

# Platelet activation in nocturnal asthma

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## Abstract

Platelet activation may be a factor in the bronchial hyperresponsiveness that characterises asthma. As hyperresponsiveness is increased at night, changes in platelet activation over 24 hours were related to the diurnal changes in peak expiratory flow and plasma catecholamine concentrations in five subjects with asthma and five normal subjects. The effect of muscarinic receptor blockade with intravenous atropine at 0400 hours on these measurements was also studied. Platelet activation, assessed as the ratio of  $\beta$  thromboglobulin to platelet factor 4, was highest when the peak expiratory flow rate was at its lowest in the asthmatic subjects. There was no correlation between platelet activation and plasma catecholamine concentrations. Intravenous atropine did not alter the ratio of  $\beta$  thromboglobulin to platelet factor 4, suggesting that parasympathetic activity is not the cause of the increased platelet activation at night.

Activated platelets have the ability to release potent bronchoconstrictor and vasoconstrictor substances such as thromboxanes, cyclic endoperoxidases, slow reacting substance, 5-hydroxytryptamine, and histamine.<sup>1</sup> Inhalation of antigen to which an asthmatic individual is sensitive causes platelet activation as measured by the release of the platelet specific proteins platelet factor 4 and  $\beta$  thromboglobulin into the circulation,<sup>2-4</sup> though this has been disputed by some authors.<sup>5,6</sup>

Platelet activating factor, which is released from many of the inflammatory cells found in asthmatic airways, causes bronchoconstriction, neutrophil and eosinophil chemotaxis,

and increased vascular permeability only in the presence of platelets.<sup>7</sup> When given by inhalation platelet activating factor in man may cause dose related bronchoconstriction and an increase in bronchial responsiveness, which may persist for one to four weeks in subjects who were previously normal.<sup>8</sup> Platelet activation has therefore been implicated in the pathogenesis of the bronchial hyperresponsiveness that characterises asthma. Platelet activation has also been implicated in acute exacerbations of asthma<sup>9,10</sup>; but there is no information on whether platelet activation occurs at night, when bronchial hyperresponsiveness is at its highest and asthma at its worst.<sup>11-13</sup>

Parasympathetic efferent pathways are implicated in the pathogenesis of nocturnal asthma,<sup>14</sup> but other factors operate in addition. We do not know whether platelets have functional acetylcholine receptors, which theoretically could link parasympathetic nervous activity and platelet activation. It is also possible that platelet activation contributes directly to nocturnal asthma through the release of platelet activating factor and other mediators.

We have therefore studied the circadian rhythm of platelet activation, as measured by platelet factor 4 and  $\beta$  thromboglobulin, and related this to the circadian rhythms of peak expiratory flow and circulating concentrations of the catecholamines adrenaline, noradrenaline, and dopamine, which cause in vitro platelet activation.<sup>15-17</sup> Adrenaline has been implicated in the pathogenesis of nocturnal asthma and has been shown to influence bronchomotor tone in physiological plasma concentrations.<sup>18</sup> The effect of atropine on platelet activation, catecholamines, and peak expiratory flow was also examined in this study.

## Methods

### SUBJECTS

We studied five asthmatic subjects with a diurnal variation in peak expiratory flow of more than 20% and five normal non-smoking subjects. All had normal renal function (table). Subjects were admitted to hospital for one day's acclimatisation and one study day. Xanthine derivatives were stopped for 48 hours before the study and no coffee or tea was allowed during the period in hospital. No asthmatic subject had been taking oral or inhaled corticosteroids for at least six months. Inhaled beta agonists and anticholinergic drugs were withheld for 10 hours before the study and throughout the study period.

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### Characteristics of the subjects

Subject No	Age (y)	Sex	Duration of asthma (y)	Atopy	Smoker	Drugs	PEF (% pred)
1	32	M	27	+	-	B	95
2	25	F	17	+	-	B	105
3	51	M	10	+	-	B	85
4	38	F	28	-	-	B	98
5	35	M	28	+	-	B	95
6	26	M	-	-	-	-	110
7	26	M	-	-	-	-	105
8	26	M	-	-	-	-	95
9	26	M	-	-	-	-	100
10	27	M	-	-	-	-	120

B—beta agonists; PEF—peak expiratory flow.

