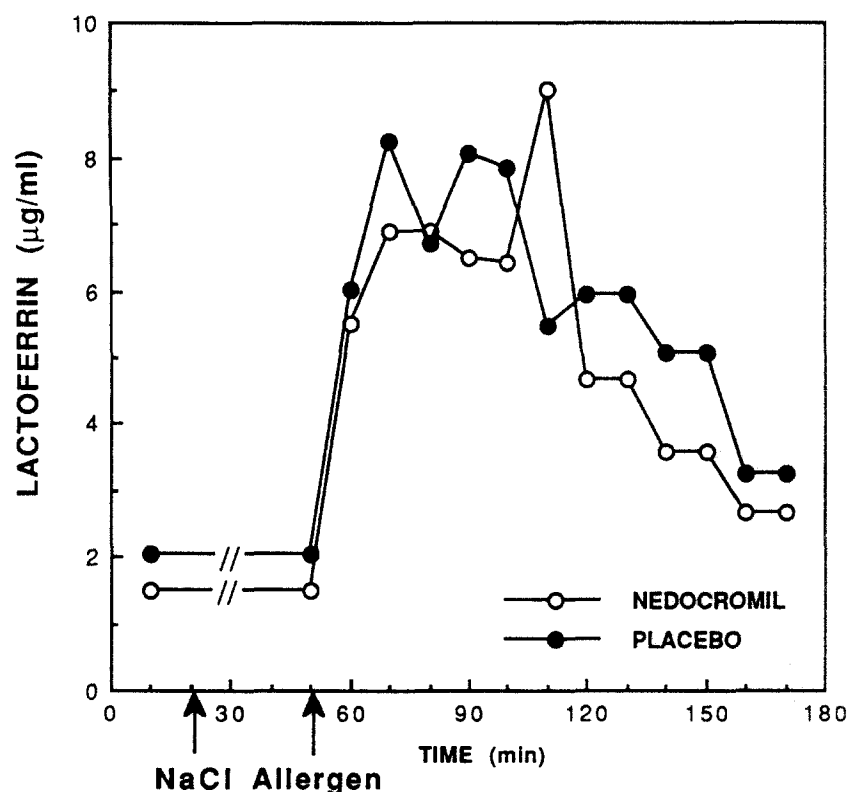


FIG. 7. Time course of mean lactoferrin levels after saline and allergen challenges. Both treatment groups experienced significant increase in lactoferrin levels after allergen challenge, which remained above the 95% CI for 100 minutes in placebo-treated subjects and 120 minutes in NS-treated subjects. No significant differences were noted between treatment groups.

lowed concomitant medications during the trial. One of the 10 patients required an antihistamine/decongestant for 36 hours after the initial nasal-provocation test. It was not specified whether placebo or NS had been administered during this test. These medications introduced a variable that makes determination of the actual effects of NS less clear. In our study, all medications were stopped before entering the study. Two hours after allergen provocation, the patients were allowed to use topical beclomethasone to inhibit LPRs as well as a short-acting oral antihistamine for 24 hours. Since we were not investigating the effect of NS on LPRs, this prophylactic medication did not interfere with the study. In previous studies, the efficacy of the drug was often based on the clinicians' and/or the patients' subjective assessment of the drug in regards to symptom reduction. However, in this study, the effect of NS on objective changes in secreted proteins and mast cell mediators was examined.

The nasal-challenge protocol performed has been used extensively during the past few years to determine and verify the physiologic effect of various drugs and mechanical stimuli on nasal mucosa. The assays are quite sensitive in detecting changes in secretions

corresponding to increased glandular or vascular secretion. In addition, assaying secretions for histamine and PGD_2 permits assessment of mast cell degranulation caused by allergen challenge.

This protocol revealed that two single doses of 130 µl of a 1% solution of NS caused no significant overall reduction in peak allergen-induced symptoms, PGD_2 generation, or secretion of the vascular proteins, albumin and IgG, or the glandular protein, lactoferrin, compared with placebo. By Wilcoxon's signed-rank test, there were only four individual statistically significant points in which NS pretreatment was associated with lower protein levels or symptom scores. Although there appeared to be a trend for lower secretion of PGD_2 and the vascular proteins, albumin and IgG, in the NS-treated group, the differences, except as mentioned above, did not achieve statistical significance. It was the intention of this study to dissect the mode of action of NS in preventing allergen-induced responses. However, NS had no clear effects on either vascular or glandular secretion or on mast cell degranulation. To observe the anti-inflammatory and mast cell-stabilizing effects of NS, perhaps more prolonged pretreatment with NS before nasal-allergen

provocation might be useful and may be attempted in the future.

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