

Nedocromil sodium blocks the early and late phases of allergen challenge in a guinea pig model of asthma

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We developed a model of asthma in conscious guinea pigs in which both early- and late-phase reductions in specific airways conductance are demonstrable after inhalation challenge. Neutrophilia that peaks 17 hours after challenge was discovered by bronchoalveolar lavage in this model, as was eosinophilia that develops more slowly, peaking at 72 hours. Nedocromil sodium blocked both the early- and late-phase reductions in airflow, but salbutamol blocked only the early phase. Both drugs blocked the neutrophilia when given before challenge, but not when given 6 hours afterward. This suggests that the neutrophilia is secondary to an intact early phase response. Nedocromil sodium, given 6 hours after challenge, reduced the eosinophilia at 72 hours but not at 17 hours. Salbutamol had no effect on the eosinophilia at either time. These results suggest that nedocromil sodium has antiinflammatory properties in the lung that are not shared by the β_2 -adrenergic stimulant drug, salbutamol. (J ALLERGY CLIN IMMUNOL 1993;92:177-82.)

After bronchial provocation with allergen, susceptible asthmatic subjects have two different airway responses. One is the early-phase response (EPR), which peaks 15 to 30 minutes after challenge, and the other is the late-phase response (LPR), which occurs spontaneously 6 to 12 hours later. Repeated exposure to allergen also leads to the development of bronchial hyperresponsiveness to nonspecific bronchial irritants. The EPR is mediated primarily by products released from mast cells,¹ but the LPR is characterized by the influx of inflammatory cells, particularly eosinophils.^{2, 3} There is a correlation between the inflammatory events initiated by the LPR and the pattern of inflammation observed in biopsy specimens, and studies of cells recovered by bronchoalveolar lavage (BAL).^{4, 5} This similarity suggests that our ability to control the pulmonary inflammation that characterizes asthma is the key to effective treatment. Understanding the mechanism of the disease process and developing appropriate therapies requires models of asthma that mimic the inflammatory changes of the asthmatic lung. Such models have been developed in

Abbreviations used:

EPR: Early-phase response
LPR: Late-phase response
BAL: Bronchoalveolar lavage

rabbits,⁶ sheep,⁷ squirrel monkeys,⁸ and guinea pigs.⁹⁻¹¹

THE GUINEA PIG MODEL OF THE LPR

In developing a guinea pig model of the LPR,¹¹ we tried to adhere to atopic asthma. This involves challenging and sensitizing animals to allergen by the aerosol route and assessing bronchial function by plethysmography while they are conscious. We recently modified the method of calculating airways function to include the correction factor suggested by Dubois et al.^{12, 13} Evidence of inflammatory changes in the lung is obtained by analysis of cells recovered by BAL and by histologic examination of airways tissue.

Challenge of sensitized guinea pigs with aerosolized ovalbumin results in three phases of airflow reduction (Fig. 1). The early phase is evident within 5 minutes, reaches a maximum at 2 hours, and resolves by 6 hours. The late phase reaches a maximum at 17 hours, and a later LPR peaks at 72 hours.¹¹ These phases are accompanied by marked changes in the granulocyte composition of the BAL (Fig. 2). The number of neutrophils

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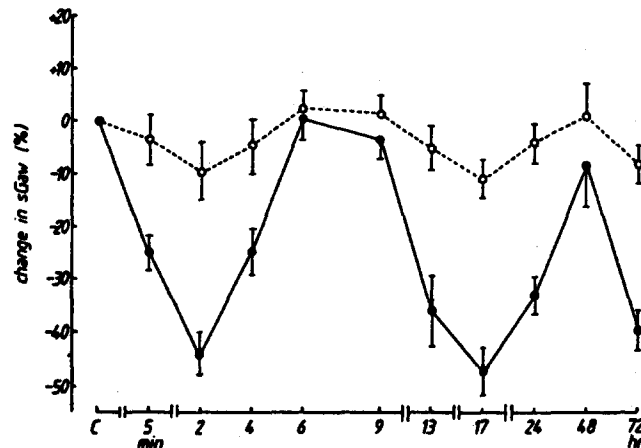


FIG. 1. The effect of saline solution (open circles) and 2% ovalbumin (closed circles) challenge on specific airways conductance (sGaw) in sensitized guinea pigs. Each point represents the mean $\pm$ standard error of the mean of results from 6 to 26 animals. (From Hutson PA, et al. Am Rev Respir Dis 1988;137:548-57.)

