

Neutrophils and mast cells: Nedocromil sodium inhibits the generation of neutrophil-derived histamine-releasing activity (HRA-N)

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The effect of nedocromil sodium on the generation of neutrophil-derived histamine-releasing activity (HRA-N) from human peripheral blood neutrophils and on the secretory effects of HRA-N and anti-IgE on rat basophil leukemia (RBL) cells was studied. Nedocromil sodium caused dose-related inhibition of HRA-N generation by human neutrophils (10^{-7} to 10^{-4} mol/L; inhibitory concentration of 50%, 1.5×10^{-8} mol/L). In contrast, the presence of nedocromil sodium had no effect on HRA-N-mediated serotonin release from RBL cells. In five experiments, with one crude and four partially purified HRA-N preparations, serotonin release from RBL cells exposed to native HRA-N or HRA-N in the presence of nedocromil sodium (10^{-4} mol/L) was $28\% \pm 6\%$ and $26\% \pm 5\%$, respectively. Similar results were found with all concentrations of nedocromil sodium studied. Preincubation of RBL cells with nedocromil sodium (10^{-4} mol/L) for 0 to 60 minutes also did not affect HRA-N-mediated serotonin release. Likewise, nedocromil sodium (10^{-7} to 10^{-4} mol/L) had no effect on anti-IgE-induced serotonin release from RBL cells. In 10 experiments, anti-IgE alone or in the presence of nedocromil sodium (10^{-4} mol/L) induced $32\% \pm 6\%$ and $32\% \pm 5\%$ serotonin release, respectively. Preincubation of RBL cells with nedocromil sodium before anti-IgE exposure had no further effect. This is the first study of pharmacologic manipulation of the generation of a histamine-releasing factor. It is hypothesized that one possible mechanism whereby nedocromil sodium protects against asthma and allergic rhinitis is through inhibition of HRA-N generation. (J ALLERGY CLIN IMMUNOL 1991;87:812-20.)

Nedocromil sodium is a new pyranoquinoline dicarboxylic acid derivative that has been demonstrated to be an effective therapeutic agent against allergic rhinitis and asthma^{1, 2} and protects against exercise-induced asthma,^{3, 4} as well as early and late asthmatic responses to inhaled antigen.^{5, 6} The exact mechanism by which nedocromil sodium works is unknown. One theory is that its major mechanism of action involves anti-inflammatory effects. In support of this theory are the observations that nedocromil sodium inhibits the activation of PMNs as well as eosinophils, monocytes, macrophages, and mast cells.⁷ Many of the

Abbreviations used

HRF:	Histamine-releasing factor
HRA-N:	Neutrophil-derived histamine-releasing activity
RBL:	Rat basophil leukemia
HBSS:	Hanks' balanced salt solution
HEPES:	N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
HHBSS-CM:	HEPES-buffered HBSS with 3 mmol/L of calcium and 2 mmol/L of magnesium
PMN:	Polymorphonuclear neutrophil
IC ₅₀ :	Inhibitory concentration of 50%
BAL:	Bronchoalveolar lavage

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inflammatory cells involved in late-phase allergic responses, including neutrophils, eosinophils, lymphocytes, monocytes, and platelets, release HRFs believed to be possibly involved in the perpetuation of the allergic response.⁸⁻¹² Spontaneous generation of

some of these HRFs correlate with asthma severity, and a subgroup of HRFs is IgE dependent.^{10, 13-15} All the HRFs induce histamine release from human basophils. The HRA-N also induces histamine release from human cutaneous mast cells in vitro, rat and guinea pig cutaneous mast cells in vivo, and from cultured mouse P cells and rat basophilic leukemia cells in vitro.¹⁶ Although the identity of many of the HRFs is unknown, one of the HRFs has been identified as connective tissue-activating peptide III, and other cytokines, such as interleukin-1, 3, and 8, and granulocyte-macrophage colony-stimulating factor, have been found to possess HRA.¹⁷⁻²¹ The effect of nedocromil sodium on this family of HRFs is unknown. Therefore, the effect of nedocromil sodium on the generation and activity of the HRA-N was examined.

