

The effect of nedocromil sodium on the early and late reaction and allergen-induced bronchial hyperresponsiveness

René Aalbers, MD,* Henk F. Kauffman, PhD,* Henk Groen, MD,**
Gerard H. Koëter, MD, PhD, and Jan G. R. de Monchy, MD, PhD*
Groningen, The Netherlands

Bronchial hyperresponsiveness (BHR) to methacholine was studied in 14 patients with asthma and five healthy control subjects, with and without pretreatment with nedocromil sodium, 3 and 24 hours after allergen challenge. Eleven patients demonstrated a dual asthmatic response. A significant decrease in the provocative concentration causing a 20% fall in FEV₁ was found from a geometric mean starting value of 1.18 mg/ml on the control day to 0.24 mg/ml ($p < 0.001$) and to 0.17 mg/ml ($p < 0.001$) 3 and 24 hours after allergen challenge. A significant correlation was observed between the increased BHR at 3 hours and the magnitude of the late response ($r = -0.57$; $p < 0.05$). Nedocromil sodium (6 mg) significantly inhibited the increase in BHR, 1 mg/ml ($p < 0.001$) at 3 hours and 0.50 mg/ml ($p < 0.001$) at 24 hours. Nedocromil sodium shifted the severity of the early allergic reaction (EAR) from mean -34.8% to -6.9% and inhibited the later allergic reaction (LAR) from -30.5% to $+0.4\%$ ($p < 0.005$). From the study can be concluded that nedocromil sodium inhibits the EAR and LAR and the allergen-induced increase in BHR. The inhibitory effect of nedocromil sodium on the LAR may be related to its ability to inhibit the increased BHR before the development of the LAR. (J ALLERGY CLIN IMMUNOL 1991;87:993-1001.)

BHR, a characteristic feature of asthma, is related to the severity of the disease and the need for therapy.^{1,2} The precise link between symptoms, BHR, and airway inflammation is still uncertain.^{3,4}

BHR can be assessed by direct and indirect stimuli. It is supposed that the bronchoconstriction induced by methacholine results from direct stimulation of the smooth muscle itself.⁵ BHR increases in sensitized patients during natural exposure to airborne allergens or occupational allergens that induce asthma. Conversely, removal from environmental exposure to domestic or occupational allergens often results in a decreased BHR.⁶⁻⁹

Abbreviations used

BHR:	Bronchial hyperresponsiveness
EAR:	Early asthmatic reaction
LAR:	Late asthmatic reaction
HDM:	House dust mite
VC:	Vital capacity
PBS:	Phosphate-buffered saline
PC ₂₀ :	Provocative concentration causing a 20% fall in FEV ₁
AUC:	Area under the curve
GM:	Geometric mean
Ag:	Antigen

From the *Departments of Allergology **and Pulmonology, Clinic for Internal Medicine, University Hospital, Groningen, The Netherlands.

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Reprint requests: R. Aalbers, MD, Department of Allergology, Clinic for Internal Medicine, University Hospital, Oostersingel 59, 9713 EZ Groningen, The Netherlands.

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Enhancement of BHR through allergen inhalation in sensitized patients has been observed by many investigators.¹⁰⁻¹³ Recently, an early increase in BHR has been linked to the severity of the LAR.^{12, 13} The mechanisms of both the LAR and Ag-induced increases in BHR are not completely understood, however. The allergen-induced increase in BHR and the development of an EAR and LAR may both depend on the release of inflammatory mediators.^{14, 15} Such mechanisms may explain the clinical useful-

