

Blood eosinophil numbers and activity during 24 hours: Effects of treatment with budesonide and bambuterol

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The effects of the inhaled corticosteroid budesonide and the oral long-acting β -agonist bambuterol on circadian variation of blood eosinophil numbers, serum levels of eosinophil cationic protein (ECP), serum eosinophil chemotactic activity (ECA), and serum neutrophil chemotactic activity (NCA) were studied in two groups of patients with allergic asthma. Group 1 ($n = 8$) had a circadian variation of peak expiratory flow (PEF) 15% or greater, and group 2 ($n = 9$) had a circadian PEF variation less than 15%. Both groups were randomized and crossover treated for 4 weeks with (A) 0.4 mg budesonide at 8 AM and 8 PM, (B) 20 mg bambuterol at 8 PM, and (C) placebo. At the end of each period blood eosinophil numbers, ECP, ECA, and NCA were measured during 24 hours at 4-hour intervals. No significant differences in the inflammatory parameters could be observed between the groups, although eosinophil numbers tended to be higher in group 1 than in group 2. Highest eosinophil numbers were observed at night. Budesonide reduced both eosinophil numbers and ECP levels, especially at night; bambuterol had no effect on both variables. No circadian variation or treatment effects were observed for ECA and NCA. This study suggests a role for the eosinophil in the nocturnal worsening of asthma, and it demonstrates that budesonide produces, in contrast to bambuterol, a reduction of (nocturnal) eosinophil numbers and activity. (J ALLERGY CLIN IMMUNOL. 1992;90:757-65.)

Key words: Asthma, nocturnal asthma, corticosteroids, β -agonists, eosinophil granulocyte

Nocturnal worsening of symptoms is a common feature in asthma and is thought to be related to a nocturnal increase in airway responsiveness.¹⁻⁴ Inflammatory mechanisms are thought to contribute to airway responsiveness, and in particular the eosinophil is regarded as an important effector cell.^{5,6} Evidence for a role of eosinophils in nocturnal worsening of airway responsiveness stems from the presence of higher numbers of eosinophils in blood and bronchoalveolar lavage at night than during daytime.^{7,8} In

Abbreviations used

ECP:	Eosinophil cationic protein
PEF:	Peak expiratory flow
FEV ₁ :	Forced expiratory volume in 1 second
PC ₂₀ :	Provocative concentration of histamine causing 20% fall in FEV ₁
ECA:	Eosinophil chemotactic activity
NCA:	Neutrophil chemotactic activity

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addition, it has been demonstrated that a large overnight decrease in lung function is associated with increased numbers of eosinophils and other inflammatory cell types in bronchoalveolar lavage at night.⁹

At this time, treatment of nocturnal symptoms of asthma includes inhaled corticosteroids and long-acting β -agonists.¹⁰⁻¹³ Inhaled corticosteroids may reduce eosinophil numbers and levels of eosinophil cationic protein (ECP) in blood or bronchoalveolar lavage.¹⁴⁻¹⁶ The studies on β -agonists are less conclu-

sive. Dahl and Venge¹⁷ reported a reduction of blood eosinophil counts and serum ECP during treatment with oral salbutamol. Other investigators measured after a wash-out period on treatment with β -agonists and found no effects on eosinophil numbers or ECP in blood or bronchoalveolar lavage.^{16, 18} So far, however, effects of antiasthma treatment on circadian patterns of number, activity, and chemotaxis of eosinophils have not been reported.

The goals of this study were to compare circadian patterns of eosinophil numbers, activity, and chemotaxis in two groups of patients with allergic asthma, selected on a large ($\geq 15\%$) and a small ($< 15\%$) variation of peak expiratory flow (PEF), and to investigate the effects of the inhaled corticosteroid budesonide and the oral long-acting β -agonist bambuterol on these patterns.

MATERIAL AND METHODS

Patients

We have prospectively selected two groups of patients, between 18 and 40 years of age, on PEF variation. Group 1 consisted of eight subjects with a circadian PEF variation 15% or greater, the nine subjects of group 2 showed a variation less than 15% (see section on data analysis). All patients had complaints of episodic wheezing consistent with the diagnosis of asthma, and they were clinically stable, without an exacerbation during 2 months before the study. Furthermore, they were selected on (1) positive allergy for house dust mite (positive skin test and elevated specific IgE), (2) forced expiratory volume in one second (FEV_1) greater than 60% of the predicted value,¹⁹ and (3) airway hyper-responsiveness, defined as a provocative concentration of histamine that caused a 20% fall in FEV_1 (PC_{20}) of less than 8 mg/ml.

Study design

Run-in period. Possible participants started with a 2-week, single-blind run-in period in which they used placebo inhalations by Turbuhaler (Astra Draco AB, Lund, Sweden) at 8 AM and 8 PM, and placebo tablets at 8 PM. Inhaled antiinflammatory drugs, including corticosteroids, cromoglycate, and nedocromil, were not allowed from 2 weeks before run-in. The only concomitant pulmonary medication was ipratropium by metered dose inhaler (Atrovent, Boehringer, Ingelheim, Germany), which was allowed when necessary. PEF measurements were performed every 4 hours on the last 3 days of the run-in period to determine circadian variation. Only PEF measurements made at least 4 hours after previous ipratropium inhalation were used for calculations. Subjects were assigned to group 1 if the mean PEF variation was 15% or greater, and if PEF variation was 15% or greater on at least 2 of the 3 days. Assignment to group 2 took place if PEF variation was less than 15% on all 3 days. Subjects who fulfilled the selection criteria at the end of the run-in period were enrolled in the double-blind part of the study.

