

# Loss of normal cyclical $\beta_2$ adrenoceptor regulation and increased premenstrual responsiveness to adenosine monophosphate in stable female asthmatic patients

Kia Soong Tan, Lesley C McFarlane, Brian J Lipworth

## Abstract

**Background** – A study was undertaken to investigate the influence of the menstrual cycle on airway responsiveness and  $\beta_2$  adrenoceptor function in female asthmatic patients. It has previously been shown that normal women exhibit cyclical changes in  $\beta_2$  adrenoceptor function with an increase in  $\beta_2$  adrenoceptor density in the luteal phase during the premenstrual period.

**Methods** – Fifteen women with stable, well controlled asthma (mean forced expiratory volume in one second (FEV<sub>1</sub>) 2.97 l (93.8% predicted)) were evaluated. Measurements were made at the follicular phase (days 1–6) and the luteal phase (days 21–24) of the menstrual cycle. Airway responsiveness was assessed using adenosine 5'-monophosphate (AMP) and expressed as PC<sub>20</sub> AMP.  $\beta_2$  adrenoceptor function was evaluated by measuring lymphocyte  $\beta_2$  adrenoceptor parameters and constructing dose-response curves to salbutamol (100–1600  $\mu$ g). The levels of female sex hormones were also measured at both phases of the cycle.

**Results** – There were significant increases in serum levels of both oestradiol (2.2-fold,  $p < 0.001$ ) and progesterone (7.2-fold,  $p < 0.05$ ) between the follicular and luteal phases. Geometric mean PC<sub>20</sub> AMP was 19.0 mg/ml and 7.6 mg/ml during the follicular and luteal phases, respectively ( $p < 0.05$ ), a 2.51-fold difference (95% CI 1.19 to 5.30) amounting to 1.33 doubling doses of AMP. There was no change in lymphocyte  $\beta_2$  adrenoceptor parameters or in airway  $\beta_2$  adrenoceptor responses to salbutamol between the two phases.

**Conclusions** – Despite an appropriate rise in female sex hormones during the luteal period,  $\beta_2$  adrenoceptor regulation in female asthmatic subjects shows a loss of the normal cyclical pattern. In addition, there were cyclical changes in airway responsiveness to AMP which was highest during the premenstrual period. Thus, drugs such as theophylline which block adenosine receptors warrant investigation in premenstrual asthma.

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Keywords: asthma,  $\beta_2$  adrenoceptor, women, AMP responsiveness.

The role of female sex steroid hormones in asthma is still unclear, although there is much circumstantial evidence to suggest that they may be important. Up to 40% of female asthmatic subjects report a premenstrual deterioration in their condition.<sup>1-4</sup> Indeed, a premenstrual fall in peak expiratory flow rate (PEFR) has been demonstrated even in those who have previously not been aware of this phenomenon.<sup>4</sup> An effect of sex steroid hormones has been shown by the observation that, in a few patients, intramuscular supplementary progesterone eliminated the premenstrual fall in PEFR and allowed better control of asthma with lower doses of systemic steroids.<sup>5</sup> However, the pathophysiology of this phenomenon remains unclear. Female sex steroid hormones have a regulatory role on  $\beta_2$  adrenoceptor function and it has been postulated that abnormal  $\beta_2$  adrenoceptor regulation may be a possible mechanism for premenstrual asthma. We have previously shown that cyclical changes in lymphocyte  $\beta_2$  adrenoceptor function occur during the menstrual cycle in normal women with greater  $\beta_2$  adrenoceptor density and isoprenaline responsiveness in the luteal phase during the premenstrual period.<sup>6</sup> Further support for this role is provided by in vitro studies which show that female sex steroid hormones potentiate the bronchorelaxant effect of catecholamines.<sup>7</sup>

In this study we have investigated  $\beta_2$  adrenoceptor regulation and airway responsiveness to adenosine 5'-monophosphate (AMP) in female asthmatic subjects. AMP was used because it is a marker of mediator release from mast cells.<sup>8</sup>

## Methods

### PATIENTS

Fifteen stable well controlled female asthmatic subjects of mean age 25 years (range 18–39) and forced expiratory volume in one second (FEV<sub>1</sub>) 2.97 (0.11) l (93.8% predicted) gave written informed consent to participate in this study which was approved by the Tayside committee on medical research ethics. All had asthma according to the criteria of the American Thoracic Society<sup>9</sup> and all were non-smokers. None had an exacerbation of asthma that required the use of oral corticosteroids or antibiotics in the preceding three months. None had any documented history of subjective pre-

Department of  
Clinical  
Pharmacology,  
University of Dundee,  
Ninewells Hospital and  
Medical School,  
Dundee DD1 9SY, UK  
K S Tan  
L C McFarlane  
B J Lipworth

Correspondence to:  
Dr K S Tan.