TABLE I. Clinical data of the patients with asthma

Pt. No.	Age (yr)	Sex	HOM		Specific IgE-HDM (PRU/ml)	Histamine PC ₂₀ (mg/ml)	VC (L)	FEV ₁ (L/sec)	% Pred	Med
			HEWS ratio	LPR in skin (mm)						
1	18	F	1.6	42	>17.5	0.75	3.4	2.9	85	B, C, I
2	18	F	1.2	30	>17.5	12.70	4.4	4.0	108	S, T
3	21	F	1.5	40	17.5	0.50	4.9	3.0	75	C, S
4	20	F	1.6	48	14.6	1.20	3.1	2.5	89	C
5	22	F	1.2	34	>17.5	0.88	3.1	2.5	73	B, C, S
6	21	F	1.6	44	>17.5	2.00	2.9	2.5	86	C, S
7	23	F	1.6	42	13.7	16.00	3.8	3.5	100	S
8	22	F	1.1	37	2.4	0.64	4.3	3.6	100	B, C, S
9	29	M	1.8	20	>17.5	1.26	5.8	4.5	93	C, S
10	19	M	1.0	40	>17.5	10.00	6.1	4.5	96	S
11	24	M	1.6	65	>17.5	0.50	5.9	3.7	80	I, N
12	22	F	1.3	46	>17.5	1.00	3.8	2.5	65	B, C, S
13	28	F	1.2	40	2.7	4.00	3.9	3.2	110	T
14	21	F	1.3	47	>17.5	2.00	3.4	2.9	88	C, S
Mean	22		1.4	41		3.80	4.2	3.3	89	
± SEM	0.87		0.06	2.70		1.37	0.29	0.20	3.5	

Pt, Patient; HEWS, histamine equivalent wheal size; PRU, Phadebas RAST unit; med, medication; B, beclomethasone or budesonide; C, sodium cromoglycate; I, ipratropium bromide; N, nedocromil sodium; S, salbutamol; T, terfenadine.

ness of anti-inflammatory drugs against LARs and BHR.¹⁶⁻¹⁸

Nedocromil sodium (Tilade; Fisons Corp., Bedford, Mass.) is the disodium salt of a pyranoquinoline dicarboxylic acid that has demonstrated activity on allergen-induced EARs and LARs, as well as bronchoconstriction induced by fog, cold air, SO₂ and adenosine monophosphate.¹⁹⁻²⁴

The objective of the present study was to investigate if a single dose of nedocromil sodium could protect against HDM-induced EAR and LAR in atopic patients with asthma.

In addition, we studied the influence of this drug on the allergen-induced changes in BHR 3 and 24 hours after allergen challenge. Inhibition of the increase in BHR at the two different time points could reveal an indirect insight into mechanisms underlying the allergen-induced increase in BHR before and after the LAR.

METHODS

Subjects

Fourteen allergic subjects with asthma (11 women and three men) and five normal, healthy, control subjects participated in the study. The clinical characteristics of the patients are presented in Table I. All subjects with asthma were selected on the basis of positive skin tests and enhanced specific IgE for HDM allergen (Phadezym RAST, Pharmacia, Uppsala, Sweden) and increased bronchial respon-

siveness to histamine inhalation. The patients had experienced no acute asthmatic attacks for at least 2 months, and there was no history of respiratory tract infections during this period. None of the patients received oral corticosteroids. All medication was withheld before the study days. Inhaled corticosteroids and sodium cromoglycate were stopped 1 week before the study; β -adrenergic drugs were stopped at least 24 hours before the study. The FEV₁ values of the subjects with asthma were >65% of their predicted values. Five normal, healthy subjects (three women and two men) participated in this study as controls. All five subjects had negative skin tests and no detectable IgE to common allergens. Inhalation challenge with methacholine and HDM allergens in the control subjects were negative. None of the participating subjects were smokers or used medication that could interfere with the results. All subjects gave written informed consent, and the study was approved by the Medical Ethics Committee of the University Hospital, Groningen, The Netherlands.

Methacholine provocation

Methacholine (Sigma Chemical Co., St. Louis, Mo.) was prepared in 0.9% sodium chloride solution to produce a range of doubling concentrations from 0.03 to 256 mg/ml. The solutions were administered with a breath-actuated dosimeter APS (E. Jaeger GmbH, Würzburg, Germany) containing 3 ml of solution, which allowed air at 20 psi into the nebulizer for 0.4 seconds. The output was 9.04 ± 0.43 μ l/0.4 sec of nebulization. With a mouthpiece and noseclip, subjects received the aerosolized solutions in five inhalations from end tidal volume to full inspiratory capacity, starting

with the lowest dose and repeating at 3-minute intervals. The PC₂₀ methacholine of the post-PBS value was considered as a threshold value and was derived by linear interpolation.