MATERIAL AND METHODS

Material

HBSS buffer without phenol red, with and without calcium and magnesium (GIBCO, Grand Island, N.Y.), heat-inactivated fetal calf serum, L-glutamine, and trypsin versene, Eagles minimum essential medium with Earle's balanced salt without L-glutamine (Biofluids, Rockville, Md., Histopaque, trypan blue, and HEPES (Sigma Chemical Co., St Louis, Mo.), 335 × 30 mm Suparose 12 fast protein liquid chromatography column (Pharmacia, Piscataway, N.J.), YM2 membranes (Amicon Corp., Danvers, Mass.), heparin sodium injection United States Pharmacopeia (Organon, Inc., Orange, N.J.), 175 cm² of Nunc tissue culture flasks (Intermed Interlab, Thousand Oaks, Calif.), 96-well U-bottomed, tissue-culture plates, Spin-x centrifuge filter units (Costar Laboratory, Cambridge, Mass.), 500 ml filters (Millipore Corp., Bedford, Mass.), 1.5 ml tubes (Sarstedt, Germany), supernatant harvesting frames (Skatron, Springfield, Va.), and polypropylene Falcon tubes (Becton Dickinson Labware, Oxnard, Calif.) were obtained from the manufacturers. RBL cells (Dr. Reuben Siraganian, NIDR), and nedocromil sodium (Dr. Francis Kane, Fisons Corp.) were each generous gifts.

Methods

Isolation of PMNs. PMNs were isolated from human venous blood by Histopaque (1.077) centrifugation, followed by dextran sedimentation as previously described.⁸ The resulting isolate was resuspended in HHBSS-CM, pH 7.3, at 5×10^7 cells per milliliter, unless otherwise stated. Purity was >94% neutrophils, and viability was >95% as determined by trypan blue dye exclusion and lactate dehydrogenase determination.

Generation of HRA-N. PMNs, 5×10^7 /ml, were incubated at 37° C for 1 hour in HBSS-CM with 0.5 mmol/L of phenylmethyl sulfonyl fluoride, 0.1 mmol/L of leupeptin, and 10 µg/ml of aprotinin, and the supernatants were collected. All experiments were completed within 4 hours of obtaining blood to ensure PMN viability. In experiments examining the effect of nedocromil sodium on HRA-N gen-

eration and on crude HRA-N activity, PMNs were incubated in HBSS-CM with or without nedocromil sodium 10^{-4} to 10^{-7} mol/L, and the supernatants were boiled for 10 minutes. HRA-N has been demonstrated to induce the release of both histamine and ³H-serotonin from RBL cells.^{8, 22} In 12 previous experiments with RBL cells, both percent serotonin and percent histamine release were measured with comparable results.²² In these experiments, HRA-N activity was determined by measuring the percent of ³H-serotonin released from duplicate aliquots of RBL cells exposed to PMN supernatants as described below.

Partial purification of HRA-N. Pooled PMN supernatants obtained from at least eight donors were concentrated over YM2 membranes (1000 molecular weight cutoff), applied to a Suparose 12 fast protein liquid chromatography column, and eluted with HHBSS, pH 7.3, running at 0.5 ml/min. Fractions were collected every minute, and calcium and magnesium were added. The operating parameters were controlled by a Hewlett-Packard (Hewlett-Packard Co., Cupertino, Calif.) high-performance liquid chromatography system with detection of proteins by absorbance at 280 nm. The HRA-N used throughout this study eluted in fractions 21 and 22.

RBL cell culture. The 2H3 subline of RBL cells, a cultured cell line probably of mucosal origin, was maintained in 175 mm² tissue-culture flasks in Eagle's minimum essential medium, supplemented with 4 mmol/L of glutamine and 15% heat-inactivated fetal calf serum. The cells were treated with trypsin-Versene, resuspended in media, and split every 3 to 4 days. Cells were maintained in a 5% CO₂ humidified atmosphere at 37° C.

Serotonin-release experiments. RBL cells (2×10^4) in 0.1 ml of culture media containing 0.1 µCi of ³H-serotonin, were plated in each well of a 96-well U-bottom culture plate and allowed to adhere overnight. Selected wells were then exposed to IgE, 5 µg, for 45 minutes, and all wells were washed three times with HHBSS-CM. Thereafter, RBL cells were incubated in duplicate with HRA-N, anti-IgE, or HHBSS-CM, 0.045 ml, for 45 minutes at 37° C. Reactions were terminated by the addition of 0.1 ml of HHBSS-CM and absorption of supernatants onto cotton wicks. The wicks were then transferred to scintillation cocktail, and radioactivity was counted. Total cell ³H-serotonin was determined in like manner from unstimulated cells incubated with 0.1% Triton-X. All counts were corrected for background radioactivity. The percent release of serotonin was defined as radioactivity in the supernatants divided by that of the cell totals. Unless otherwise specified, net serotonin release, corrected for release induced by the buffer control, is presented.

Statistics. The one- and two-tailed Student's *t* test was used to analyze the results.