Double-blind periods. After enrollment subjects were treated in randomized order, double-blind and crossover during 4 weeks with (A) budesonide (Pulmicort, Astra Draco AB), two inhalations by Turbuhaler of 0.2 mg at 8 AM and 8 PM (0.8 mg daily), and placebo tablets at 8 PM, (B) placebo inhalations at 8 AM and 8 PM and a bambuterol tablet (Bambec, Astra Draco AB) of 20 mg at 8 PM, and (C) placebo inhalations at 8 AM and 8 PM and a placebo tablet at 8 PM. As in the run-in period, ipratropium was the only allowed concomitant pulmonary medication.

At the end of each period subjects were hospitalized for 3 days. They arrived at the clinic at 8 PM (day 1). Blood samples for eosinophil count and determination of ECP and chemotactic activity for eosinophils (ECA) and neutrophils (NCA) were taken during 24 hours at 4-hour intervals from 8 AM (day 2) until 8 AM (day 3). All trial medication was continued during the hospitalization, and the use of ipratropium was not allowed.

The study was approved by the hospital medical ethics committee, and written informed consent was obtained before the start of the study.

Laboratory techniques

Blood eosinophil numbers were counted in a Bürker chamber after staining with eosine, reference values being 0 to 400 $\cdot 10^6/l$. Leukocyte counts were performed with a Counter Counter, model S-plus VI (Coulter Electronics, Hialeah, Fla.).

Serum for measurement of ECP, ECA, and NCA was prepared after coagulation of the blood for 1 hour at room temperature. The supernatant was centrifuged twice for 10 minutes at 2000 g to eliminate any remaining cells and stored at -70°C until analysis. ECP was determined by radioimmunoassay.²⁰ Chemotactic activity of the serum on eosinophils and neutrophils was assayed as described previously,^{21, 22} by means of the leading-front method. Unfortunately, several serum samples were defrosted during transport to Sweden, where analysis of ECP, ECA, and NCA took place. Results from these samples were not included in the analysis. Serum samples were available of 16 (placebo), 15 (budesonide), and 15 (bambuterol) subjects for determination of ECP. The available samples for measurement of chemotactic activity were 16 (placebo), 14 (budesonide), and 15 (bambuterol).

Pulmonary function tests

FEV_1 was measured with a Microspiro HI-298 dry spirometer (Chest, Tokyo, Japan); the best of three measurements was used for analysis. Airway responsiveness was measured according to the method described by Cockcroft et al.²³ with use of a Wiesbadener Doppel nebulizer (Wiesbadener Inhalatoren-Vertrieb, Wiesbaden, Germany) with an output of 0.12 ml/min. A 0.9% phosphate-buffered saline solution and doubling histamine diphosphate concentrations from 0.03 to 16 mg/ml were inhaled for 2 minutes at 5-minute intervals. The test was stopped when FEV_1 had fallen by 20% from the presaline value, and PC_{20} was calculated by linear interpolation of the last two points of the log-dose response curve.

TABLE I. Subject characteristics after run-in

	Age (yr)	Gender	Smoking status	FEV ₁ (l)	FEV ₁ %pred	PC ₂₀ histamine (mg/ml)	Variation of PEF (%)*	Treatment before study
Group 1: PEF variation ≥15%						†		
1	21	m	n	5.25	117	2.22	29.7	ba
2	29	f	n	2.35	96	1.27	31.4	ic
3	40	m	e	2.00	59	0.82	26.7	ic,ba
4	20	m	n	3.10	62	0.59	27.1	ic,ba
5	28	f	e	2.00	65	0.25	40.0	cr,ic,ba
6	29	m	e	3.65	74	0.46	37.5	ic,ac,ba
7	21	f	n	2.70	70	0.43	16.4	ba
8	21	m	n	3.55	78	0.13	21.7	cr,ac,ba
‡mean	26			3.08	77.5	0.55	26.5	
Group 2: PEF variation <15%								
9	22	m	e	4.15	86	1.42	9.8	ba
10	39	m	n	4.30	111	1.23	5.8	cr,ic,ac,ba
11	29	f	n	3.70	117	1.19	10.5	cr,ic,ba
12	18	m	n	3.00	81	0.93	8.5	cr,ic,ac
13	24	m	n	4.00	83	7.51	8.0	ba
14	22	f	n	2.60	83	0.42	4.7	cr,ac
15	19	m	n	3.30	80	2.78	5.3	ba
16	18	f	n	3.00	102	2.30	7.1	ba
17	25	m	n	4.50	91	1.53	6.4	cr,ic,ac
‡mean	24			3.62	92.5	1.57	7.2	

f, Female; m, male; e, exsmoker; n, nonsmoker; cr, cromoglycate or nedocromil; ic, inhaled corticosteroids; ac, ipratropium bromide; ba, inhaled β-agonists.

*(PEF_{4 PM} - PEF_{4 AM})/PEF_{4 PM} · 100%.

†*p* < 0.05 from *t* test for comparison of groups.

‡Geometric mean for PC₂₀.

