

Circadian rhythm of cutaneous hypersensitivity reactions in nocturnal asthma

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Background: Diurnal variation in mast cell discharge may play a central role in the early morning fall in peak expiratory flow rate (PEFR) in nocturnal asthmatic patients.

Methods: We tested the hypothesis that there is a circadian rhythm in mast cell response to allergen in 15 patients with nocturnal asthma by measuring the magnitude of cutaneous hypersensitivity reactions at 0600, 1200, 1800, and 2400 hours. Pre-admission, prick skin testing on the ventral aspect of the forearm to various allergens was performed. The allergen producing the largest wheal was tested at six sites on one forearm. Response was quantified after 20 minutes by measuring the area of the wheal produced using planimetry. Every six hours the skin testing was repeated at six new sites on alternating forearms. The average area of the six wheals was calculated and recorded at each time. The prick skin technique was used at all times.

Results: Maximal reactions occurred in 10 of the 15 patients at noon ($P = .031$, Friedman's two way analysis of variance). In these 10 patients wheal area at the time of maximum reactivity was on average 3.3-fold higher than at the time of minimum reactivity. The mean wheal areas for all 15 patients at 0600, 1200, 1800, and 2400 hours were 34 mm², 42 mm², 34 mm², and 35 mm² respectively.

Conclusions: These observations support the concept of a circadian rhythm in mast cell activity in patients with severe nocturnal asthma.

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INTRODUCTION

Diurnal variation in airway caliber, usually measured as change in peak expiratory flow rate (PEFR), is a common phenomenon in asthma. Airway narrowing is consistently maximal in the early morning.¹ This diurnal variation is markedly exaggerated in a subgroup of asthmatic patients, resulting in severe early morning symptoms. The mechanisms responsible for the nocturnal decrease in airway calibre in asthma are unknown but may relate to various circadian rhythms all of which become unfavorable as regards the asthmatic airway at night.²

Atopy is a common finding in asthma, occurring in 60% to 90% of patients with the condition.^{3,4} There is

evidence that the allergen-induced late phase response plays a role in nocturnal wheezing in a proportion of patients with asthma.⁵ It has been argued that a diurnal variation in the level of pulmonary mast cell discharge in response to allergen may be involved in generating a diurnal rhythm in airway inflammation.² A rise in the number of inflammatory cells in bronchoalveolar lavage fluid obtained at 4 AM from patients with severe asthma has been reported.⁶

Direct measurement of airway inflammation and pulmonary mast cell discharge at multiple time points over a 24-hour period is limited by obvious ethical and technical difficulties. The immediate hypersensitivity reaction in response to allergen is mediated by several products released on mast cell discharge.² By measuring the magnitude of acute cutaneous hypersensitivity reactions, we tested the hypothesis that there is a circadian rhythm in mast

cell response to allergen in patients with nocturnal asthma.

MATERIALS AND METHODS

Ethics committee approval was obtained from Ealing Local Regional Ethics Committee. All patients gave written informed consent. Fifteen asthmatic patients, ten women and five men, were recruited from hospital asthma clinics. All patients were atopic and had nocturnal asthma. Nocturnal asthma was defined by the presence of both the following two criteria:

- At least one early morning awakening per week due to shortness of breath, wheeze, or cough.
- An average overnight fall in PEFR > 20%, on home monitoring, over a 2-week period prior to admission.

Maintenance treatment at the time of study is detailed in Table 1. No patients were taking antihistamines at the time of study. Four patients were taking oral prednisolone (patients 7, 8, 9, and 10 in Table 1). In general, this group of patients had severe asthma as judged by treatment requirements.