#### SAMPLES AND PROCEDURES

The study started at 0400 hours, when the subject was woken and immediately recorded three peak expiratory flow measurements from a sitting position. Venepuncture was carried out with a 19G butterfly, with minimal trauma and avoidance of suction in the syringe. The first 10 ml of blood was taken into a syringe and transferred to an ice cooled lithium heparin tube for catecholamine analysis. The next 5 ml of blood was run down the side of an ice cooled test tube containing EDTA, theophylline, and prostaglandin  $E_1$  to inhibit platelet activation. Blood in both tubes was mixed by gentle inversion. The platelet sample tube was kept in melting ice for 30 minutes before centrifugation.<sup>19</sup> The catecholamine tube was centrifuged immediately. Both tubes were centrifuged at 2200  $g$  and 4°C for 30 minutes. Aliquots of platelet poor plasma were removed from the middle layer of the supernatant plasma and both catecholamine and platelet aliquots stored at -70°C until they were assayed. The procedure was repeated at 0800, 1200, 1600, 2000, and 2400 h. During the day the subjects rested supine for one hour before samples were taken. An intravenous cannula was inserted after the 2400 h blood sample and at 0400 h an intravenous injection of 30  $\mu\text{g}/\text{kg}$  of atropine was given 30 minutes before the subject was wakened to block postganglionic muscarinic receptor activity.<sup>14</sup>

We determined catecholamines radioenzymatically, using the modification of McKechnie *et al*<sup>20</sup> to the procedure of DaPrada and Zurcher.<sup>21</sup> Platelet factor 4 and  $\beta$  thromboglobulin were assayed by radioimmunoassay.<sup>19</sup>

#### STATISTICAL ANALYSIS

Data on the patients were analysed initially by multivariate analysis of variance to see whether there was any difference between asthmatic and normal subjects in any of the platelet or catecholamine variables in relation to time. Any variable that differed significantly ( $p < 0.05$ ) was subjected to post hoc analysis by means of the Neumann Keuls test. Thus some variables—those with values higher at only one time and lower at only one time than at other times—show a peak and a trough, whereas other variables show a peak value only. Finally, some variables—for example, peak flow—had a peak phase because values for several times were significantly higher than the others. Correlations between variables were performed by linear regression analysis.

#### Results

No significant difference was found in the mean values for platelet factor 4,  $\beta$  thromboglobulin, adrenaline, noradrenaline or dopamine between normal and asthmatic subjects. Subsequent analyses therefore used the combined data from the two groups for these variables. Mean peak expiratory flow was lower in the asthmatic subjects (392 l/min) than in the normal subjects (598 l/min;  $p < 0.01$ ).

#### CIRCADIAN CHANGES

There was a significant circadian rhythm ( $p < 0.01$ ) for  $\beta$  thromboglobulin (peak value 0800 h) and for the ratio of  $\beta$  thromboglobulin to platelet factor 4 (peak value 0400 h). No circadian rhythm for platelet factor 4 was seen.

Plasma adrenaline showed a circadian rhythm ( $p < 0.05$ ) with a peak at 1600 h and a trough at 0400 h; the other catecholamines did not show any significant circadian rhythm.

The peak expiratory flow of the asthmatic subjects showed a circadian rhythm ( $p < 0.05$ ), with a peak of 433 l/min between 1200 and 2000 h and a trough of 350 l/min at 0400 h. The amplitude % mean of the rhythm was 24%. No rhythm in peak expiratory flow was seen in normal subjects (mean peak flow 598 l/min).

Atropine caused a rise in the peak expiratory flow rate of the asthmatic subjects at 0400 h from 108 l/min ( $p < 0.01$ ) to 358 l/min. This did not significantly differ from the peak phase of expiratory flow seen between 1200 and 2000 h. In the normal subjects atropine caused a rise in PEF peak flow of 36 l/min at 0400 h ( $p < 0.01$ ).

Atropine did not alter platelet activation as measured by platelet factor 4 and  $\beta$  thromboglobulin concentrations or their ratio, and also did not alter catecholamine concentrations (figs 1-3).