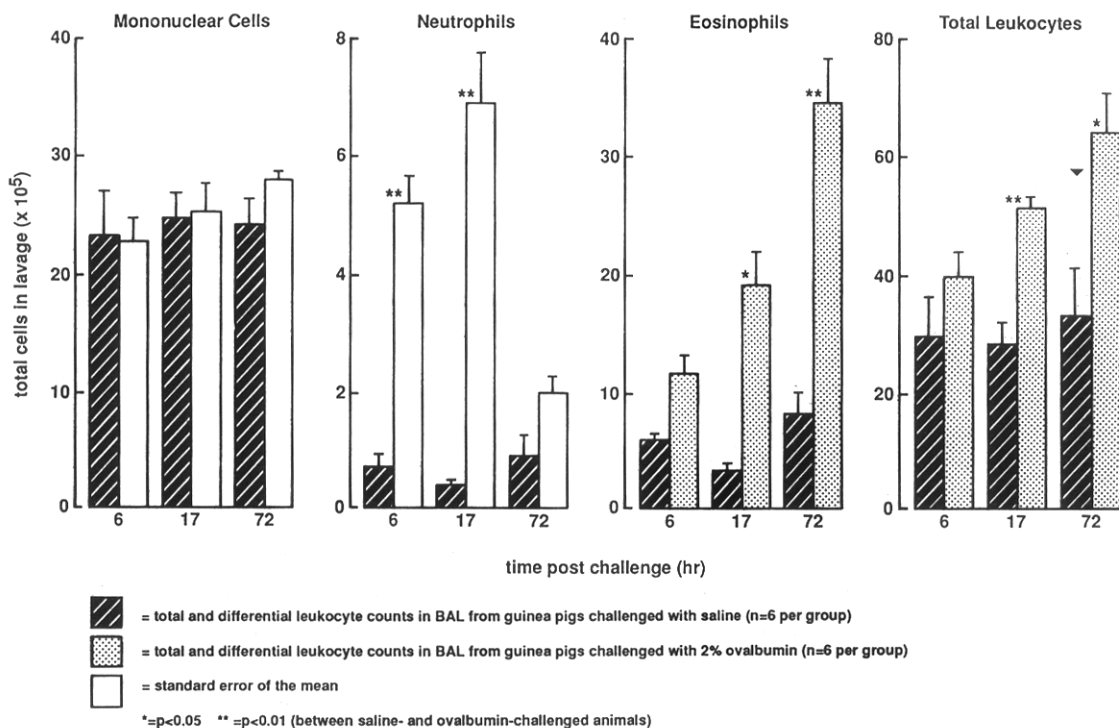


FIG. 2. Total and differential leukocyte counts in bronchoalveolar lavage from sensitized guinea pigs challenged with saline solution (hatched bars) and 2% ovalbumin (dotted bars). Each bar represents the mean $\pm$ standard error of the mean of results from six animals. The significance of difference between saline solution- and ovalbumin-challenged groups are indicated by * $p < 0.05$ and ** $p < 0.01$. (From Hutson PA, et al. Am Rev Respir Dis 1988;137:548-57.)

increases rapidly after challenge, reaching a peak 17 hours later when they are 17-fold the number of neutrophils in animals exposed to saline solution. By 72 hours, the number of neutrophils falls to a level that is not significantly different from

that in control animals. In contrast, the number of eosinophils rises slowly and reaches a maximum at 72 hours, at which time they comprise almost 50% of the total nucleated cells recovered by BAL. The number of macrophages and lymphocytes

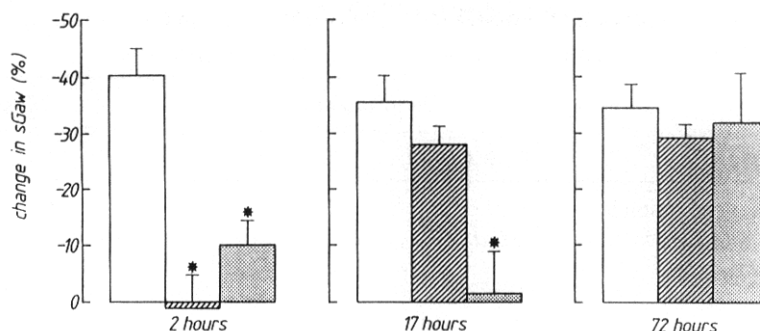


FIG. 3. The effect on specified airways conductance (*sGaw*) of saline solution (open bars), 0.1 mg/ml salbutamol (hatched bars), and 10 mg/ml nedocromil sodium (stippled bars) inhaled 15 minutes before 2% ovalbumin challenge. Each bar represents mean $\pm$ standard error of the mean of data from 6 to 16 animals. * Indicates statistical significance ($p < 0.05$) between saline solution- and drug-treated animals.