Study Design

Patients were admitted overnight and studied at 6 AM, noon, 6 PM, and midnight. Pre-admission, prick skin testing on the ventral aspect of the forearm to house dust (150% wt/vol), house dust mite (1.2% wt/vol), grass pollen (2.5% wt/vol), tree pollen (2.5% wt/vol), Aspergillus (5% wt/vol), dog hair (150% wt/vol), histamine (10 mg/mL), and saline (Bencard, Brentford, England) was performed. Response was quantified after 20 minutes by tracing around the wheal onto transparent plastic and then calculating the area by planimetry. The allergen that produced the largest response was then used for multiple serial testing (Table 1). The

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Table 1. Patient Characteristics and Treatment Requirements*

Patient	Age, yr	Sex	AN†	M‡	Treatment		
					Prednisolone	Inhaled Steroids	Theophylline
1	40	F	HD	J	–	beclomethasone: 2 mg	450 mg
2	25	F	DH	J	–	beclomethasone: 2 mg	–
3	27	F	HD	J	–	budesonide: 400 µg	–
4	52	F	HD	J	–	beclomethasone: 800 µg	225 mg
5	56	F	HD	J	–	beclomethasone: 2 mg	–
6	36	M	HD	J	–	beclomethasone: 1 mg	–
7	27	F	HD	J	30 mg	beclomethasone: 1 mg	400 mg
8	23	M	HD	J	35 mg	–	450 mg
9	25	M	HDM	O	30 mg	beclomethasone: 2 mg	–
10	58	M	GP	O	30 mg	beclomethasone: 2 mg	–
11	45	M	HD	J	–	fluticasone: 500 µg	–
12	40	F	HDM	J	–	beclomethasone: 2 mg	–
13	20	F	HDM	J	–	beclomethasone: 1 mg	–
14	56	F	GP	J	–	beclomethasone: 1 mg	–
15	71	F	AF	J	–	budesonide: 400 µg	–

* Drug dosages quoted in the table represent the total daily requirements in patients participating in the study. All patients were taking inhaled beta-agonists as required. Steroids were taken at 8 AM.

† AN, allergen used for prick skin testing; HD, house dust; DH, dog hair; HDM, house dust mite; GP, grass pollen; and AF, Aspergillus.

‡ M, month in which tests were carried out; J, January; and O, October.

same allergen was tested at six sites on one forearm. Every six hours the skin testing was repeated at six new sites on alternating forearms. The average area of the six wheals was calculated and recorded at each time. The prick skin technique was used throughout the study and the wheal area calculated by planimetry 20 minutes after allergen application at all time points.

As a control, prick skin testing was carried out according to the same protocol using histamine (10 mg/mL) in one additional patient who satisfied the above criteria for nocturnal asthma. The patient, a 30-year-old woman was taking inhaled steroids and beta-agonists at the time of study.

All tests on an individual patient were carried out by the same physician. The time of the initial test (midnight, 6 AM, noon, 6 PM) was varied among subjects in an attempt to minimize cumulative effects upon a given test time (Table 2).

Peak expiratory flow rate was measured with a mini-Wright peak flow meter while the patient was seated and before prick skin testing was performed; the best of three attempts was recorded every six hours.

Statistics

Non-parametric statistical analysis was employed as no assumptions could be made with regard to data distribution. The magnitude of skin test responses to allergen and histamine at 0600, 1200, 1800, and 2400 hours were compared using Friedman's two way analysis of variance (Unistat, statistical package, Unistat Limited, London, England). The possibility of a sequence effect of repeated testing on a given test time was analyzed using Friedman's two way analysis of variance after regrouping the data according to the order in which tests were carried out (1st to 4th). All the patients had 12 prick skin tests carried out on each arm. Left versus right arm reactivity in each individual was compared using the Mann Whitney U test.