HDM inhalation

Allergen solutions were prepared from stock solutions of *Dermatophagoides pteronyssinus* (HDM), diluted in PBS with 0.03% human serum albumin and with 0.5% phenol (Diephuis Laboratories, Groningen, The Netherlands) to produce a range of fivefold increasing concentrations from 80 to 10,000 Ne/ml. The allergen solutions were administered in 10 inhalations with the dosimeter, as described for methacholine, and inhaling at 15-minute intervals starting with the lowest dose. The dose of PC₂₀ HDM of post-PBS value was considered as a threshold value and was derived by linear interpolation.

Study design

Subjects attended the laboratory on 3 consecutive days with an additional visit (allergen challenge with nedocromil sodium) 8 to 10 days later. Pulmonary function was measured as VC and FEV₁ with a water-sealed spirometer. The patients used a Schiller spirometer, type SP-1A (Schiller AG, Baar, Switzerland) for FEV₁ measurement during the evening hours on the day of the challenge.

On the first day, control solution was inhaled four times at 15-minute intervals, measuring VC and FEV₁ immediately and 15 minutes after each inhalation. After the fourth inhalation of control solution, spirometry was recorded at 10-minute intervals for the first hour, and hourly for 8 hours. Three hours after the last inhalation of the control solution, a methacholine inhalation test was performed. At 5 PM, the patients went home after having received instructions on how to use the Schiller spirometer for measurements every hour until the subject retired to bed. Patients also received instructions of what to do in case of chest complaints. The subjects returned to the hospital the next morning at 8 AM. Allergen challenge with HDM was started at 8:30 AM if the VC and FEV₁ had returned to baseline values. Spirometry was performed as described. At precisely 3 hours after the last administered dose of allergen, methacholine inhalation tests were performed, again only if the VC and FEV₁ had returned to baseline values. At 5 PM, the subjects went home with the same instructions. On the morning of the third day, patients attended the hospital at 9 AM and after VC and FEV₁ had been assessed as having returned to baseline values, a methacholine inhalation test was again performed 24 hours after the HDM challenge. When a decrease in FEV₁ of 20% or more was observed, all patients received interventional inhalation therapy.

Between 8 and 10 days later, the subjects returned to the hospital at 8 AM, and allergen challenge was performed again, now preceded by three inhalations of nedocromil sodium (2 mg/puff) 15 minutes before the inhalation provocation. On the fifth visit, 24 hours after the second allergen challenge, a methacholine inhalation test was again performed, as described for the third day. None of the subjects

was aware of the type of drug and its influence on the allergen challenge. To prevent an increase in the complexity and duration of this part of the study, we chose a single-blind, not placebo-controlled, design.

Data analysis

Statistical analyses were performed with the SPSS/PC + statistical package (Spectrum Medical Industries, Inc., Los Angeles, Calif.). Values refer to the mean $\pm$ SEM unless it is otherwise stated, and the $p < 0.05$ level of significance was accepted. Baseline values on all study days and the changes in PC₂₀ values after allergen challenge were compared with Wilcoxon's matched-pairs, signed-ranks test.

The mean changes in methacholine two-log PC₂₀ after allergen for the subjects with a definite LAR were compared with the corresponding values for the whole group and for subjects with only an EAR. Group comparisons were performed by use of the two-tailed Mann-Whitney U test.

The following indices were selected to describe the FEV₁ time-response curves after administration of the PD₂₀ HDM compared to the FEV₁ after control solution: (1) maximal decrease in FEV₁ from the postsaline baseline value and (2) the overall bronchoconstrictor response described as the area under the percentage fall in FEV₁ time curve (AUC) during the EAR and the LAR, derived by trapezoid integration. The Schiller spirometer measurements were used as supplementary data in the analysis of the LAR.

Subjects were considered to have an LAR if their maximal decrease in FEV₁, measured from 4 hours after the onset of the EAR, was $>20\%$ and/or a decrease of $>20\%$ was measured by Schiller spirometer values at home. Any relation between the percent decrease in FEV₁ and AUC of the EAR and LAR and the two-log PC₂₀ at 3 and 24 hours after HDM was sought by least-squares linear-regression analysis.

RESULTS

There were no changes in PC₂₀ methacholine after HDM challenge with and without nedocromil sodium in the normal healthy control subjects; nor did these subjects demonstrate a bronchoconstriction on challenge with HDM that was measured by FEV₁ and AUC.