#### RELATION OF PLATELET ACTIVATION FACTORS TO OTHER VARIABLES

Platelet factor 4 and  $\beta$  thromboglobulin concentrations showed a correlation with each

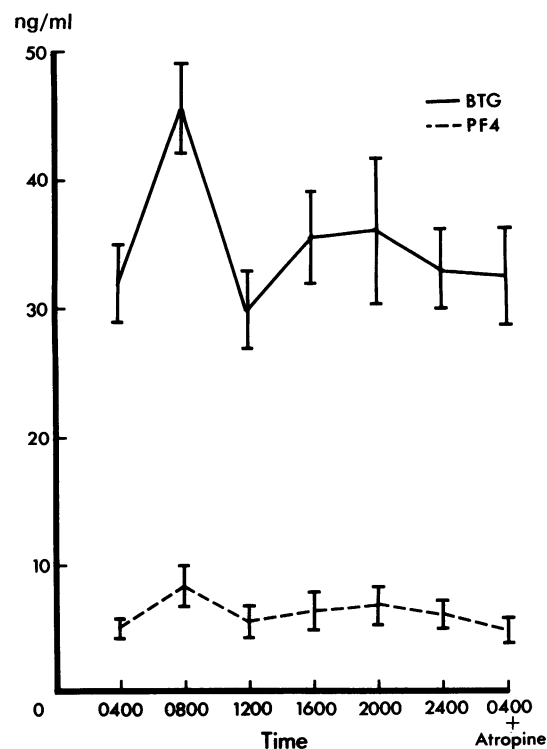


Figure 1 Plot of  $\beta$  thromboglobulin (BTG) and platelet factor 4 (PF4) values over 24 hours and the effect of atropine. The value for  $\beta$  thromboglobulin at 0800 h is significantly higher than at other times ( $p < 0.01$ ). Values are means and standard errors.

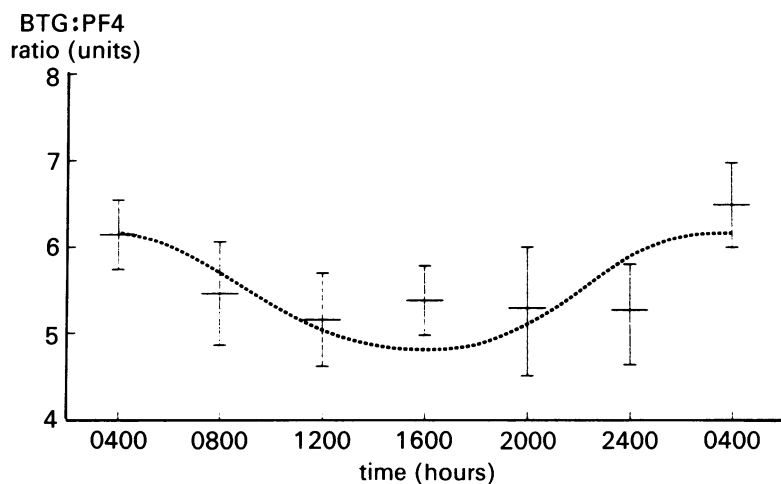


Figure 2 Plot of the ratio of  $\beta$  thromboglobulin (BTG) to platelet factor 4 (PF4): mean (SEM) values over 24 hours, with a cosine regression curve superimposed. The ratio is significantly higher at 0400 h, when peak flow is at its lowest.

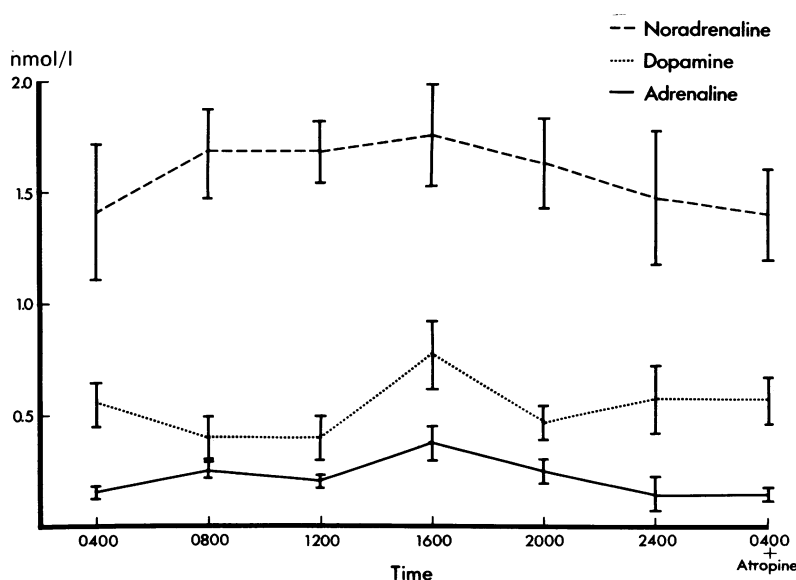


Figure 3 Plot of the plasma concentrations of adrenaline, noradrenaline, and dopamine over 24 hours and the effect of atropine. The 1600 values for adrenaline are significantly higher and the 0400 h values significantly lower than the other values. No significant rhythm is seen for the other catecholamines.