RESULTS

All patients completed the protocol. There were no adverse effects. Table 2 shows the variation in PEFr and skin tests over time in the 15 subjects. There was a large degree of variability in the magnitude of skin test reactivity between patients. However, reproducibility of measured wheal area at each

time point in a given individual was generally good (Table 2). Cutaneous hypersensitivity reactions were significantly greater at 1200 hours than at all other times in the 15 patients studied ($P = .031$). Ten patients had maximum cutaneous responses at noon. In these 10 patients wheal size at noon was on average 3.3-fold higher (range 1.4 to 17.8) than at the time of minimum reactivity. The mean ($\pm$ standard error of the mean) wheal areas for all 15 patients at 0600, 1200, 1800, and 2400 hours were $34(\pm 9)$ mm², $42(\pm 6)$ mm², $34(\pm 5)$ mm², and $35(\pm 9)$ mm², respectively. The relatively large standard errors reflect intra-subject variability in the magnitude of skin test reactions.

Eleven patients had significant falls in PEFr at 6 AM on the actual day of study, though all had previously shown an average fall of greater than 20% overnight on home peak flow monitoring and had been waking on at least one night per week with asthmatic symptoms.

In one patient, skin tests were carried out simultaneously on both arms; no significant difference was seen in the size of the response between the

Table 2. Patient Skin Test Results* and PEFR

Time	Wheal Area, mm ²				PEFR, L/min			
	1200	1800	2400	0600	1200	1800	2400	0600
Patient								
1	7 (0.4)†	6 (1.6)	4 (0.9)	6 (0.9)	370	310	260	280
2	44 (5.5)†	28 (8.5)	34 (5.2)	30 (1.4)	500	460	330	300
3	50 (7.7)†	39 (5.0)	28 (4.8)	28 (1.8)	460	440	440	410
4	34 (1.4)	31 (1.6)†	24 (0.7)	31 (1.7)	270	200	280	200
5	44 (2.7)	43 (5.9)†	30 (3.5)	38 (2.7)	280	265	240	165
6	43 (3.5)	41 (3.7)†	26 (0.9)	26 (1.2)	450	450	480	490
7	45 (6.8)	28 (2.7)	37 (2.9)†	34 (1.0)	410	400	300	140
8	91 (19.3)	42 (3.8)†	52 (2.6)	51 (5.3)	290	310	320	180
9	71 (7.1)	66 (2.9)†	9 (0.5)	4 (0.3)	330	250	140	140
10	24 (1.1)	13 (1.1)	21 (1.6)	19 (2.0)†	230	220	280	200
11	48 (2.8)†	61 (6.3)	51 (2.9)	33 (3.6)	310	370	310	360
12	22 (0.3)	31 (2.8)†	29 (2.2)	18 (1.9)	400	480	430	230
13	13 (1.4)†	10 (0.6)	11 (0.4)	19 (1.7)	525	490	440	350
14	79 (7.6)†	57 (5.6)	156 (28.3)	156 (21.0)	430	430	340	340
15	8 (1.8)	14 (1.4)	10 (1.3)	12 (1.8)†	250	190	235	190
MWA ± SEM	42±6	34±5	35±9	34±9				
MWD ± SEM	7±0.7	6±0.5	6±0.7	6±0.7				

* Each skin measurement represents the mean of six tests. The standard deviation of the mean is quoted in brackets. Wheal area was significantly greater at 12 noon than at all other times ($P = .031$).

† Time of initial skin test. MWA, mean wheal area (mm²); MWD, mean wheal diameter (mm); and SEM, standard error of the mean.

two arms whether or not that arm had been used for prick skin testing previously (data not shown). In addition, the time of the initial test (midnight, 6 AM, noon, 6 PM) was varied among subjects in an attempt to minimize cumulative effects upon a given test time. Randomization was not equal with initial tests carried out at 1200, 1800, 2400, and 0600 hours in six, six, one and two patients respectively (Table 2). This was for reasons of patient convenience and as previous studies had shown no evidence of cumulative effects of repeated skin testing.⁷ In our study, statistical analysis carried out after regrouping the data in terms of the time of initial skin testing showed no evidence of a sequence effect ($P = .10$). One patient out of 15 showed a significant difference in overall right versus left arm reactivity (patient 7, Tables 1 and 2, $P = .01$). The arm with higher wheal areas had been used for testing at 1200 hours. The remaining 14 patients showed no evidence of a difference in right versus left arm wheal area.