The clinical data of the patients are presented in Table I. After the first unprotected HDM challenge, four patients (Nos. 3, 4, 5, and 9) required salbutamol (100 μ g) at night because of dyspnea. None of the patients required additional medication after the second challenge, which was preceded by nedocromil sodium. There were no significant differences in baseline values of FEV₁ between any of the study days. Thus, comparisons of data obtained on different study days are valid.

Data are presented in GM; values in parentheses are the logarithmic standard errors of the mean.

TABLE II. Obstructive reactions after HDM challenge before and after nedocromil sodium

Pt. No.	EAR		LAR		EAR		LAR	
	% FEV ₁	AUC (without nedocromil sodium)	% FEV ₁	AUC	% FEV ₁	AUC (with nedocromil sodium)	% FEV ₁	AUC
1	-54	47.4	-68	205.0	-11	4.8	-17	23.0
2	-21	14.8	-8	8.0	-22	7.3	0	-16.5
3	-20	8.1	-32	38.6	-1	-4.9	+4	-24.4
4	-33	18.2	-22	43.0	-7	2.8	-9	25.0
5	-52	36.3	-50	134.2	-2	-6.7	-2	25.8
6	-41	29.7	-41	120.0	-11	2.8	-16	52.0
7	-34	15.2	-4	4.2	-1	-1.1	+3	-22.7
8	-22	8.4	-22	21.5	-6	-4.2	+10	-52.3
9	-50	27.4	+2	-78.5	-15	-20.0	+23	-129.0
10	-22	8.7	-36	64.0	-5	-1.9	-5	4.0
11	-43	25.9	-16	43.1	+5	-6.3	-5	5.8
12	-25	14.1	-42	118.0	-7	-1.6	+13	-92.0
13	-45	24.8	-8	5.3	-7	2.0	+2	-12.4
14	-20	10.1	-9	13.0	-6	-3.1	+5	-30.0
Mean	-35	20.77	-25	52.8	-7	-2.1	+0.4	-21.0
± SEM	3.5	3.2	5.4	19.0	1.8	1.8	3.0	12.6

Unprotected allergen challenge

Eleven of the 14 patients had an EAR and LAR (dual responders) after HDM challenge (Table II). For three patients (Nos. 9, 11, and 14), the LAR was measured with the Schiller spirometer at home. The mean early percentage fall in FEV₁ after the allergen challenge was 34.8% of the initial value (range, 20% to 54%). The mean early AUC was 20.7 (range, 8.1 to 47.4). This initial fall had resolved completely by 3 hours and was followed by a late response (except for patients 2, 7, and 13) with a peak between 4 and 11 hours after allergen challenge. The mean fall in FEV₁ for the late response was 30.5% (range, 20% to 68%), and the mean AUC was 65.6 (range, 21.5 to 205) (Table II).

Bronchial responsiveness after allergen challenge

After the first unprotected HDM challenge, there was a significant decrease in the PC₂₀ for all patients, from a GM value of 1.18 mg/ml (0.63) for the PC₂₀ on the control day after PBS to 0.24 mg/ml (0.74) ($p < 0.001$) at 3 hours and to 0.17 mg/ml (0.69) ($p < 0.001$) at 24 hours after HDM challenge. Comparing the decrease in PC₂₀ relative to baseline at 3 hours with that at 24 hours after the first challenge, there was a difference, although this was not significant. At 24 hours after the allergen challenge, nine of the 14 patients were even more hyperresponsive than at 3 hours. Details of the PC₂₀ values are presented in Table III.

When the PC₂₀ values of the dual responders were analyzed separately, the PC₂₀ after PBS demonstrated a GM of 0.67 mg/ml (0.57); at 3 hours after HDM, 0.13 mg/ml (0.68); and at 24 hours, 0.09 mg/ml (0.63). Thus, the dual responders proved to be more hyperresponsive than the whole group of patients. This difference was significant after PBS and at 3 hours and 24 hours after challenge ($p < 0.05$).

The magnitude of the changes in PC₂₀ after the allergen challenge (expressed as prechallenge two-log PC₂₀ minus postchallenge two-log PC₂₀ in all patients) was compared with the magnitude of the LAR with the percent decrease in FEV₁ and the AUC. There was a significant correlation between the change in magnitude of the PC₂₀ at 3 hours, based on all data and the severity of the LAR: $r = -0.57$ and $p < 0.05$ for the decrease in FEV₁ and $r = 0.55$ and $p < 0.05$ for the AUC. No significant correlation could be demonstrated between the PC₂₀ at 24 hours and the LAR.