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menstrual deterioration in asthma control. The median consumption of  $\beta_2$  agonist was 0.8 puffs/day and 10 subjects were receiving inhaled corticosteroid in a median dose of 800  $\mu\text{g}$  beclomethasone daily (range 200–2000  $\mu\text{g}$ ). Two subjects were on oral theophyllines (250 mg and 600 mg daily).

#### STUDY DESIGN

Subjects kept a diary record of morning and evening PEFr using a Wright's peak flow meter (Airmed, London, UK). The best of three successive readings was recorded. Subjects attended the laboratory on two separate days, during the follicular (days 1–6) and luteal (days 21–24) phases of the menstrual cycle, day 1 being the first day of the menses. Before each visit inhaled bronchodilators were withheld for eight hours and long acting  $\beta_2$  agonists and oral theophyllines for 24 hours. Inhaled corticosteroids were continued unchanged. Both visits were made at the same time of day at 09.00 hours.

At each visit an intravenous cannula was inserted into an antecubital vein, and after 30 minutes rest blood was removed for measurement of serum levels of oestradiol and progesterone, eosinophil cationic protein (ECP) and lymphocyte  $\beta_2$  adrenoceptor parameters. Airway responsiveness to AMP was evaluated using a standard challenge protocol. After bronchial provocation subjects were rested until FEV<sub>1</sub> returned to within  $\pm 5\%$  of the baseline value. A dose-response curve to inhaled salbutamol via a spacing device (Ventolin metered dose inhaler, Volumatic, Allen & Hanburys, Uxbridge, UK) was then constructed by giving cumulative doubling doses of 100  $\mu\text{g}$ , 200  $\mu\text{g}$ , 400  $\mu\text{g}$ , 800  $\mu\text{g}$ , and 1600  $\mu\text{g}$ . Measurements of FEV<sub>1</sub>, FEF<sub>25–75</sub>, plasma potassium levels and postural finger tremor were made at baseline and 20 minutes after each dose increment, with each dose increment given every 30 minutes.

#### MEASUREMENTS

FEV<sub>1</sub> and FEF<sub>25–75</sub> were measured according to American Thoracic Society criteria<sup>10</sup> with a Vitalograph compact spirometer equipped with a pneumotachograph head and pressure transducer, and on-line computer-assisted determination of FEV<sub>1</sub> and FEF<sub>25–75</sub>. Forced expiratory manoeuvres were performed from total lung capacity to residual volume. The best FEV<sub>1</sub> value was taken from three consistent measurements, and the FEF<sub>25–75</sub> was taken from the best test of three consistent forced expiratory curves. A coefficient of variation of less than 3% was considered as acceptable.

Bronchial challenge was performed using a Nebicheck nebuliser controller (PK Morgan Ltd) with a System 22 Acorn nebuliser (Medic-Aid Ltd) with a driving pressure of 20 psi (138 kPa). The nebuliser was activated for 1.2 seconds from the initiation of inspiration. Fresh solutions of adenosine 5'-monophosphate (AMP; Sigma, Poole, UK) were made up in normal saline on each study day in a range of concentrations from 0.04 mg/ml to 800 mg/ml.

Subjects inhaled five breaths of normal saline control solution followed by sequential doubling concentrations of AMP given at three minute intervals. FEV<sub>1</sub> was measured one minute after each administration of saline and AMP. The test was terminated when a 20% fall in FEV<sub>1</sub> from the post-saline value was attained. A log-dose response curve was constructed and PC<sub>20</sub> was calculated by linear interpolation.

Plasma levels of potassium (K) were measured by flame photometry (IL943 Analyser, Instrumentation Laboratory Ltd, Warrington, UK) with analysis done at the end of the study and samples assayed in duplicate. The intra-assay and interassay coefficients of variation for analytical imprecision were 0.93% and 0.79%, respectively.

Postural finger tremor was measured by a previously validated method using an accelerometer transducer (Entran Ltd, Ealing, UK).<sup>11</sup> Four recordings were made and the results stored on a computer for subsequent spectral analysis of total tremor power of more than 2 Hz (mg<sup>2</sup>/s) with computer assisted autocovariance. The mean of three consistent recordings was subsequently analysed.