RESULTS

To determine whether nedocromil sodium affects HRA-N generation, it was first necessary to investigate whether nedocromil sodium interfered with the assay for HRA-N or with the positive control, IgE-mediated serotonin release of RBL cells.

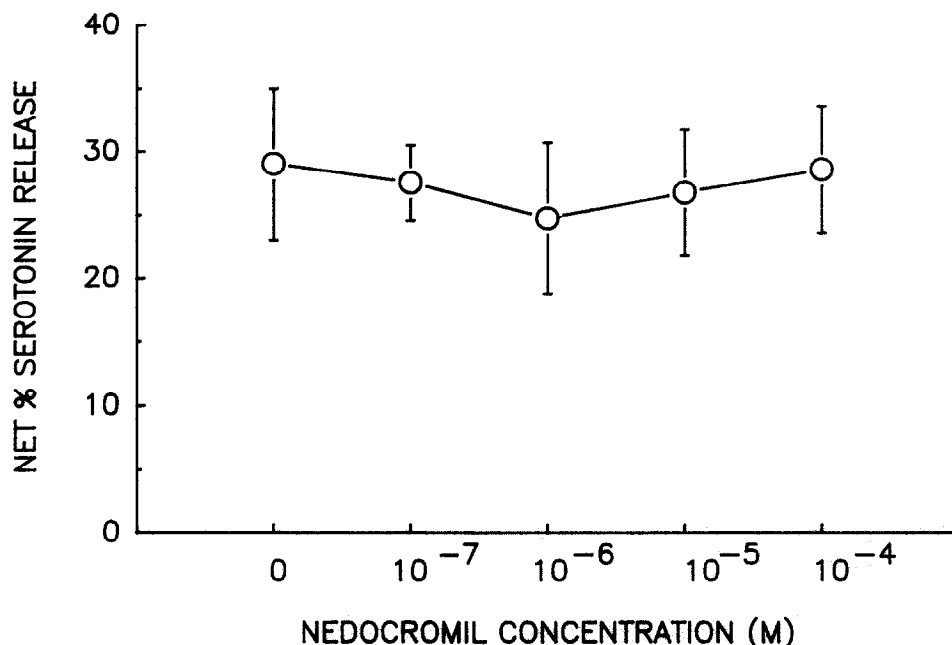


FIG. 1. The effect of acute exposure to nedocromil sodium on HRA-N-mediated serotonin release from RBL cells. RBL cells were incubated for 45 minutes at 37° C with HRA-N premixed with equal volumes of buffer or nedocromil sodium to yield the stated concentrations of nedocromil sodium, and serotonin release was measured. Results are expressed as the mean $\pm$ SEM (*p*, not significant). Three experiments were performed in duplicate with one crude and two partially purified preparations of HRA-N.

TABLE I. The effect of acute exposure to nedocromil sodium on HRA-N-mediated serotonin release from RBL cells

Experiment No.*	Concentration of nedocromil sodium present during RBL cell stimulation with HRA-N	
	None	10 ⁻⁴ mol/L
	(Net % serotonin release from RBL cells)	
1	24	21
2	16	18
3	49	46
4	22	19
5	27	25
Mean $\pm$ SEM	28 $\pm$ 5.6	26 $\pm$ 5.2

*Crude neutrophil supernatants were used in experiment 1, whereas partially purified HRA-N was used in experiments 2 to 5.

Effect of nedocromil sodium on HRA-N-induced serotonin release

To determine whether nedocromil sodium affected HRA-N-mediated serotonin release from RBL cells, RBL cells were exposed to PMN supernatants diluted with buffer or nedocromil sodium to yield final nedocromil sodium concentrations ranging from 10⁻⁷ to

10⁻⁴ mol/L, and serotonin release was measured. One crude and two partially purified preparations of HRA-N were used. HRA-N alone induced 29% $\pm$ 6% serotonin release from RBL cells. Serotonin release was not affected by any concentration of nedocromil sodium used (Fig. 1). In five separate experiments with one crude and four partially purified preparations, serotonin released from RBL cells exposed to native HRA-N and HRA-N in the presence of a single dose of nedocromil sodium (10⁻⁴ mol/L) was 28% $\pm$ 6% and 26% $\pm$ 5%, respectively (Table I). Thus, nedocromil sodium, present only during stimulation at the doses tested, does not inhibit HRA-N-mediated serotonin release.