Protection with nedocromil sodium

The second HDM challenge was performed between 8 and 10 days after the first challenge and was preceded at 15 minutes by a single-blind inhalation of 6 mg of nedocromil sodium. The patients were unaware of the possible effects of the drug administered.

The mean early fall in FEV₁ after the protected challenge was 6.9% (range, +5% to -22%). The mean early AUC was -2.1 (range, -20 to +7). No late fall in FEV₁ could be demonstrated, mean,

TABLE III. PC₂₀ (milligrams per milliliter) methacholine, 3 hours after control solution (PBS) and 3 and 24 hours after HDM challenge with and without protection of nedocromil sodium

Control solution		HDM (3 hr) (without nedocromil sodium)	HDM (24 hr) (without nedocromil sodium)	HDM (3 hr) (with nedocromil sodium)	HDM (24 hr) (with nedocromil sodium)
1	0.40	0.03	0.03	0.25	0.12
2	19.20	5.04	1.30	20.20	4.10
3	1.00	0.12	0.02	0.41	0.40
4	0.78	0.12	0.12	1.26	0.28
5	0.76	0.12	0.11	0.79	0.23
6	0.82	0.09	0.06	0.14	0.11
7	9.50	6.18	5.66	13.39	13.39
8	0.18	0.04	0.09	0.35	0.21
9	0.14	0.05	0.02	0.20	0.09
10	18.90	6.90	2.30	10.60	3.70
11	0.71	0.19	0.52	1.10	0.62
12	0.22	0.03	0.03	0.20	0.11
13	5.09	0.33	0.32	2.17	1.45
14	0.80	0.48	0.17	0.72	0.46
GM	1.18	0.24	0.17	1.00	0.50
± SEM	0.63	0.74	0.69	0.63	0.60

SEM of the logarithmic value of the mean.

+0.43% (range, -17% to +23%). This was also found for the late AUC, mean, -21.02 (range, 52.0 to -129.0) (see Figs. 2 and 3). (A negative value in the case of the AUC means a negative area and therefore better than after PBS.) Nedocromil sodium protected against both allergen-induced EAR and LAR, with the LAR being prevented more fully than the EAR. This protective effect was significant for both the EAR and LAR ($p < 0.005$).

Bronchial responsiveness after protection

The PC₂₀ for all patients at 3 hours after the second (protected) HDM challenge, GM 1.0 mg/ml (0.63), did not differ significantly from the PC₂₀ on day 1 after the control solution, 1.18 mg/ml (0.63). Seven of the 14 patients even demonstrated an improved PC₂₀ after the protected challenge (Table III). When the PC₂₀ at 3 hours after the first (unprotected) HDM challenge was compared with the PC₂₀ at 3 hours after the protected HDM challenge, a significant inhibitory effect of nedocromil sodium was demonstrated ($p < 0.001$) (Fig. 1). This finding strongly suggests a protective effect of nedocromil sodium on the allergen-induced increase in BHR at 3 hours after HDM challenge.

The observations at 24 hours after HDM challenge demonstrated comparable results. Twenty-four hours after the second (protected) challenge, there was a decrease in PC₂₀ compared with the PC₂₀ after the control solution on day 1, 0.50 mg/ml (0.60) ($p < 0.01$), but when the PC₂₀s at 24 hours after the

first (unprotected) and after the second (protected) HDM challenge were compared, a significant protective effect of nedocromil sodium was again found ($p < 0.001$) (Fig. 1). All 14 patients were less hyperresponsive after pretreatment (second HDM challenge) than after the first HDM challenge (Table III).

Analysis of the PC₂₀ values of the 11 dual responders again demonstrated a significant protective effect of nedocromil sodium. The GM PC₂₀ after the protected challenge in the dual responders was 0.56 mg/ml (0.53) ($p < 0.005$) at 3 hours and 0.28 mg/ml (0.47) ($p < 0.005$) at 24 hours, compared to the corresponding measurements of the first, unprotected HDM challenge in the dual responders.

After treatment with nedocromil sodium, no significant correlation could be demonstrated between the change in magnitude of the PC₂₀ at 3 hours and the development of the LAR.