Data analysis

Circadian PEF variation was calculated according to the formula: (PEF_{4 PM} - PEF_{4 AM})/PEF_{4 PM} · 100%. Circadian amplitude in eosinophil numbers, ECP, ECA, and NCA was defined as the difference between 4 PM and 4 AM. Multivariate analysis of repeated measurements (MANOVA) was applied to test whether these amplitudes were different from zero (within-subject factor time) and whether they were different between the groups (between-subject factor study group). Effects of treatment were also evaluated with MANOVA: models included the between-subjects factor study group, the within-subjects factor treatment, and the two-way interaction between study group and treatment. The interaction factor was used to test the presence of different treatment responses in the two groups. Paired *t* tests were used for comparisons between treatments in case of a significant treatment effect. Differences between groups in baseline characteristics and in variables at specific time points were tested with Student's *t* tests for unpaired and paired data, respectively. Twenty-four-hour mean values for leukocytes and eosinophils were logarithmically transformed before analysis to normalize the distribution. Results from parametric analyses were checked with nonparametric tests (Mann-Whitney U test for unpaired data, and Wilcoxon's signed rank sum test for paired data).

We considered a *p* value of <0.05 to be significant. Data are presented as means (SEM). For analyses we used SPSS/PC+, V4.0 (SPSS Inc., Chicago, Ill.).

RESULTS

Differences between the groups

Baseline characteristics. Baseline characteristics of the subjects are listed in Table I; group 1 was more responsive to histamine (*p* = 0.02) and had lower values of FEV₁ (*p* = 0.09) than group 2 after run-in. All subjects completed the study.

During the placebo period. Individual 24-hour mean values for eosinophil numbers and ECP levels during placebo are plotted in Fig. 1 (eosinophils) and Fig. 2 (ECP). It appeared that group 1 had somewhat higher eosinophil numbers and ECP levels than group 2, especially at night (Fig. 3). However, differences between the two groups in 24-hour mean values or at specific time points, for example, at 4 AM, were not statistically significant. In addition, no significant differences were observed for ECA and NCA.

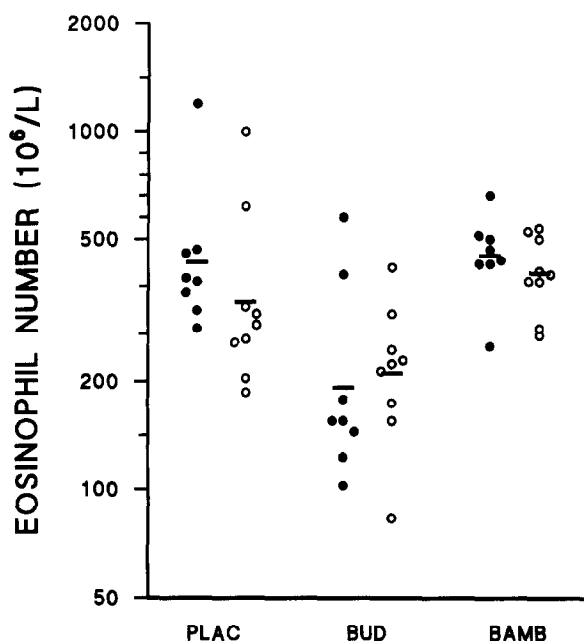


FIG. 1. 24-hour mean individual blood eosinophil numbers during the placebo (*plac*), budesonide (*bud*), and bambuterol (*bamb*) period. Horizontal lines: geometric mean group value. Closed circles: group 1 (PEF variation $\geq 15\%$); open circles: group 2 (PEF variation $< 15\%$).

Response to treatment. No statistically significant difference in treatment response in the two groups was observed for any of the studied variables. Mean data are therefore presented for all 17 subjects in the text, table, and figures.

Circadian variation during placebo

Circadian amplitude of eosinophil number for all 17 subjects (Fig. 4) was $109 (39) \cdot 10^6/l$, which was significantly different from zero ($p = 0.012$). The circadian amplitude of ECP levels (Fig. 5) was $9.1 (7.8) \mu g/L$, which was not different from zero; the change from noon to midnight was not statistically significant. Leukocyte numbers were lower at 4 PM ($7.53 [0.59] \cdot 10^9/l$) than at 4 AM ($8.02 [0.46] \cdot 10^9/l$), the difference, however, not being significant. No significant circadian amplitudes were observed for ECA ($5.0 [5.2] \%$, Fig. 6) and NCA ($-4.3 [6.0] \%$, Fig. 7).

Effects of treatment (Table II)

Treatment was a statistically significant factor in the analysis of 24-hour mean eosinophil numbers ($p < 0.001$) and ECP levels ($p = 0.003$). Budesonide reduced 24-hour mean eosinophil numbers, compared with placebo ($p = 0.002$) and bambuterol

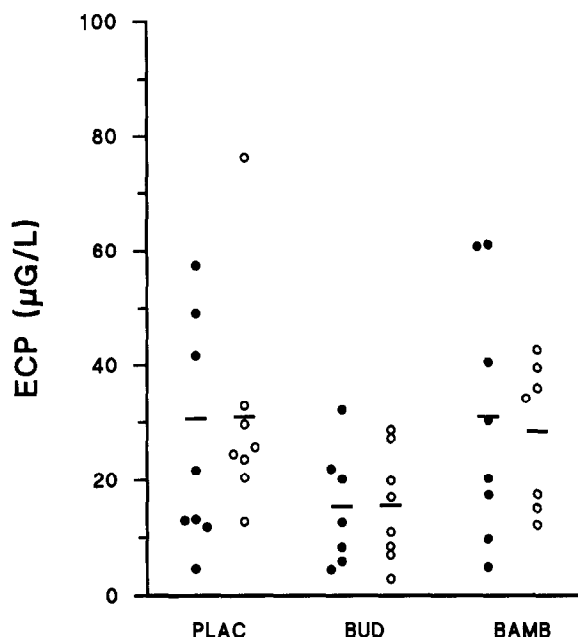


FIG. 2. 24-hour mean individual serum ECP levels. Symbols as in Fig. 1.