To rule out the possibility that preincubation is necessary for nedocromil sodium to inhibit HRA-N-mediated serotonin release, RBL cells were incubated with or without nedocromil sodium (10⁻⁴ mol/L) between 5 and 60 minutes before stimulation with HRA-N alone or HRA-N plus nedocromil sodium (10⁻⁴ mol/L), and serotonin release was measured (Fig. 2). One crude and three partially purified preparations of HRA-N were used. HRA-N alone or containing nedocromil sodium (10⁻⁴ mol/L) induced 22% $\pm$ 2% and 21% $\pm$ 2% serotonin release from RBL cells, respectively. Preincubation with nedocromil sodium did not affect HRA-N-induced serotonin release. Thus, neither acute nor prolonged

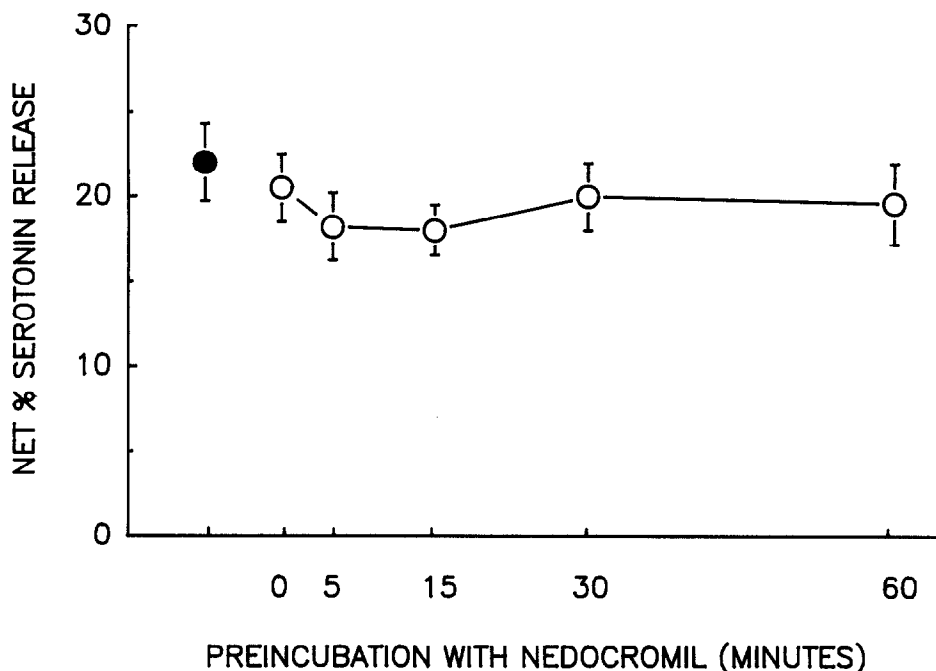


FIG. 2. Effect of preincubation with nedocromil sodium on HRA-N-mediated serotonin release from RBL cells. Aliquots of RBL cells were incubated with nedocromil sodium (10^{-4} mol/L) for 0 to 60 minutes before stimulation with HRA-N premixed with equal volumes of either buffer or nedocromil sodium (10^{-4} mol/L final concentration) for 45 minutes at 37° C, and serotonin release was measured (○). Four experiments were performed in duplicate with one crude and three partially purified preparations of HRA-N. HRA-N-mediated serotonin release in the absence of nedocromil sodium (●). Results are expressed as the mean $\pm$ SEM. (p was not significant.)

exposure to nedocromil sodium inhibits HRA-N-mediated serotonin release from RBL cells.

Effect of nedocromil sodium on IgE-mediated serotonin release

Since HRA-N-induced serotonin release is independent of IgE, the effect of acute exposure to nedocromil sodium on IgE-mediated serotonin release was next examined. In five separate experiments, IgE-sensitized RBL cells were exposed to anti-IgE diluted with buffer or nedocromil sodium to yield final nedocromil sodium concentrations ranging from 10^{-7} to 10^{-4} mol/L, and serotonin release was measured. Anti-IgE alone induced $31\% \pm 12\%$ serotonin release from RBL cells. Serotonin release was not affected by any concentration of nedocromil sodium used (Fig. 3). In 10 separate experiments, anti-IgE alone or containing nedocromil sodium (10^{-4} mol/L) induced $32\% \pm 6\%$ and $32\% \pm 5\%$ serotonin release from RBL cells, respectively (Table II). Thus, acute exposure to nedocromil sodium in the doses tested does not inhibit IgE-mediated serotonin release from RBL cells.