DISCUSSION

The results of this study demonstrate that there is a protective effect of nedocromil sodium on the EAR, LAR, and on the allergen-induced increase in BHR also, when it is administered as a single dose before allergen challenge.

We have demonstrated that BHR does increase 3 hours after allergen challenge when the FEV₁ has returned to baseline values. We were also able to demonstrate a significant correlation between the increase in BHR at 3 hours and the severity of the LAR that was not demonstrated after pretreatment with nedo-

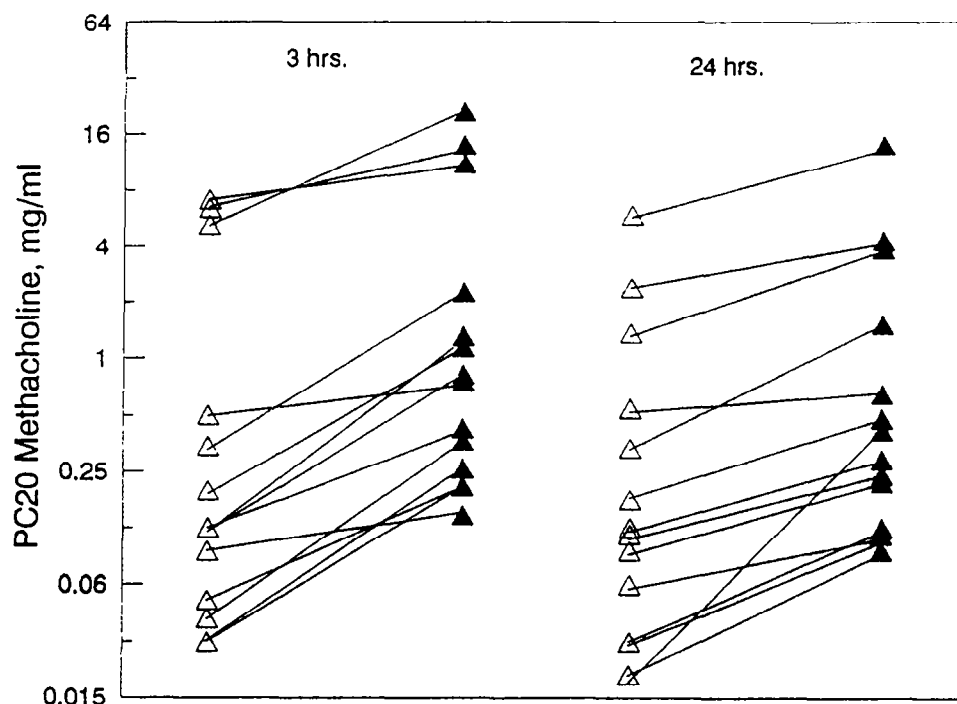


FIG. 1. Represented are the PC₂₀ 3 and 24 hours after allergen challenge, untreated (Δ) and preceded by nedocromil sodium ($\blacktriangle$) (N = 14). The difference was significant both at 3 and at 24 hours ($p < 0.001$).

cromil sodium. Durham et al.^{12, 13} first described, in two studies, increases in airway histamine responsiveness at 3 hours before the late response that was highly correlated with the magnitude of the late response. In our study, using methacholine instead of histamine, we were able to confirm their findings.

The differences found before and after nedocromil sodium cannot be explained by different basal measurements, since comparable baseline values for VC and FEV₁ were obtained on all study days. In this study we did not check the PC₂₀ methacholine before the second (protected) allergen challenge; however, in previous work, the 8- to 10-day time interval has proved to be adequate.¹¹

In earlier *in vivo* studies, in humans as well as in animals, nedocromil sodium has been demonstrated to inhibit allergen-induced immediate and late bronchoconstriction when it is administered as an aerosol before provocation.^{19, 20, 25, 39} It has also been demonstrated that nedocromil sodium exhibits inhibitory activity generally superior to that of sodium cromoglycate against bronchoconstriction provoked by various nonspecific stimuli, such as sulfur dioxide, adenosine monophosphate and neurokinin A.²²⁻²⁷ Protective effects of nedocromil sodium on Ag-induced increases in BHR have been demonstrated in a

sheep model and in humans during the grass-pollen season.^{20, 28}

In the present study, inhibition of EAR and LAR together with a significant improvement in BHR was observed after HDM challenge preceded by nedocromil sodium (Figs. 1 to 3). A single-blind study design was chosen, but since the changes were large and equivocal, the results appear to be valid.