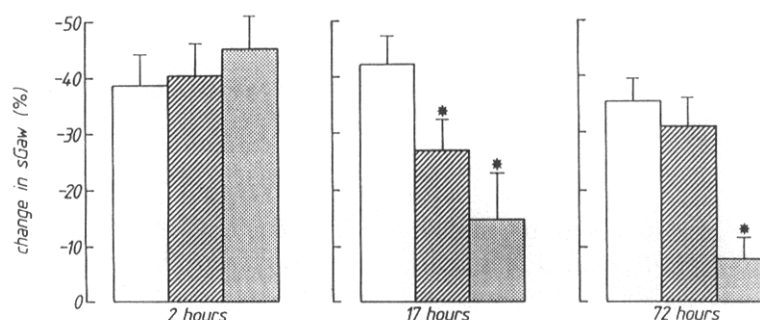


FIG. 4. The effect of specific airways conductance (*sGaw*) of saline solution (open bars), 0.1 mg/ml salbutamol (hatched bars), and 10 mg/ml nedocromil sodium (stippled bars) inhaled 6 hours after 2% ovalbumin challenge. Each bar represents mean $\pm$ standard error of the mean of data from 6 to 16 animals. * Indicates statistical significance ($p < 0.05$) between saline solution- and drug-treated animals.

recovered by BAL shows no obvious changes after challenge. However, we have recently shown¹⁴ that the number of CD3+/CD8- lymphocytes rises in the submucosal tissues, which suggests that CD4+ helper T lymphocytes maybe involved in the inflammatory response after antigen challenge.

DRUG MODULATION OF THE LATE RESPONSE

Prophylactic treatment with antiallergic drugs creates very different effects in the early- and late-phase responses to allergen challenge in human asthma. The EPR but not the LPR can be prevented by β_2 -adrenergic stimulant drugs such as salbutamol (albuterol in the United States) or terbutaline,^{15, 16} which are potent inhibitors of mast cell mediator release.¹⁷ Corticosteroids, which do not prevent mast cell mediator release¹⁸

but have widespread antiinflammatory properties, inhibit only the LPR.^{16, 17} Cromolyn sodium and nedocromil sodium, however, have effects on both phases of the response^{15, 16, 19, 20} because they modify mast cell mediator release²¹ and have antiinflammatory properties.²²

To examine the effects of nedocromil sodium in our guinea pig model, a 10 mg/ml solution of the drug was aerosolized and inhaled for 2 minutes by sensitized guinea pigs. Inhalation occurred 15 minutes before allergen challenge, 6 hours after challenge, or at both times.²³ The effect of nedocromil sodium on airway changes after ovalbumin challenge differed from results obtained in animals who inhaled 0.9% sodium chloride saline solution or salbutamol.²⁴

When administered before challenge (Fig. 3), both nedocromil sodium and salbutamol significantly reduced the early phase of bronchocon-

TABLE I. The effect of nedocromil sodium and salbutamol on neutrophil and eosinophil accumulation in BAL fluid after antigen challenge of sensitized guinea pigs*

Treatment	Neutrophils		Eosinophils	
	17 Hr ($\times 10^5$)	72 Hr ($\times 10^5$)	17 Hr ($\times 10^5$)	72 Hr ($\times 10^5$)
Sensitized nonchallenged animals	0.5 $\pm$ 0.1		4.9 $\pm$ 0.9	
Control (challenge and saline solution treatment)	6.6 $\pm$ 0.8	1.5 $\pm$ 0.3	19.6 $\pm$ 2.7	32.9 $\pm$ 2.8
Nedocromil				
0	2.4 $\pm$ 0.3†	1.9 $\pm$ 0.5	16.9 $\pm$ 4.2	33.6 $\pm$ 5.1
6	4.7 $\pm$ 2.4	2.0 $\pm$ 0.6	11.6 $\pm$ 2.9	21.0 $\pm$ 3.8†
0 + 6	2.0 $\pm$ 0.4†	1.9 $\pm$ 0.4	17.6 $\pm$ 5.1	14.9 $\pm$ 5.3†
Salbutamol				
0	2.6 $\pm$ 0.4†	2.2 $\pm$ 0.6	16.5 $\pm$ 5.2	27.6 $\pm$ 5.8
6	5.3 $\pm$ 0.6	2.0 $\pm$ 0.4	19.2 $\pm$ 5.5	29.9 $\pm$ 6.1
0 + 6	2.2 $\pm$ 0.5†	1.9 $\pm$ 0.5	17.6 $\pm$ 5.3	29.8 $\pm$ 6.1

BAL, Bronchoalveolar lavage.