The effect of preincubation with nedocromil sodium on IgE-mediated serotonin release from RBL cells was

TABLE II. The effect of acute exposure to nedocromil sodium on IgE-mediated RBL serotonin release from RBL cells

Experiment No.	Concentration of nedocromil sodium present during stimulation of RBL cells with anti-IgE	
	None	10^{-4} mol/L
(Net % serotonin release from RBL cells)		
1	15	20
2	21	40
3	77	58
4	27	25
5	24	21
6	46	46
7	46	47
8	22	19
9	22	30
10	17	17
11	48	48
12	32	32
13	51	47
14	48	57
Mean $\pm$ SEM	35 ± 4.7	36 ± 3.9

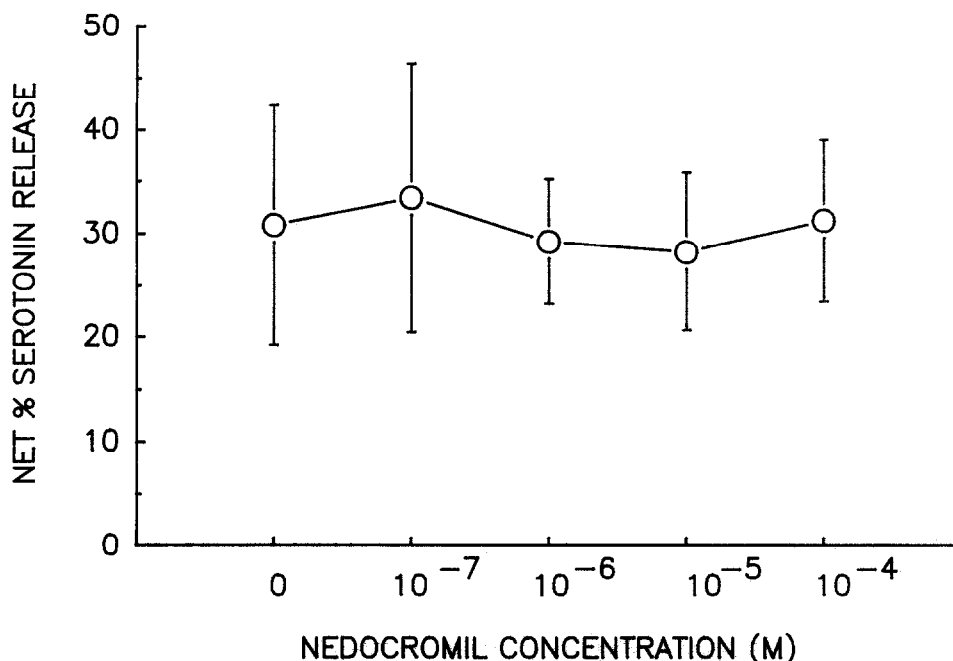


FIG. 3. Effect of acute exposure to nedocromil sodium on IgE-mediated serotonin release from RBL cells. IgE-sensitized RBL cells were incubated for 45 minutes at 37° C with antirat IgE premixed with either equal volumes of buffer or nedocromil sodium to yield the stated concentrations of nedocromil sodium, and serotonin release was measured. Results are expressed as the mean $\pm$ SEM (p , not significant). Five experiments were performed in duplicate.

next examined. In six experiments, RBL cells were incubated with or without nedocromil sodium (10^{-4} mol/L) between 5 and 60 minutes before stimulation with anti-IgE alone or with HRA-N containing nedocromil sodium (10^{-4} mol/L), and serotonin release was measured (Fig. 4). Anti-IgE alone or containing nedocromil sodium (10^{-4} mol/L) induced $36\% \pm 6\%$ and $38\% \pm 6\%$ serotonin release, respectively. Preincubation with nedocromil sodium did not affect IgE-mediated serotonin release from RBL cells. Thus, neither acute nor prolonged exposure to nedocromil sodium inhibits IgE-mediated serotonin release from RBL cells.

Effect of nedocromil sodium on HRA-N production

Nedocromil sodium inhibits *N*-formyl-methionyl-leucyl-phenylalanine-induced cytotoxicity and up regulation of human neutrophil complement receptor and E rosetting.⁹ The effect of nedocromil sodium on HRA-N generation or release from human neutrophils was therefore examined. Neutrophils were isolated from the peripheral blood of 10 individuals and incubated in HHBSS-CM with or without nedocromil sodium, 10^{-7} to 10^{-4} mol/L for 1 hour. The supernatants were collected, boiled for 10 minutes, and

assayed on RBL cells for HRA-N activity. Crude supernatants from seven of the 10 donors' neutrophils incubated in the absence of nedocromil sodium induced 13% to 32% net serotonin release from RBL cells. Nedocromil sodium (10^{-7} to 10^{-4} mol/L), added to the neutrophil preparations during HRA-N generation, caused dose-related inhibition of HRA-N activity in the neutrophil supernatants (individual data presented in Table III).