($p < 0.001$). In addition, budesonide produced a reduction in 24-hour mean ECP levels, compared with placebo ($p = 0.02$) and bambuterol ($p = 0.001$). Eosinophil numbers and ECP levels during bambuterol did not differ from those during placebo. Twenty-four-hour patterns after treatment are plotted in Fig. 4 (eosinophils) and Fig. 5 (ECP). The observed reduction in eosinophil numbers by budesonide was also significant at 4 AM, compared with placebo ($p = 0.001$) and bambuterol ($p = 0.001$). No significant treatment effects were observed for ECA (Fig. 6) and NCA (Fig. 7).

DISCUSSION

This study was carried out to investigate 24-hour patterns of blood eosinophil numbers and serum levels of ECP, ECA, and NCA. It demonstrates a circadian variation in eosinophil numbers, with peak values at night. The two groups did not significantly differ in any of the studied inflammatory variables, although the subjects with a large PEF variation tended to have higher eosinophil numbers. The study further elicits that budesonide reduced both 24-hour mean eosinophil numbers and ECP levels. Bambuterol had no effects on eosinophil numbers and ECP levels. No circadian variation nor treatment effects could be observed for ECA and NCA.

The role of the eosinophil in asthma and airway responsiveness is reasonably well established. The

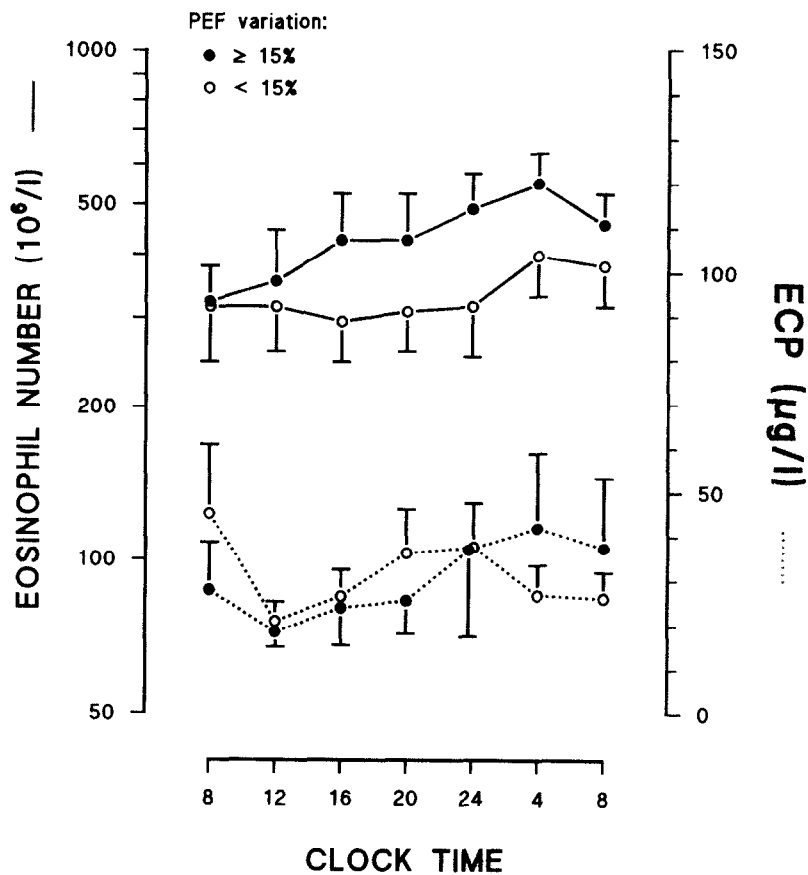


FIG. 3. Blood eosinophil numbers and ECP levels in the placebo period. Differences between groups in the 24-hour means or at specific time points were not significant.

TABLE II. Effects of treatment on 24-hour mean blood leukocyte and eosinophil count and serum levels of ECP, ECA, and NCA*

	Placebo	Budesonide	Bambuterol	Significant differences between treatments†
Leukocyte count ($\times 10^9/L$)‡	7.0 (4.3 - 11.4)	7.2 (5.1 - 10.3)	7.2 (4.8 - 10.8)	
Eosinophil count ($\times 10^6/L$)‡	372 (212 - 1496)	202 (73 - 559)	419 (251 - 698)	bud-plac; bud-bamb
ECP ($\mu g/L$)	30.5 (5.8)	15.2 (2.4)	29.5 (4.5)	bud-plac; bud-bamb
ECA (% of control)	99.4 (3.5)	103.4 (2.9)	100.2 (2.9)	
NCA (% of control)	105.4 (2.5)	108.6 (2.8)	106.2 (2.3)	

Bud, Budesonide; plac, placebo; bamb, bambuterol.

*Mean (SEM).

†Statistically significant differences between treatments in case of a significant treatment effect in MANOVA.