It is uncertain what determines the changes in BHR between the EAR and LAR. The contribution of inflammatory cells and their mediators to the pathophysiology of the LAR may play a role in the allergen-induced increase in BHR as well. We were not able to demonstrate an increase in inflammatory cells in the lavage fluid before LAR after allergen challenge in patients who developed an LAR.²⁹ This may suggest that the increased BHR at 3 hours is not the result of inflammatory changes. It is attractive, however, to suppose that inflammatory cells are already present and activated in the bronchial wall between the EAR and LAR but have not yet infiltrated the bronchial lumen. Studies of biopsy specimens have not yet been able to demonstrate a significant correlation between inflammatory cell numbers and BHR.^{30, 31} However, these observations do not exclude a role for inflammatory cells and their products in an allergen-induced

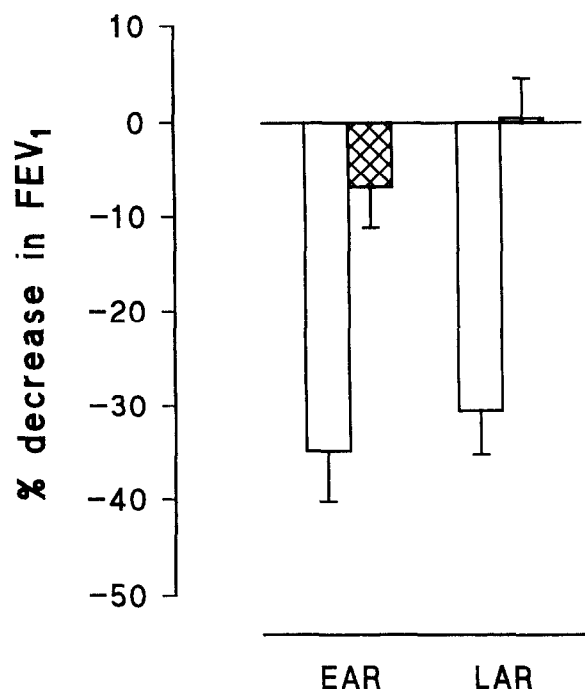


FIG. 2. Represented are the EAR and LAR untreated (*open bars*) and preceded by nedocromil sodium (*cross bars*), expressed as percentage decrease in FEV₁ after HDM challenge ($p < 0.005$).

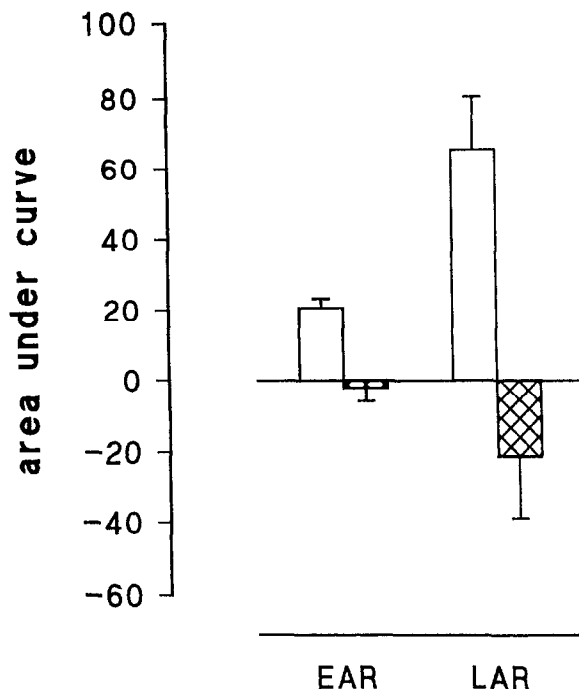


FIG. 3. Represented are the EAR and LAR untreated (*open bars*) and preceded by nedocromil sodium (*cross bars*), expressed as the AUC percentage fall in FEV₁ time curve after HDM challenge ($p < 0.005$).

increase in BHR between the EAR and LAR. Priming, cell to cell interaction, and increased cellular activity may all play a part in producing a cascade of changes. Thus, cell activation may be at least as important as the presence of increased numbers of inflammatory cells as such.