As a control, prick skin testing was carried out over a 24-period in one

additional patient with nocturnal asthma using histamine (10 mg/mL). The mean ($\pm$ standard error of the mean) wheal areas for this patient at 0600, 1200, 1800, and 2400 hours were 15($\pm$ 0) mm², 9($\pm$ 1) mm², 9($\pm$ 1) mm², and 11($\pm$ 1) mm², respectively. Mean wheal area at 0600 hours was significantly greater than at all other times ($P = .014$).

DISCUSSION

This is the first study to report cutaneous allergic responses over a 24-hour period in atopic patients with nocturnal asthma. Peak wheal responses occurred at 12 noon in two-thirds (10/15) of the patients. Mean response in the whole group was statistically significantly greater at 12 noon compared with other time points. Wheal area was examined as an index of cutaneous allergic response: flare being more reliant on local neuronal (axon reflex) activity. Reproducibility within a subject, at any one time, was generally satisfactory (Table 2).

We studied cutaneous hypersensitivity reactions as a marker of generalized mast cell activity. The immediate and

late phase responses to allergen are known to be mast cell dependent in both lung and skin. While there is no doubt that cutaneous and bronchial mast cells differ in terms of several important properties, the available evidence suggests that the time of onset and peak occurrence of mast cell-mediated hypersensitivity reactions are very similar at the two sites.^{8,9}

Bronchoalveolar lavage studies indicate that a circadian rhythm in airway inflammation can only be demonstrated in patients with severe nocturnal asthma.^{6,10} For this reason we wished to study patients with severe disease and did not exclude those on oral steroids or theophylline. Systemic steroids have little effect on immediate hypersensitivity reactions.¹¹ In addition, several studies have shown that theophylline has no effect on either the immediate or late phase cutaneous response to antigen.^{12,13}

The reason for increased immediate hypersensitivity reactions at 12 noon in this group of patients remains to be elucidated. A circadian rhythm in mast cell degranulation, peaking at noon, has been demonstrated in the Hard-

erian gland of the Syrian hamster, although factors responsible for this rhythm have not been determined.¹⁴ Our finding of a maximal cutaneous response to allergen at 12 noon is at variance with the results of three other studies all of which found peak responses to allergen (and histamine) between 1800 and 2300 hours.^{7,15,16} None of these studies investigated patients with asthma. It remains to be established whether this phase shift, compared with atopic nonasthmatics, reflects the presence of asthma or more specifically patients with nocturnal worsening of their asthma.

The importance of allergen exposure in the pathogenesis of nocturnal asthma has been demonstrated in several studies. Cockcroft et al, reported that a single exposure to allergen resulted not only in an immediate bronchial reaction but also in recurrent nocturnal wheezing episodes.⁵ Neutrophil infiltration in the airways and the late phase response to inhaled allergen peaks some 17 hours after exposure.¹⁷ For this reason it seems likely that if a circadian rhythm in the mast cell response to allergen is involved in the pathogenesis of nocturnal asthma, peak mast cell activity should occur at least 12 to 16 hours before maximal airway resistance as implied by the present study.

Our clinical study shows cutaneous immediate hypersensitivity responses to antigen are maximal at 12 noon in patients with severe nocturnal asthma. It is possible that diurnal changes in the vascular reactivity to histamine may contribute to this finding. Diurnal variation in vascular smooth muscle reactivity to various vasoconstrictor and vasodilator agents has been demonstrated. Maximal reactivity to both endothelium-dependent and independent vasodilators has been shown to occur at 0300 hours.¹⁸ This may explain our demonstration in a control subject of a significant circadian

rhythm in the cutaneous response to histamine with peak reaction at 0600 hours. It would not, however, account for increased immediate hypersensitivity reactions at noon. Our observations are, therefore, at least consistent with the existence of a circadian rhythm in allergen-induced mast cell discharge in patients with nocturnal asthma.

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