‡Geometric mean (95% range).

number of eosinophils is increased in blood and bronchoalveolar lavage fluid of asthmatic patients,²⁴⁻²⁶ and is related to lung function,²⁷ level of airway responsiveness,²⁸ and symptom scores.²⁶ Higher levels of eosinophil cytotoxic proteins, such as major basic protein and ECP, are found in asthmatic patients in bronchoalveolar lavage fluid and serum,^{16, 25, 29} compared

with normal controls. Higher levels of ECP are associated with lower PEF and worse symptom scores.^{26, 30} And recently, inhalation of major basic protein has been shown to increase airway responsiveness in monkeys.³¹ Moreover, increased chemotactic activity on eosinophils in serum (ECA) has been demonstrated in situations of increased airway re-

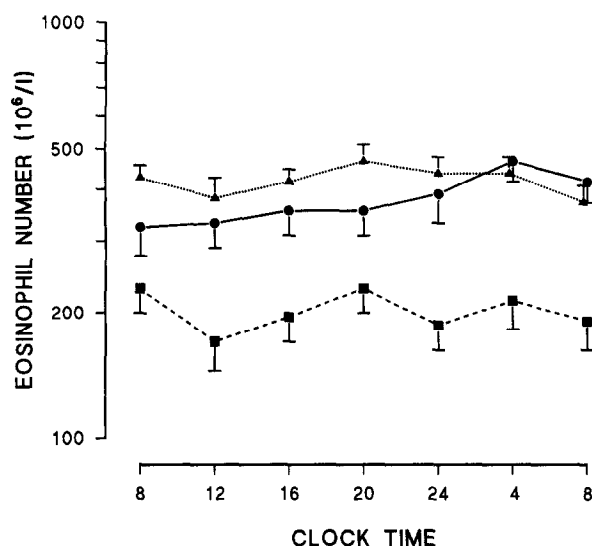


FIG. 4. Blood eosinophil numbers of all 17 subjects during the placebo (circle), budesonide (square), and bambuterol (triangle) period.

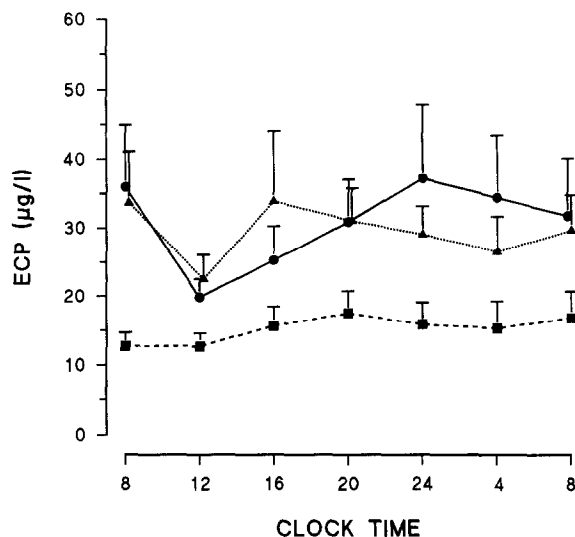


FIG. 5. ECP levels of the available samples of all subjects during the placebo (circle), budesonide (square), and bambuterol (triangle) period.

sponsiveness, that is, after allergen challenge and during the pollen season.^{22, 33}

Two findings in our study suggest that the eosinophil contributes to the nocturnal worsening of asthma. First, the observed 24-hour variation of eosinophil numbers with highest levels occurring at night are a mirror image of the patterns of lung function and airway responsiveness that have been demonstrated before.^{34, 35} And second, the group with a larger nocturnal fall in PEF (group 1) has higher eosinophil numbers and levels of ECP at 4 AM than group 2. This difference between the two groups is in our study not statistically significant, probably because of a large between-subject variation, but may be of clinical relevance. In this respect, several endogenous and exogenous mechanisms are likely to contribute to the nocturnal increase in inflammatory activity. The nocturnal dip in endogenous cortisol levels and β -adrenergic activity may result in larger inflammatory responses.³⁶⁻³⁸ In addition, exposure to allergens may be increased at night as mattress and bedding usually contain considerable amounts of house dust mites.

So far, data are scarce on the effect of treatment on the eosinophil. In general, corticosteroids reduce the number of peripheral eosinophils.^{15, 39} This reduction may be a consequence of dampening of inflammatory processes in the airways, or a systemic side effect. Budesonide has probably no or very little systemic action in the administered dose (0.8 mg), as measured in bone turnover, adrenal function, or white blood counts.⁴⁰⁻⁴³ Moreover, the amount of blood leu-

kocytes, which tends to increase after oral glucocorticosteroids, is unchanged after budesonide. A local effect of inhaled corticosteroids is therefore more likely. Gibson et al.⁴⁴ found a reduction in circulating progenitors of eosinophils and basophils in subjects who had recovered from an asthma exacerbation with use of inhaled corticosteroids and suggested a reduced production in the airways of colony-stimulating factors, such as IL-5 and granulocyte-macrophage colony-stimulating factor, which leads to reduced numbers of circulating eosinophils. If an inhaled corticosteroid acts in this way, its effect is remarkably fast as suggested by the decrease in eosinophil numbers in our subjects 4 hours after each inhalation.

The precise mechanism of corticosteroid action on eosinophil activity is as yet unknown. Eosinophils possess glucocorticosteroid-receptors,⁴⁵ but it has been suggested that the effect of corticosteroids is indirect by influencing other cell types contributing to the inflammatory responses.⁴⁶ The decrease in ECP levels by budesonide in our study is in agreement with previous studies.^{16, 47, 48} Our results suggest, however, that budesonide reduces not only mean ECP levels, but especially the nocturnal values (Fig. 5), which is an important finding from clinical viewpoint.