other ( $r = 0.6$ ,  $p < 0.01$ ), but neither was correlated with catecholamine concentration. The correlations between the ratio of the platelet specific proteins and adrenaline ( $r = -0.71$ ,  $p = 0.07$ ) and noradrenaline ( $r = -0.73$ ,  $p = 0.06$ ) were not quite significant; but the correlation with PEF in the asthmatic patients was significant ( $r = -0.77$ ,  $p = 0.04$ ).

### Discussion

In this study a circadian rhythm was detected for adrenaline as in previous studies,<sup>18</sup> but not for dopamine and noradrenaline concentrations, in contrast to previous data.<sup>22,23</sup> A circadian rhythm in peak expiratory flow was seen in the asthmatic subjects,<sup>24</sup> but not in the normal subjects.

Blood samples were taken 30 minutes after intravenous atropine on the basis that maximum pulmonary and cardiac muscarinic recep-

tor blockade is achieved five to 15 minutes after injection with a plateau of effect of at least one hour.<sup>25</sup> The plasma half life for the measures we examined varied, being several minutes for catecholamines,<sup>26</sup> four to eight minutes for platelet factor 4<sup>27</sup> and 10 minutes for  $\beta$  thromboglobulin.<sup>28</sup> Any effect from muscarinic receptor blockade should have been seen for catecholamines and platelet factor 4 by 30 minutes after atropine administration. The effect of atropine on  $\beta$  thromboglobulin would not be maximal at 30 minutes; some effect would be expected, however, at 30 minutes if muscarinic stimulation was affecting the secretion of  $\beta$  thromboglobulin. As atropine did not affect platelet proteins or their ratio we may reasonably presume that muscarinic receptors do not mediate platelet activation as measured by these two platelet specific proteins.

Platelet activation, as measured by platelet aggregation, has been found to be increased in the morning in normal subjects.<sup>29</sup> In our study we found peak platelet activation at times ranging from 0400 h for the ratio of  $\beta$  thromboglobulin to platelet factor 4 to 0800 h for  $\beta$  thromboglobulin. No circadian rhythm, however, was seen for platelet factor 4. Although there is some dispute about whether raised absolute values of platelet factor 4 and  $\beta$  thromboglobulin or the ratio of the two more accurately reflects in vivo platelet activation, the ratio is generally regarded as the better index.<sup>30</sup> There was an inverse correlation between peak expiratory flow and the ratio of  $\beta$  thromboglobulin to platelet factor 4. This implies that platelet activation is highest at the time of the lowest peak flow rate, on the assumption that the ratio is a better indicator of in vivo platelet activation than the individual concentrations of  $\beta$  thromboglobulin and platelet factor 4.

This association does not, however, necessarily imply a causal relation in nocturnal asthma. Although platelet activation has the potential to contribute to the nocturnal fall in peak expiratory flow rate in asthma, an alternative explanation would be that the diurnal variation in adrenaline causes diurnal change in platelet activation and that this is a coincidental epiphenomenon.

The diurnal variation in peak expiratory flow was in phase with that of adrenaline. Alterations of plasma adrenaline within the physiological range have been shown to have a significant effect on bronchomotor tone in both normal<sup>31</sup> and asthmatic subjects.<sup>32</sup> Although alterations in plasma dopamine have no direct bronchodilator action, dopamine has been shown to decrease the bronchial response to inhaled histamine,<sup>33</sup> and may therefore have a permissive role in the control of bronchomotor tone.

Parasympathetic nervous blockade with atropine abolished the nocturnal fall in peak expiratory flow rate that occurred in the asthmatic subjects, PEF values returning to close to the peak values seen at 1600 h. These data confirm that parasympathetic pathways are important in the pathogenesis of nocturnal asthma.<sup>14</sup>

Our studies suggest that platelet activation

may be an aetiological factor in nocturnal asthma. We need further research, using other indexes of in vivo platelet activation and agents that inhibit platelet activation, for establishing causality.

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