To quantitate the inhibition of HRA-N generation by nedocromil sodium, a dose-response curve of HRA-N-induced serotonin release from RBL cells was constructed by exposing RBL cells to various dilutions of the seven HRA-N-containing supernatants generated in the absence of nedocromil sodium. The resulting curve was linear with a slope of 12, and 1 U of HRA-N was arbitrarily defined as that concentration of HRA-N inducing 10% net serotonin release. Units of HRA-N activity per milliliter of neutrophil supernatants generated in the presence or absence of nedocromil sodium were then determined by the net percent of serotonin release from RBL cells induced by the supernatants. Neutrophils incubated in the absence of nedocromil sodium generated 1.25 to 2.8 U of HRA-N per milliliter. Nedocromil sodium, at the doses tested, caused significant inhibition of HRA-N

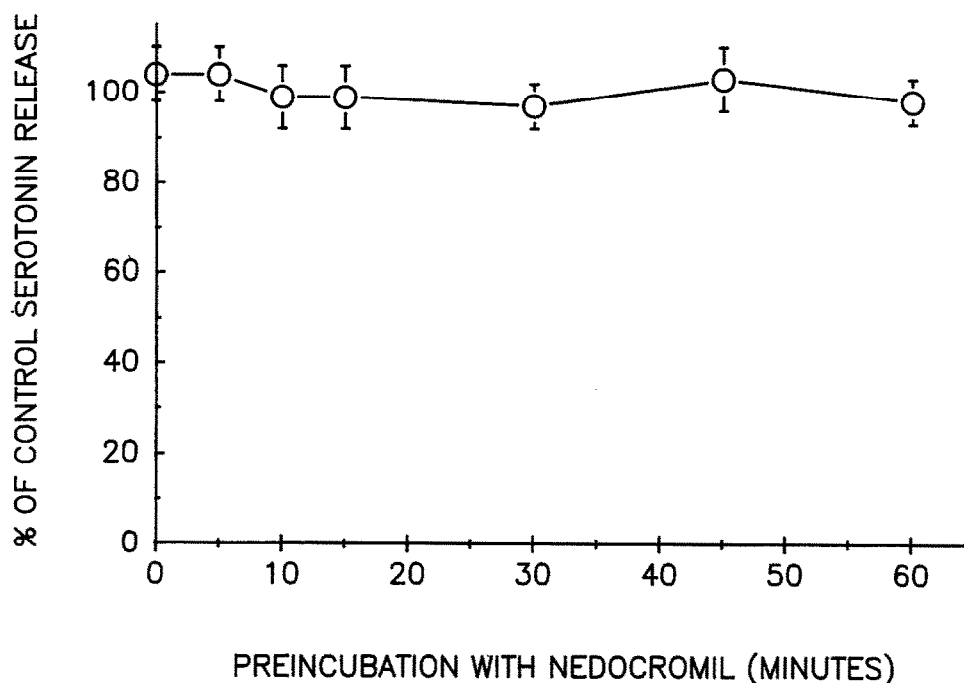


FIG. 4. Effect of preincubation with nedocromil sodium on IgE-mediated serotonin release from RBL cells. Aliquots of IgE-sensitized RBL cells were incubated with nedocromil sodium (10^{-4} mol/L) for 0 to 60 minutes before stimulation with anti-IgE premixed with equal volumes of either buffer or nedocromil sodium (10^{-4} mol/L final concentration) for 45 minutes at 37°C and serotonin release was measured. Six experiments were performed in duplicate. Anti-IgE-mediated serotonin release in the absence of nedocromil sodium ranged from 24% to 50%. Results are expressed as the mean $\pm$ SEM (p , not significant).

TABLE III. Inhibition of HRA-N generation from human neutrophils by nedocromil sodium

Donor	Nedocromil sodium concentration during HRA-N generation (mol/L)				
	None	10^{-7}	10^{-6}	10^{-5}	10^{-4}
(Net % serotonin release from RBL cells induced by HRA-N)					
2	13	3	3	0	4
5	16	ND	0	5	0
6	15	ND	ND	3	0
7	14	0	1	0	0
8	30	16	0	0	0
9	15	ND	7	0	0
10	32	12	0	0	0
Mean $\pm$ SEM	19 $\pm$ 3	8 $\pm$ 3	2 $\pm$ 1	1 $\pm$ 0.1	0.5 $\pm$ 0.5

ND, Not done.

generation (68% and 95% by nedocromil sodium, 10^{-7} and 10^{-4} mol/L, respectively ($p < 0.001$; Fig. 5). Thus, nedocromil sodium causes significant dose-related inhibition of HRA-N generation by human neutrophils.