The action of β -agonists on eosinophil number and activity is as yet not clear. In our study, bambuterol has no effect on eosinophil number, which agrees with other studies on β -agonists.^{16, 18} In these latter studies, however, eosinophil counts in blood or bronchoalveolar lavage fluid were performed after wash-out of

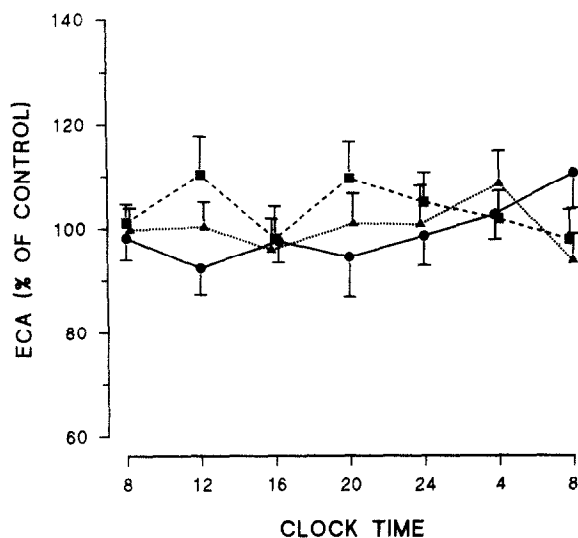


FIG. 6. ECA levels of the available samples of all subjects during the placebo (circle), budesonide (square), and bambuterol (triangle) period.

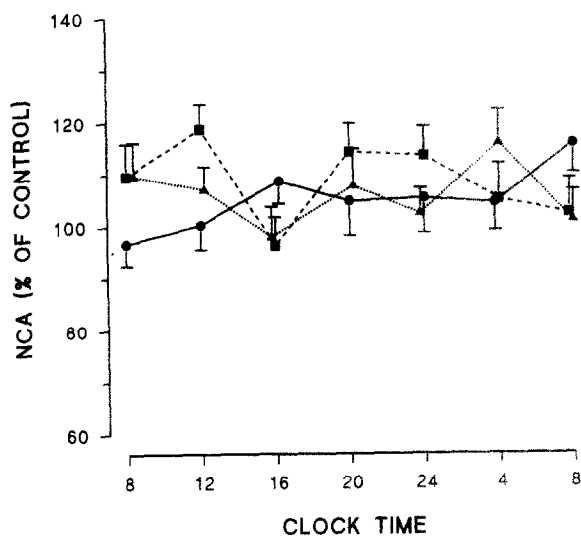


FIG. 7. NCA levels of the available samples of all subjects during the placebo (circle), budesonide (square), and bambuterol (triangle) period.

the last dose of (inhaled) terbutaline. In contrast, Dahl and Venge¹⁷ reported a reduction in eosinophil count during treatment with oral salbutamol. In our study bambuterol has no statistically significant effect on ECP, although a slight reduction in ECP level seems to occur at midnight and 4 AM. This nocturnal effect agrees with data of Dahl and Venge,¹⁷ who reported some effect of oral salbutamol on ECP levels in serum. In addition, Adelr  th et al.¹⁶ recently reported a small but not significant reduction of ECP levels in bronchoalveolar lavage fluid by terbutaline. A modulation of eosinophil activity by β -agonists is not or only marginally supported by in vitro and guinea pig model studies: β -agonists have some or no effect on chemotaxis of eosinophils,^{49, 50} no effect on phagocytosis,⁴⁹ and do not inhibit the production of oxygen radicals and eosinophil peroxidase.⁵¹ More research is needed to elucidate direct or indirect effects of β -agonists on eosinophil activity.

Changes in ECA and NCA have previously been demonstrated after allergen challenge and during the pollen season.^{22, 32, 52, 53} Moreover, increases in ECA and NCA were blocked by immunotherapy and treatment with terbutaline.^{21, 33} These findings suggest that relatively large changes in allergen load may be required for measurable changes in ECA and NCA, and the absence of a 24-hour variation in ours and one other study (for NCA⁵⁴) may be due to too small changes in allergen load over the day. The measurements in our study were performed during 36 hours in the hospital, and measuring in home conditions with

different allergen loads might have provided different results.

In conclusion, eosinophils are likely to contribute to the nocturnal worsening of asthma and may be an important target of intervention. In this respect, the inhaled corticosteroid budesonide reduces both eosinophil number and ECP levels, whereas the β -agonist bambuterol has no effect on these two variables.

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REFERENCES

1. Turner-Warwick M. Epidemiology of nocturnal asthma. *Am J Med* 1988;85(suppl 1b):6-8.
2. Asthma at night [Editorial]. *Lancet* 1983;1:220-2.
3. Barnes PJ. Inflammatory mechanisms and nocturnal asthma. *Am J Med* 1988;85(suppl 1B):64-70.
4. Aalderen WMC van, Postma DS, Ko  ter GH, Knol K. Circadian change in bronchial responsiveness and airflow obstruction in asthmatic children. *Thorax* 1989;44:803-7.
5. Gleich GJ. The eosinophil in bronchial asthma: current understanding. *J ALLERGY CLIN IMMUNOL* 1990;85:422-36.
6. Holgate ST, Roche WR, Church MK. The role of the eosinophil in asthma. *Am Rev Respir Dis* 1991;143:S66-70.
7. Dahl R, Venge P, Olsson I. Variations of blood eosinophils and eosinophilic cationic protein in serum in patients with bronchial asthma. *Allergy* 1978;33:211-5.
8. Martin RJ, Cicutto LC, Smith HR, Ballard RD, Szefer SJ. Airway inflammation in nocturnal asthma. *Am Rev Respir Dis* 1991;143:351-7.
9. Martin RJ, Cicutto LC, Ballard RD. Factors related to the