*Solutions of nedocromil sodium (10 mg/ml) and salbutamol (0.1 mg/ml) were aerosolized and inhaled at 15 minutes before challenge (0); 6 hours after challenge (6); or at both 15 minutes before and 6 hours after challenge (0 + 6). Groups of control animals inhaled saline solution at the same times, but because the differences between these groups were not statistically different, a single mean result is shown ($n = 22$). For all other groups, results are the mean of observation in six to nine animals.

†Significantly different from control ($p < 0.05$).

striction. This result is consistent with the ability of both drugs to inhibit IgE-dependent mast cell mediator release^{17, 21} and the ability of salbutamol to bronchodilate. However, as in humans, the drugs had different effects on the LPR; nedocromil sodium inhibited the LPR, whereas salbutamol had no significant effect. When given 6 hours after challenge (Fig. 4), after the completion of the EPR, nedocromil sodium caused a marked inhibition of the LPR at both 17 and 24 hours. In contrast, salbutamol had only a small but statistically significant effect on the 17-hour LPR.

Both salbutamol and nedocromil sodium have mast cell-stabilizing properties. That they have different effects on the LPR suggests that mast cell mediators are not responsible for the initiation of the LPR. This conclusion must carry the caveat that the drugs inhibit the generation of cytokines by mast cells after activation²⁵ in addition to inhibiting histamine release and eicosanoid generation. Studies demonstrating this have not been reported.

Ovalbumin challenge of sensitized guinea pigs in the absence of drug treatment induced a marked neutrophilia (Table I), which coincided with the lowest point in specific airways conductance measured at 17 hours. This response was suppressed by both salbutamol and nedocromil sodium given prophylactically, but not by either drug given 6 hours after challenge.

Two conclusions can be drawn from these observations. The first is that the neutrophilia is induced probably as a direct result of the actions of mediators released during the EPR. This conclusion is supported by reports that neutrophil chemotactic factor is released after allergen challenge in both human and guinea pig lung.^{26, 27} Further support comes from clinical observations that pretreatment of patients with either cromolyn sodium or nedocromil sodium prevents the release of both histamine and neutrophil chemotactic factor.²⁷⁻²⁹ The second conclusion is that although there is a temporal association between the neutrophil rise and the LPR at 17 hours, these events do not appear to be linked functionally. This was suggested by the fact that abolition of the neutrophilia by salbutamol was not accompanied by a reduction in the LPR. The lack of a functional relationship was also supported by experiments in which we have depleted the neutrophils with a specific anti-serum. This treatment failed to modulate the early- or late-phase changes in specific airways conductance.¹³

The eosinophil has been attracting a great deal of attention as a possible mediator of the LPR and the acquisition of bronchial hyperresponsiveness.^{4, 30} Asthma is characterized by marked eosinophilia; large numbers of these cells are present in the sputum and bronchial biopsy specimens. When appropriately stimulated, eosino-

phils have the capacity to secrete a range of preformed cytotoxic proteins, including major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxic protein, and eosinophil peroxidase. They also release newly generated mediators such as leukotriene C₄, platelet-activating factor, and active species of oxygen.³¹

In our guinea pig model of asthma there is no temporal relationship between the degree of bronchial eosinophilia, which increases progressively over the 72 hours after challenge, and the LPR to allergen challenge. Furthermore, a single dose of nedocromil sodium given either 15 minutes before or 6 hours after challenge ablates the 17-hour LPR without significantly reducing eosinophil accumulation at this time (Fig. 3, Fig. 4, and Table I). In contrast, a significant fall in bronchial eosinophilia accompanied the inhibition of the 72-hour LPR produced by nedocromil sodium when given either as a single dose 6 hours after challenge or as two doses, before and after challenge. Clearly, further experiments are necessary to define the role of eosinophils in the two phases of nonimmediate bronchoconstriction.³² These experiments do show, however, an anti-inflammatory effect with nedocromil sodium that was not seen with salbutamol in the same model.^{23, 24}

CONCLUSIONS

With use of a model of asthma in the guinea pig we demonstrated the ability of nedocromil sodium to inhibit both the early and late phases of reduction in specific airways conductance after allergen challenge. Inhibition of the early phase is likely to represent an effect on mast cells, whereas the influence on the LPRs appears to be the result of antiinflammatory properties. These results agree with Auty's description of nedocromil sodium as a drug to treat airway inflammation.²² They also demonstrate the usefulness of the guinea pig model of asthma to study the mechanism of action of antiasthmatic drugs.