DISCUSSION

In these studies, nedocromil sodium was a potent inhibitor of HRA-N generation from human neutrophils. Neutrophils incubated in buffer alone released 1.25 to 2.8 U of HRA-N per milliliter (mean $\pm$ SEM,

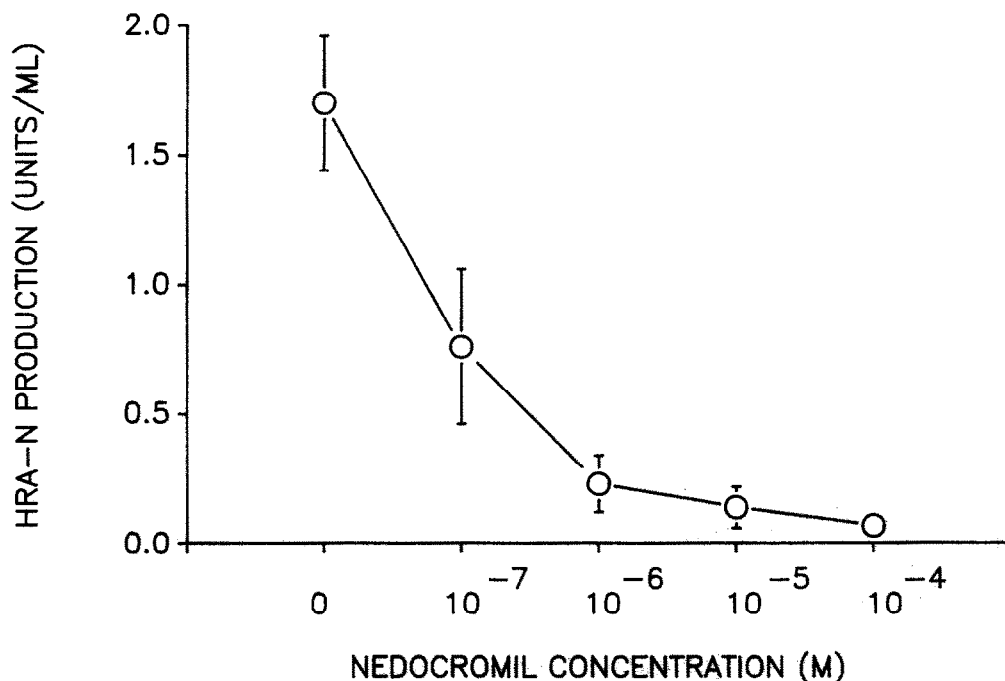


FIG. 5. Effect of nedocromil sodium on HRA-N generation by human neutrophils. Isolated human peripheral blood neutrophils from seven donors were incubated for 1 hour at 37° C in buffer with or without the stated concentrations of nedocromil sodium, and their supernatants were assayed for serotonin-releasing activity on RBL cells. Various dilutions of HRA-N generated from these donors in the absence of nedocromil sodium were also tested for HRA-N activity, and a standard dose-response curve of HRA-N concentration versus net percent serotonin release was constructed. The resultant curve was linear with a slope of 12, and 1 U of HRA-N activity was arbitrarily defined as that concentration required to yield 10% net serotonin release. The concentration of HRA-N present in neutrophil supernatants generated in the presence or absence of nedocromil sodium was determined from the standard curve. Results are expressed as the mean $\pm$ SEM of seven experiments assayed in duplicate. All serotonin-release assays were performed on the same day.

1.8 ± 0.75). Coincubation of human neutrophils with nedocromil sodium caused significant dose-related inhibition of HRA-N release into the supernatants (IC_{50} , 1.5×10^{-8} mol/L), and inhibition was $>90\%$ at doses of 10^{-5} mol/L nedocromil sodium or more. These results are not unexpected because nedocromil sodium also inhibits FMLP-induced enhancement of complement C3b (IC_{50} , 9.5×10^{-10} mol/L) and IgG1 Fc (IC_{50} , 2.2×10^{-9} mol/L) receptor expression on normal human neutrophils as well as *N*-formyl-methionyl-leucyl-phenylalanine-stimulated neutrophil killing of complement-coated schistosomula of *Schistosoma mansoni*.²³

In contrast, acute exposure to nedocromil sodium (10^{-7} to 10^{-4} mol/L) had no effect on serotonin release from RBL cells induced by HRA-N. In five separate experiments, HRA-N alone induced 28% serotonin release as opposed to 26% serotonin release in the presence of nedocromil sodium, 10^{-4} mol/L; nor did preincubation with nedocromil sodium, 10^{-4}

mol/L, for between 5 and 60 minutes, cause inhibition of HRA-N-mediated serotonin release. Thus, although nedocromil sodium inhibits the generation of HRA-N, it does not inhibit the effects of HRA-N on serotonin release from RBL cells.