- nocturnal worsening of asthma. *Am Rev Respir Dis* 1990;141:33-8.
10. Dahl R, Pedersen B, Hägglöf B. Nocturnal asthma: effect of treatment with oral sustained-release terbutaline, inhaled budesonide, and the two in combination. *J ALLERGY CLIN IMMUNOL* 1989;83:811-5.
 11. Lorentzon S, Boe J, Eriksson G, Persson G. Use of inhaled corticosteroids in patients with mild asthma. *Thorax* 1990;45:733-5.
 12. Fairfax AJ, McNabb WR, Davies HJ, Spiro SG. Slow-release oral salbutamol and aminophylline in nocturnal asthma: relation of overnight changes in lung function and plasma drug levels. *Thorax* 1980;35:526-30.
 13. Maesen FPV, Smeets JJ, Gubbelmans HLL, Zweers PGMA. Formoterol in the treatment of nocturnal asthma. *Chest* 1990;98:866-70.
 14. Yager D, Shore S, Drazen JM. Airway luminal liquid. Sources and role as an amplifier of bronchoconstriction. *Am Rev Respir Dis* 1991;143:S52-4.
 15. Kraan J, Koëter GH, Mark ThW van der, et al. Dosage and time effects of inhaled budesonide on bronchial hyperactivity. *Am Rev Respir Dis* 1988;137:44-8.
 16. Ådelroth E, Rosenhall L, Johansson S, Linden M, Venge P. Inflammatory cells and eosinophilic activity in asthmatics investigated by bronchoalveolar lavage. *Am Rev Respir Dis* 1990;142:91-9.
 17. Dahl R, Venge P. Blood eosinophil leucocyte and eosinophil cationic protein. In vivo study of the influence of beta-2-adrenergic drugs and steroid medication. *Scand J Respir Dis* 1978;59:319-22.
 18. Kraan J, Koëter GH, Mark ThW van der, Sluiter HJ, Vries K de. Changes in bronchial hyperactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma: a comparison between budesonide and terbutaline. *J ALLERGY CLIN IMMUNOL* 1985;76:628-36.
 19. Quanjer PhH, ed. Standardized lung function testing. *Bull Eur Physiopathol Respir* 1983;19(suppl 5):1-93.
 20. Venge P, Roxin LE, Olsson I. Radioimmunoassay of human eosinophil cationic protein. *Br J Haematol* 1977;37:331-5.
 21. Venge P, Dahl R, Håkansson L. Heat-labile neutrophil chemotactic activity in subjects with asthma after allergen inhalation: relation to the late asthmatic reaction and effects of asthma medication. *J ALLERGY CLIN IMMUNOL* 1987;80:679-88.
 22. Håkansson L, Rak S, Dahl R, Venge P. The formation of eosinophil and neutrophil chemotactic activity during a pollen season and after allergen challenge. *J ALLERGY CLIN IMMUNOL* 1989;83:933-9.
 23. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;7:235-43.
 24. Schatz M, Wasserman S, Patterson R. The eosinophil and the lung. *Ann Intern Med* 1982;142:1515-9.
 25. Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma: relationship to bronchial hyperactivity. *Am Rev Respir Dis* 1988;137:62-9.
 26. Bousquet J, Chanez P, Lacoste JY, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
 27. Horn BR, Robin ED, Theodore J, Van Kessel A. Total eosinophil counts in the management of bronchial asthma. *N Engl J Med* 1975;292:1152-5.
 28. Taylor KJ, Luksza AR. Peripheral blood eosinophil counts and bronchial responsiveness. *Thorax* 1987;42:452-6.
 29. Carlson M, Håkansson L, Peterson C, Stålenheim G, Venge P. Secretion of granule proteins from eosinophils and neutrophils is increased in asthma. *J ALLERGY CLIN IMMUNOL* 1991;87:24-33.
 30. Griffin E, Håkansson L, Formgren H, Jörgensen K, Peterson C, Venge P. Blood eosinophil number and activity in relation to lung function in patients with asthma and with eosinophilia. *J ALLERGY CLIN IMMUNOL* 1991;87:548-57.
 31. Gundel RH, Letts LG, Gleich GJ. Human eosinophil major basic protein induces airway constriction and airway hyper-responsiveness in primates. *J Clin Invest* 1991;87:1470-3.
 32. Metzger WJ, Richerson HB, Wasserman SI. Generation and partial characterization of eosinophil chemotactic activity and neutrophil chemotactic activity during early and late-phase asthmatic response. *J ALLERGY CLIN IMMUNOL* 1986;78:282-90.
 33. Rak S, Håkansson L, Venge P. Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during pollen season. *J ALLERGY CLIN IMMUNOL* 1990;86:706-13.
 34. Vries K de, Goei JT, Booy-Noord H, Orie NGM. Changes during 24 hours in the lung function and histamine hyperactivity of the bronchial tree in asthmatic and bronchitic patients. *Int Arch Allergy Appl Immunol* 1962;20:93-101.
 35. Gervais P, Reinberg A, Gervais C, Smolensky M, DeFrance O. Twenty-four hour rhythm in the bronchial hyperactivity to house dust in asthmatics. *J ALLERGY CLIN IMMUNOL* 1977;59:207-13.
 36. Szefer SJ, Ando R, Cicutto LC, Surs W, Hill MR, Martin RJ. Plasma histamine, epinephrine, cortisol, and leukocyte β -adrenergic receptors in nocturnal asthma. *Clin Pharmacol Ther* 1991;49:59-68.
 37. Barnes P, Fitzgerald G, Brown M, Dollery C. Nocturnal asthma and changes in circulating epinephrine, histamine, and cortisol. *N Engl J Med* 1980;303:263-7.
 38. Morrison JFJ, Teale C, Pearson SB, Marshall P, Dwyer NM, Jones S, Dean HG. Adrenaline and nocturnal asthma. *BMJ* 1990;301:473-6.
 39. Toogood JH, Baskerville J, Jennings B, Lefcoe NM, Johansson S-A. Bioequivalent doses of budesonide and prednisone in moderate and severe asthma. *J ALLERGY CLIN IMMUNOL* 1989;84:688-700.
 40. Jennings BH, Andersson K-E, Johansson SÅ. Assessment of systemic effects of inhaled glucocorticosteroids: comparison of the effects of inhaled budesonide and oral prednisolone on adrenal function and markers of bone turnover. *Eur J Clin Pharmacol* 1991;40:77-82.
 41. Ali NJ, Capewell S, Ward MJ. Bone turnover during high dose inhaled corticosteroid treatment. *Thorax* 1991;46:160-4.
 42. Toogood JH, Crilly RG, Jones G, Nadeau J, Wells GA. Effect of high-dose inhaled budesonide on calcium and phosphate metabolism and the risk of osteoporosis. *Am Rev Respir Dis* 1988;138:57-61.
 43. Johansson S-Å, Andersson K-E, Brattsand R, Gruvstad E, Hedner P. Topical and systemic glucocorticoid properties of budesonide and beclomethasone dipropionate in man. *Eur J Clin Pharmacol* 1982;22:523-9.
 44. Gibson PG, Dolovich J, Girgis-Gabardo A, et al. The inflammatory response in asthma exacerbation: changes in circulating eosinophils, basophils and their progenitors. *Clin Exp Allergy* 1990;20:661-8.
 45. Peterson AP, Altman LC, Hill JS, Gosney K, Kadin ME. Glucocorticoid receptors in human eosinophils: comparison with neutrophils. *J ALLERGY CLIN IMMUNOL* 1981;68:212-7.
 46. Kita H, Abu-Ghazaleh R, Sanderson CJ, Gleich GJ. Effect of steroids on immunoglobulin-induced eosinophil degranulation. *J ALLERGY CLIN IMMUNOL* 1991;87:70-7.
 47. Venge P, Dahl R, Peterson CGB. Eosinophil granule proteins