REFERENCES

- Holgate ST, Benyon RC, Howarth PH, et al. Relationship between mediator release from human lung mast cells in vitro and in vivo. *Int Arch Allergy Appl Immunol* 1985; 77:47-56.
- Metzger WJ, Richerson HB, Worden K, Monick M, Hunninghake GW. Bronchoalveolar lavage of allergic asthmatic patients following allergen provocation. *Chest* 1986; 89:477-83.
- deMonchy JGR, Kauffman HF, Venge P, et al. Bronchoalveolar eosinophils during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985;131:373-6.
- Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. *Clin Allergy* 1985;15:411-8.
- Beasley CRW, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial challenge. *Am Rev Respir Dis* 1989;139:806-17.
- Shampain MP, Behrens BL, Larsen GL, Henson PM. An animal model of late pulmonary responses to *Alternaria* challenge. *Am Rev Respir Dis* 1983;126:493-8.
- Abraham WM, Delehunt JC, Yerger L, Marchette B. Characterization of a late phase pulmonary response following antigen challenge in allergic sheep. *Am Rev Respir Dis* 1983;128:839-44.
- Hamel R, McFarlane CS, Ford-Hutchinson AW. Late pulmonary responses induced by *Ascaris* allergen in conscious squirrel monkeys. *J Appl Physiol* 1986;61:2081-7.
- Wieslander E, Andersson P, Linden M, et al. Importance of particulate antigen for the induction of dual bronchial reaction in guinea pigs. *Agents Actions* 1985;16:37-8.
- Iijima H, Ishii M, Yamamuchi K, et al. Bronchoalveolar lavage and histologic characterization of late asthmatic response in guinea pigs. *Am Rev Respir Dis* 1987;136: 922-9.
- Hutson PA, Church MK, Clay TP, Miller P, Holgate ST. Early and late phase bronchoconstriction following allergen challenge of non-anesthetized guinea pigs: I. The association of disordered airway physiology to leukocyte infiltration. *Am Rev Respir Dis* 1988;137:548-57.
- Dubois AB, Botelho SY, Comroe JHJ. A new method for measuring airways resistance in man using a body plethysmograph: values in normal subjects and patients with respiratory disease. *J Clin Invest* 1956;35:327-35.
- Hutson PA, Sanjar S, Kings M, Hogate ST, Church MK. Evidence that neutrophils do not participate in the late phase airways response provoked by ovalbumin inhalation in conscious sensitized guinea pigs. *Am Rev Respir Dis* 1990;141:535-9.
- Frew AJ, Moqbel R, Azzawi M, et al. T-lymphocytes and eosinophils in allergen-induced late phase asthmatic reactions in the guinea pig. *Am Rev Respir Dis* 1990;141:407-13.
- Hegardt B, Pauwels R, van der Straeten M. Inhibitory effect of KWD 2131, terbutaline, and DSCG on the immediate and late allergen-induced bronchoconstriction. *Allergy* 1981;36:115-22.
- Cockcroft DW, Murdock KY. Comparative effects of inhaled salbutamol, sodium cromoglycate, and beclomethasone dipropionate on allergen-induced early asthmatic responses, late asthmatic responses, increased bronchial responsiveness to histamine. *J ALLERGY CLIN IMMUNOL* 1987;79:734-40.
- Church MK, Hiroi J. Inhibition of IgE-dependent histamine release from human dispersed lung mast cells by anti-allergic drugs and salbutamol. *Br J Pharmacol* 1987; 90:421-9.
- Church MK. The biochemical basis of pulmonary and anti-allergic drugs. In: Devlin JP, ed. *Pulmonary and anti-allergic drugs*. New York: John Wiley & Sons, 1985: 43-121.
- Booij Noord H, Orie NGM, de Vries K. Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. *J ALLERGY CLIN IMMUNOL* 1971;48:344-54.