Lymphocyte  $\beta_2$  adrenoceptor parameters included receptor density (Bmax), binding affinity (Kd), and maximal cAMP response to isoprenaline (Emax), measured as previously described.<sup>6</sup> In brief, 40 ml whole blood was collected into tubes containing ethylenediamine tetraacetic acid diluted to 50 ml with phosphate buffered saline (PBS) and then two equal portions were centrifuged with 15 ml Lymphoprep (Nycomed Pharma AS, Oslo, Norway). The lymphocyte layer was removed and, after two further washes with PBS and centrifugation, the lymphocyte pellet was removed and resuspended in 5 ml PBS. Lymphocyte Bmax and Kd were determined with <sup>125</sup>I-iodocyanopindolol (ICYP, NEN-DuPont (UK) Ltd, Stevenage, UK) at eight concentrations between 5 and 160 pmol/l. CGP 12177 (Ciba-Geigy, Basle, Switzerland) was added to half the tubes to prevent ICYP binding to receptor sites, thus allowing non-specific binding to be evaluated. Counts were determined with a gamma camera (LKB Wallac, Wallac OY Pharmacia, Turku, Finland) and specific binding was calculated from total binding minus non-specific binding. The interassay coefficients of variation for analytical imprecision were 10.3% for Bmax and 5.9% for Kd. A radioimmunoassay technique (Incstar, Stillwater, USA) was used to evaluate cyclic AMP levels (Emax) following suspension in PBS containing theophylline (100  $\mu\text{M}$ ) and bovine serum albumin and stimulation with isoprenaline  $10^{-4}$  M. The intra-assay and inter-assay coefficients of variation for analytical imprecision were 2.7% and 10.2%, respectively.

Serum levels of oestradiol (Sorin Biomedica, Saluggia, Italy), progesterone (Incstar, Stillwater, USA) and ECP (Pharmacia AB, Uppsala, Sweden) were measured by radioimmunoassay. The intra-assay coefficients of variation for analytical imprecision were 2.9%, 6.3% and 6.3%, respectively.

Table 1 Mean (95% CI) values for sex hormones, AMP airway reactivity, lymphocyte  $\beta_2$  adrenoceptor parameters, eosinophil cationic protein (ECP), peak responses for salbutamol dose-response curve, and peak flow readings during the follicular and luteal phases of the menstrual cycle ( $n=15$ )

|                                       | Follicular             | Luteal                   |
|---------------------------------------|------------------------|--------------------------|
| Progesterone (nmol/l)                 | 1.9 (-2.9 to 6.6)      | 13.7 (8.9 to 18.5)*      |
| Oestradiol (pmol/l)                   | 151.4 (104.1 to 198.6) | 330.6 (283.3 to 377.9)** |
| PC <sub>20</sub> AMP (mg/ml)†         | 19.0 (11.2 to 32.2)    | 7.6 (4.5 to 12.8)*       |
| Bmax (fmol/10 <sup>6</sup> cells)†    | 1.70 (1.4 to 2.0)      | 1.80 (1.5 to 2.1)        |
| Kd (pmol/l)                           | 12.92 (8.35 to 17.49)  | 13.79 (9.22 to 18.37)    |
| Emax (pmol/10 <sup>6</sup> cells)     | 6.65 (4.30 to 9.00)    | 5.69 (3.18 to 8.21)      |
| ECP ( $\mu$ g/l)                      | 4.43 (2.87 to 6.00)    | 4.78 (3.21 to 6.34)      |
| Peak delta FEV <sub>1</sub> (l)       | 0.48 (0.40 to 0.55)    | 0.43 (0.36 to 0.51)      |
| Peak delta FEF <sub>25-75</sub> (l/s) | 1.31 (1.12 to 1.51)    | 1.10 (0.91 to 1.30)      |
| Peak delta K (mmol/l)                 | -0.62 (-0.76 to -0.47) | -0.56 (-0.71 to -0.41)   |
| Peak delta tremor (log units)         | 0.74 (0.50 to 0.98)    | 0.53 (0.28 to 0.79)      |
| Morning PEFr (l/min)                  | 402 (388 to 416)       | 409 (395 to 423)         |
| Evening PEFr (l/min)                  | 424 (410 to 437)       | 426 (412 to 439)         |
| Diurnal variability (l/min)           | 21 (15 to 27)          | 18 (12 to 24)            |

Bmax = receptor density; Kd = binding affinity; Emax = maximal cAMP response to isoprenaline; ECP = eosinophil cationic protein; FEV<sub>1</sub> = forced expiratory volume in one second; FEF<sub>25-75</sub> = mid forced expiratory flow; PEFr = peak expiratory flow rate.

\*  $p < 0.05$ ; \*\*  $p < 0.001$ .

† Geometric mean values.

#### STATISTICAL ANALYSIS

PC<sub>20</sub>, Bmax, and finger tremor were log-transformed for analysis as these are not normally distributed. Effects on salbutamol dose-response curves were analysed as peak responses and area under the curve (AUC). Comparisons between the two phases were made by multifactorial analysis of variance (MANOVA). A probability value of  $p < 0.05$  (two tailed) was considered significant.