- in serum after allergen challenge of asthmatic patients and the effects of anti-asthmatic medication. *Int Arch Allergy Appl Immunol* 1988;87:306-12.
48. Bisgaard H, Groenborg H, Mygind N, Dahl R, Lindqvist N, Venge P. Allergen-induced increase of eosinophil cationic protein in nasal lavage fluid: effect of the glucocorticoid budesonide. *J ALLERGY CLIN IMMUNOL* 1990;85:891-5.
49. Masuyama K, Ishikawa T. Direct interaction of guinea pig eosinophils and adrenergic agents. *Int Arch Allergy Appl Immunol* 1985;78:243-8.
50. Hutson PA, Holgate ST, Church MK. The effect of cromolyn sodium and albuterol on early and late phase bronchoconstriction and airway leucocyte infiltration after allergen challenge of nonanesthetized guinea pigs. *Am Rev Respir Dis* 1988;138:1157-63.
51. Yukawa T, Ukena D, Kroegel C, et al. Beta₂-adrenergic receptors on eosinophils. Binding and functional studies. *Am Rev Respir Dis* 1990;141:1446-52.
52. Atkins PC, Norman ME, Zweiman B. Antigen-induced neutrophil chemotactic activity in man: correlation with bronchospasm and inhibition by disodium cromoglycate. *J ALLERGY CLIN IMMUNOL* 1978;62:149-55.
53. Nagy L, Lee TH, Kay AB. Neutrophil chemotactic activity in antigen-induced late asthmatic reactions. *N Engl J Med* 1982;306:497-501.
54. Kallenbach JM, Panz VR, Joffe BI, et al. Nocturnal events related to 'morning dipping' in bronchial asthma. *Chest* 1988;93:751-7.

IgE and adenosine 5' triphosphate receptors on immature murine mast cells are functionally linked to signal transduction mechanisms

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Calcium mobilization in response to IgE-receptor cross-linking by antigen was assessed in immature murine mast cells cultured from bone marrow to determine whether the early expression of IgE receptors on such cells may be of functional significance. IgE receptors were expressed by approximately 30% of cells after 1 week in culture and by an increased proportion at 2 and 3 weeks. The ability of a non-IgE-dependent stimulus, adenosine 5' triphosphate (ATP), to increase intracellular calcium in these cells was also tested. Calcium mobilization in large numbers of individual cells was monitored with use of a fluorimetric reagent and flow cytometry. Both antigen and ATP had significant effects on intracellular calcium in cells cultured for as little as 1 week with interleukin-3, when few cells exhibited morphologic or functional characteristics of mast cells. Longer times in culture were associated with an increase in the proportion of cells responding to these stimuli with calcium mobilization, but not with a change in the magnitude of the response. We conclude that the early expression of IgE receptors during mast cell development may be functionally significant, since these receptors appear to be linked to cellular signal transduction mechanisms. The data additionally imply a possible role for ATP in mast cell development. (J ALLERGY CLIN IMMUNOL 1992;90:765-71.)

Key words: Mast cells, calcium, signal transduction, IgE receptors, ATP

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New aspects of mast cell biology have recently been elucidated, supporting the opinion that mast cells could play important roles in normal physiology in addition to their well-recognized involvement in disease.¹ Although a body of data exists regarding the biologic activity of mature mast cells and the various stimuli and factors that lead to mast cell differentiation, growth, and activation,¹ comparatively little is known about the functional capabilities of immature mast cells or mast cell precursors undergoing differ-