20. Holgate ST. Clinical evaluation of nedocromil sodium in the treatment of asthma. *Eur J Respir Dis* 1986;69(suppl 147):149-59.
21. Leung KBP, Flint KC, Brostoff J, Hudspeth BN, Johnson NMCI, Pearce FL. A comparison of nedocromil sodium and sodium cromoglycate on human mast cells obtained by bronchoalveolar lavage and by dispersion of lung fragments. *Eur J Respir Dis* 1986;69(suppl 147):223-6.
22. Auty RM. The clinical development of a new agent for the treatment of airway inflammation, nedocromol sodium (Tilade). *Eur J Respir Dis* 1986;69(suppl 147):120-31.
23. Hutson PA, Holgate ST, Church MK. Inhibition by nedocromil sodium of early and late bronchoconstriction and airways cellular infiltration provoked by ovalbumin in conscious sensitized guinea pigs. *Br J Pharmacol* 1988;94:6-8.
24. Hutson PA, Holgate ST, Church MK. The effect of Cromolyn sodium and albuterol on early and late phase bronchoconstriction and airway leukocyte infiltration following allergen challenge of non-anesthetized guinea pigs. *Am Rev Respir Dis* 1988;138:839-45.
25. Wodnar-Filipowicz A, Heusser CH, Moroni C. Production of haemopoietic growth factors GM-CSF and interleukin 3 by mast cells in response to IgE-receptor mediated activation. *Nature* 1989;339:150-2.
26. Schenkel E, Atkins PC, Yost R, Zweiman B. Antigen-induced neutrophil chemotactic activity from sensitized lung. *J ALLERGY CLIN IMMUNOL* 1982;70:321-5.
27. O'Driscoll BRC, Lee TH, Cromwell O, Kay AB. Immunologic release of neutrophil chemotactic activity from human lung tissue. *J ALLERGY CLIN IMMUNOL* 1983;72:695-701.
28. Atkins PC, Norman ME, Zwieman B. Antigen-induced neutrophil chemotactic activity in man: correlation with bronchospasm and inhibition by disodium cromoglycate. *J ALLERGY CLIN IMMUNOL* 1978;62:149-55.
29. Howarth PH, Durham SR, Lee TH, Kay AB, Church MK, Holgate ST. Influence of albuterol, cromolyn sodium and ipratropium bromide on the airway and circulating mediator responses in antigen bronchial provocation in asthma. *Am Rev Respir Dis* 1985;132:986-92.
30. Frigas E, Gleich GJ. The eosinophil and the pathophysiology of asthma. *J ALLERGY CLIN IMMUNOL* 1986;77:527-37.
31. Slifman NR, Adolphson CR, Gleich GJ. Eosinophils: biochemical and cellular aspects. In: Middleton E, Reed CE, Ellis EF, Adkinson NF, Yunginger JW, eds. *Allergy: principles and practice*. 3rd ed. St. Louis: The C V Mosby Co, 1988:179-205.
32. Sanjar S, Aoki S, Boubekur K, et al. Eosinophil accumulation in pulmonary airways of guinea pigs induced by exposure to an aerosol of platelet activating factor: effect of anti-asthma drugs. *Br J Pharmacol* 1990;99:267-72.

Effect of nedocromil sodium on airway sensory nerves

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There is increasing evidence that the sensory nerves of the airway play a role in the asthmatic response. Nerve endings are exposed by the epithelial shedding that occurs with asthma. They may become sensitized and activated by inflammatory mediators and may release neuropeptides that then spread and amplify the inflammatory process in the airways. Nedocromil sodium may prevent the sensory nerves from becoming sensitized and inhibit their activation. This possibility is suggested because nedocromil is highly effective against several indirect challenges that involve sensory nerve stimulation. Nedocromil sodium was able to inhibit the bronchoconstriction induced in patients with asthma by exposure to bradykinin, sulfur dioxide, metabisulfite, and ultrasonically nebulized water. Cough, which is a prominent symptom of asthma, is believed to be a result of sensory nerve activation. In several long-term clinical studies, nedocromil sodium reduces the severity of cough among patients with asthma. Studies are needed to define how nedocromil sodium acts on the sensory nerves. (J ALLERGY CLIN IMMUNOL 1993;92:182-6.)

Neural regulation of the airways is a complex process, and there is increasing evidence that neural mechanisms may contribute to some of the features of asthma.¹ For example, hyperalgesia is

Abbreviations used

SP: Substance P
SO₂: Sulfur dioxide

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a common feature of inflammation at many sites in the body and may well be present in the inflamed airways of patients with asthma. Until now, this aspect of asthma has not been emphasized.