Loss of epithelium is a consistent finding in the airways of subjects with asthma and may contribute to BHR. Even subjects with stable asthma demonstrate evidence of airway epithelial desquamation. Jeffery et al.³⁰ demonstrated that the degree of epithelial loss correlated with the degree of airway reactivity, and epithelial damage can expose afferent nerve endings to inflammatory mediators.³² Airway epithelial cell arachidonic acid metabolites may also cause contraction of airway smooth muscle.³³ Recent studies have demonstrated that eosinophils and their products play a role in tissue injury during the LAR.²⁹ In addition, significant increases in lymphocytes and neutrophils have been demonstrated to occur.³⁴

Considering these observations, it is reasonable to speculate that the relative contributions of the several mechanisms involved might differ between the allergen-induced increase in BHR at 3 hours and the development of the LAR.

Although generally the development of an increased

BHR is related to the LAR, the results of this study may demonstrate that the development of an increased BHR at 24 hours after allergen challenge does not necessarily depend on the development of the LAR, since three subjects with only an EAR demonstrated an increase in BHR at 24 hours as well. In this study, no correlation could be demonstrated between the magnitude of the LAR and the increased BHR at 24 hours. This has been found in both human and animal studies. In sheep, the 24-hour Ag-induced BHR was blocked by treating the animals before Ag challenge with either indomethacin or FPL-57231 (a sulphido-peptide leukotriene antagonist)¹⁵ and nedocromil sodium.²⁰ In these studies, indomethacin did not block the LAR, indicating the different mechanisms may be responsible for the LAR and the 24-hour increase in BHR. Similar findings have been reported in patients with asthma.¹⁴ The above findings and the results of this study may thus imply indirectly that the allergen-induced increase in BHR at 3 hours is mediated by other mechanisms than the allergen-induced increase in BHR at 24 hours.

To date, the precise mechanism by which nedocromil sodium acts is unclear. Several modes of action of nedocromil sodium have been proposed and demonstrated in clinical pharmacology studies and from

preclinical in vitro and in vivo studies including neuronal activity in addition to effects on inflammatory cells.^{35-36,40}

In the present study, nedocromil sodium blocked the EAR, LAR, and the allergen-induced increase in BHR at 3 and at 24 hours. Single-dose inhalation of salbutamol inhibits the EAR but not the LAR nor the allergen-induced increase in BHR. The LAR and allergen-induced increase in BHR, but not the EAR, however, can be inhibited by beclomethasone, although the EAR, LAR, and allergen-induced increase in BHR are inhibited by sodium cromoglycate.³⁷ Several studies of the effects of sodium cromoglycate and nedocromil sodium on allergen-induced asthmatic responses have been published,^{16, 19, 20, 37-39} however, eliciting difficulties in quantitative comparison. Administered before provocation, nedocromil sodium appears to be equipotent or slightly more potent than sodium cromoglycate.²⁵ A direct comparison between the effects of nedocromil sodium and sodium cromoglycate on the early increase in BHR has not been published. Nedocromil sodium and not sodium cromoglycate inhibited the release of histamine, leukotriene C₄, and prostaglandin D₂ from primate bronchoalveolar mast cells after challenge with Ag in vitro.⁴⁰

It is therefore attractive to speculate that by preventing the initial Ag-induced mediator release and by inhibiting the increased BHR at 3 hours, nedocromil sodium inhibits the subsequent inflammatory response and elaboration of mediators leading to the LAR and increased BHR at 24 hours. Additional studies with nedocromil sodium, especially on specimens of BAL and bronchial biopsies, performed at these critical time points, are needed to investigate these possibilities.

In conclusion, the significant correlation between increased BHR at 3 hours and the magnitude of the LAR, also demonstrated in this study, suggests that increased BHR at this time may predict whether patients will develop an LAR. The fact that patients with only an EAR can develop an increased BHR at 24 hours, as well as dual responders, and that in our study no significant correlation of BHR with the LAR could be demonstrated at this time may indicate an independent phenomenon, not directly related to the LAR. A significant inhibitory effect of nedocromil sodium was demonstrated on the EAR, LAR, and allergen-induced increase in BHR. These results confirm in an indirect manner the several modes of action attributed to nedocromil sodium. The inhibitory effect of nedocromil sodium on the allergen-induced increase in BHR at 3 hours may underlie its ability to block the LAR and the allergen-induced increase in BHR at 24 hours.

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