#### Results

The results of all parameters are summarised in table 1. There were significant increases in serum levels of both oestradiol (2.2-fold,  $p < 0.001$ ) and progesterone (7.2-fold,  $p < 0.05$ ) between the follicular and luteal phases. Mean differences between the follicular and luteal phases were 179.3 pmol/l for oestradiol (95% confidence interval (CI) 112.4 to 246.1) and 11.8 nmol/l for progesterone (95% CI 5.1 to 18.6). Baseline FEV<sub>1</sub> (l) and FEF<sub>25-75</sub> (l/s) were not significantly different (2.77 versus 2.78 and 2.16 versus 2.34 (follicular versus luteal), respectively). There were significant changes in AMP responsiveness during the menstrual cycle with geometric mean PC<sub>20</sub> 19.0 mg/ml during the follicular phase and 7.6 mg/ml during the luteal phase ( $p < 0.05$ ), a 2.51-fold difference (95% CI 1.19 to 5.30) amounting to 1.33 doubling doses. There was no significant correlation between the cyclical change in PC<sub>20</sub> AMP and the changes in serum levels of oestradiol ( $r^2 = 0.17$ ) or progesterone ( $r^2 = 0.08$ ).

Lymphocyte  $\beta_2$  adrenoceptor parameters and  $\beta_2$  adrenoceptor responses to salbutamol (as peak or AUC) were not altered during the menstrual cycle. Serum ECP levels did not differ between the two phases of the cycle and domiciliary peak flow recordings did not show any changes during the menstrual cycle.

#### Discussion

The subjects in our study showed a 2.5-fold increase in airway responsiveness to AMP in the luteal phase during the premenstrual period. Previous studies have not shown any changes in airway responsiveness during the menstrual cycle. These have used methacholine<sup>4 12</sup> and histamine<sup>13</sup> which are agents that act directly on airway smooth muscle. AMP, on the other hand, induces bronchoconstriction indirectly by activating mast cells to release bronchoconstrictor mediators.<sup>14</sup> It may be that increased AMP responsiveness during the luteal period is caused by sensitisation of adenosine receptors on mast cells by sex hormones resulting in a lower threshold for mediator release in response to adenosine. Against this hypothesis is our finding of no correlation between hormone levels and PC<sub>20</sub>. The influence of female sex hormones is not only confined to airway responsiveness changes but is also evident in skin prick test reactions to histamine and allergen with greater weal and flare reactions during the early luteal phase.<sup>15</sup> Furthermore, it has recently been reported that hormone replacement therapy may increase the risk of developing asthma in postmenopausal women and this may be related to the dose of the oestrogen component and duration of use.<sup>16</sup>

Our study has limitations which we recognise. Firstly, we did not have a control group of non-asthmatic subjects for comparison. The reason for this was that it would not be possible to compare PC<sub>20</sub> values in normal versus asthmatic airways because of confounding effects of airway geometry, even when using airways conductance. Secondly, our patients were stable and did not exhibit premenstrual asthma as such. Nonetheless, we felt at the outset that it would be valid to look at such patients as a way of investigating possible mechanisms for sex hormone modulation of airway and receptor function.

Despite an appropriate rise in luteal phase sex hormones in our group of asthmatic subjects,  $\beta_2$  adrenoceptor parameters exhibited a loss of normal cyclical regulation previously described in normal women.<sup>6</sup> It is unlikely that this is due to the effects of exposure to exogenous  $\beta_2$  agonist as their drug consumption was minimal. In this respect, we have recently shown that exogenous progesterone but not oestrogen up-regulates follicular phase lymphocyte  $\beta_2$  adrenoceptor in normal women<sup>17</sup> and this raises the possibility that, in asthmatic patients, there may be subsensitivity to the postovulatory rise in endogenous progesterone. The lack of difference in serum ECP levels probably reflects the low levels observed in this group of stable patients with mild disease.

What is the clinical implication of our findings? In patients with stable asthma it is important to appreciate that the intrinsic variability of bronchial hyperresponsiveness is such that PC<sub>20</sub> values can vary by up to twofold – that is, one doubling dose.<sup>18</sup> Our findings in this study are therefore likely to have clinical relevance as it exceeds the limit of biological variability. Hence, this change in airway responsiveness from the follicular to the luteal

phase may possibly explain the worsening symptoms observed during the premenstrual period, although the latter was not experienced by our group of mild asthmatic subjects. It is conceivable that these changes may be more clinically significant in patients with more severe disease. Whether treatment for premenstrual asthma will involve drugs such as theophylline,<sup>19</sup> sodium cromoglycate,<sup>20</sup> or nedocromil sodium<sup>21</sup> which antagonise adenosine receptors requires further research.

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