Similar results were found when the effect of nedocromil sodium on anti-IgE-mediated serotonin release from RBL cells was studied. Anti-IgE-induced serotonin release from RBL cells was not inhibited by acute exposure to any dose of nedocromil sodium tested (10^{-7} to 10^{-4} mol/L). In 10 separate experiments, anti-IgE alone induced 32% serotonin release as compared with 32% serotonin release in the presence of anti-IgE mixed with nedocromil sodium, 10^{-4} mol/L; nor did preincubation with nedocromil sodium for between 5 and 60 minutes cause inhibition of anti-IgE-mediated serotonin release from RBL cells. Thus, nedocromil sodium does not inhibit anti-IgE-mediated serotonin release from RBL cells.

These data, however, do not necessarily imply that

nedocromil sodium has no effect on histamine release from RBL cells, since other studies have demonstrated differential inhibition of rat peritoneal mast cell histamine and ^3H -serotonin release by amitriptyline²⁴; nor do the data suggest that nedocromil sodium is ineffective in inhibiting the secretory effect of HRA-N and anti-IgE on other histamine-containing cells, since considerable heterogeneity exists in the response of mast cells and basophils to the inhibitory effects of nedocromil sodium. Indeed, nedocromil sodium inhibits the release of histamine, leukotriene C_4 , and prostaglandin D_2 from *Ascaris*-sensitive monkey BAL cells challenged in vitro with *Ascaris* antigen or with antihuman IgE as well as from rat peritoneal mast cells challenged with antirat IgE.²⁵ Nedocromil sodium also inhibits anti-IgE-induced histamine release from human BAL cells at doses considerably larger (2×10^{-6} mol/L) than required to inhibit neutrophil activation,²³ as well as A23187-induced histamine release from BAL cells obtained from patients with asthma.²⁶ In a comparative study, nedocromil sodium was found to be significantly more active inhibiting histamine release from human BAL cells as compared with enzymatically dispersed human lung parenchymal cells.²⁷ In another comparative study, nedocromil sodium inhibited antigen-induced secretion from rat pleural and peritoneal mast cells, but not from mouse peritoneal mast cells, human basophils, rat intestinal mast cells, or guinea pig mesentery or lung mast cells, and had intermediate activity against hamster peritoneal mast cells.²⁸ Thus, although nedocromil sodium does not inhibit HRA-N-induced serotonin release from RBL cells, its effect on HRA-N-induced stimulation of human mast cells and basophils cannot be implied from these data and remains to be investigated.

This study is the first reported pharmacologic manipulation of the generation of an HRF. Similar inhibitory effects have been reported after dietary manipulation in food-allergic subjects²⁹ and after immunotherapy in subjects with allergic asthma.¹³ Nedocromil sodium has been demonstrated in numerous studies to be an effective therapeutic agent against allergic rhinitis and asthma^{1, 2} and protects against exercise-induced asthma,^{3, 4} as well as early and late asthmatic responses to inhaled antigen.^{5, 6} One possible mechanism by which nedocromil sodium may exert its antiallergic effect is through inhibition of inflammatory cells participating in late-phase allergic reactions. Indeed, nedocromil sodium has also been demonstrated to inhibit the activation of neutrophils as well as eosinophils, monocytes, and macrophages. All these inflammatory cells release HRFs. Evidence has been presented in this study demonstrat-

ing that nedocromil sodium is a potent inhibitor of HRA-N generation. Its effect on the generation of other HRFs is unknown. However, one mechanism whereby nedocromil sodium inhibits late-phase allergic reactions may be by inhibiting the generation of HRA-N and perhaps other HRFs as well.

In summary, nedocromil sodium is a potent inhibitor of HRA-N generation by human neutrophils but has no effect on serotonin release from RBL cells induced by either HRA-N or by anti-IgE. One of the major effects of nedocromil sodium may be the inhibition of HRA-N. The effect of nedocromil sodium on HRA-N-induced activation of human mast cells and basophils is unknown as is its effect on the generation of other HRFs. It would be of interest to study these questions, since the inhibition of the generation and/or the activity of the various HRFs may be one target of activity of nedocromil sodium.

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