Influence of sex-steroid hormones on the regulation of lymphocyte β_2 -adrenoceptors during the menstrual cycle

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- 1 Up to 40% of female asthmatic subjects suffer a premenstrual deterioration in their condition which may be ameliorated by progesterone supplementation, although the mechanism responsible for this phenomenon is not understood. *In vitro* studies have shown that female sex-steroid hormones potentiate the bronchorelaxant effect of isoprenaline, whilst *in vivo* it has been shown that females exhibit greater sensitivity of systemic β_2 -adrenoceptor responses.
- 2 The aim of the present study was to determine whether cyclical alterations in β_2 -adrenoceptor expression, occurring under the influence of ovarian sex-steroid hormones, may offer an explanation for these findings. *In vitro* parameters of lymphocyte β_2 -adrenoceptor function were investigated in nine normal female subjects (aged 24 ± 2 years) during the follicular (day 2–4) and luteal (day 21–23) phases of their menstrual cycle, and results were compared with those of nine age-matched healthy male controls studied at the same time intervals.
- 3 In female subjects there were significant increases in serum concentrations of oestradiol (3.3-fold) and progesterone (10.6-fold) between the follicular and luteal phases of the menstrual cycle, whereas no changes occurred in males.
- 4 In females during the luteal phase, the increase in sex-steroid hormones was mirrored by an increase in lymphocyte β_2 -adrenoceptor density ($B_{\max}$) and in maximal cyclic AMP response to isoprenaline ($E_{\max}$), which were significantly higher than in male subjects. Mean differences (95% CI) between male and female subjects on visit 2 were 1.09 (0.49 to 1.69) fmol/ 10^6 cells ($P = 0.001$) for $B_{\max}$, and 3.42 (0.80 to 6.04) pmol/ 10^6 cells ($P = 0.02$) for $E_{\max}$. The mean difference (95% CI) in $E_{\max}$ between visits 1 and 2 in females was 2.57 (0.32 to 4.82) pmol/ 10^6 cells ($P = 0.03$). The receptor affinity (K_d) remained unchanged in both sexes.
- 5 These findings suggest that lymphocyte β_2 -adrenoceptors are regulated under the influence of ovarian sex-steroid hormones during the menstrual cycle, and may account for previously observed gender differences of *in vivo* β_2 -responses. Furthermore, the rapid hormone flux and fall in β_2 -adrenoceptor density and cyclic AMP response between luteal and follicular phases may also provide a possible mechanism for premenstrual deterioration in asthma and its response to progesterone.

Keywords β_2 -adrenoceptor oestradiol progesterone lymphocyte menstrual cycle

Introduction

We have previously demonstrated that female subjects exhibit enhanced sensitivity for a variety of systemic agonist β_2 -adrenoceptor responses [1]. Gender differences in the chronotropic effects of

the β -adrenoceptor agonist isoprenaline have also been observed, which are independent of body weight and which appear to represent true differences in β -adrenergic sensitivity at the tissue level [2]. These

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findings may reflect either inherent biological differences in β -adrenoceptor expression between male and female subjects, or the effect of hormonal influences on β -adrenoceptor function in target tissues.

A role for sex-steroid hormones in the regulation of β_2 -adrenoceptor function is further suggested by the observation that a proportion of female asthmatic patients are known to suffer a premenstrual deterioration in their condition [3–5]. The premenstrual period of the cycle is characterised by rapidly falling concentrations of ovarian hormones, and supplemental progesterone at this time has been shown to improve asthma control and reduce the need for systemic steroid therapy in such patients [6]. Since no changes in bronchial hyper-reactivity are seen during the menstrual cycle [7,8], the possibility exists that cyclical changes in sex-steroid production might directly influence airway β_2 -adrenoceptors, altering either receptor numbers or their response to endogenous catecholamines or inhaled β_2 -adrenoceptor agonists. The idea that steroid hormones may modulate female β_2 -receptor function is supported by data from *in vitro* studies which have shown that sex-steroid hormones potentiate the bronchorelaxant and vaso-relaxant effect of catecholamines [9,10], and that corticosteroids increase the density and adenylate-cyclase activity of β_2 -adrenoceptors on human lymphocytes [11–13].

The aim of the present study was to determine whether differences are apparent between the sexes in the expression and function of β_2 -adrenoceptors and whether cyclical changes in these parameters occur under the influence of ovarian hormones during the menstrual cycle. Circulating lymphocytes were studied since these provide a pure and easily obtainable source of peripheral β_2 -adrenoceptors, and appear to reflect the changes which occur in the β_2 -receptors of other tissues including myocardial and bronchial smooth muscle [14,15].

Methods

Blood samples were taken from 18 normal healthy volunteers, of which nine were female. Mean ($\pm$ s.e. mean) ages were 25 ± 2 years (males) and 24 ± 2 years (females). All subjects had a normal cardio-respiratory examination, full blood count, biochemical profile, 12-lead electrocardiogram and urinalysis prior to entry.

None of the subjects had any significant past medical history, nor were any taking regular medication. All female volunteers were nulliparous, with a normal menstrual history and regular cycles, and none had recently (<3 months) taken oral contraceptives. Male and female subjects were paired and each pair attended the laboratory on two occasions, governed by the timing of the female menstrual cycle. Visit 1 occurred in the follicular phase, between days 2 and 4 of the cycle (day 1 being the first day of the menstrual period), and visit 2 in the luteal phase, between days 21 and 23. These periods were chosen to correspond with low and high serum concentrations respectively of oestrogen and progesterone in the

female subjects. On each occasion subjects rested supine for a period of 30 min to minimise possible effects of endogenous catecholamines on lymphocyte β_2 -receptors, after which 50 ml of blood was withdrawn for the lymphocyte β -receptor and sex steroid assays.

Lymphocyte preparation

Whole blood (30 ml) was collected into tubes containing ethylenediamine tetra acid (EDTA) and diluted to 50 ml with phosphate buffered saline (PBS). Equal aliquots of diluted blood were then layered carefully onto 15 ml of 'Lymphoprep' (Nycomed Pharma AS, Oslo, Norway) and centrifuged at $1500 \text{ rev min}^{-1}$ for 30 min. The lymphocyte layer was removed from each tube and combined, before being twice washed with PBS and centrifuged. The resulting lymphocyte pellet was removed and resuspended in 5 ml and PBS. Lymphocyte numbers in this preparation were counted using a Coulter counter and a sufficient volume was removed in order to obtain a total of 5×10^6 lymphocytes, which were required for cyclic AMP (cAMP) stimulation. The remainder of the suspension was re-centrifuged and the lymphocyte pellet resuspended in lymphocyte resuspension buffer to give a concentration of $5 \times 10^6 \text{ cells ml}^{-1}$.

Receptor density and binding affinity

β_2 -adrenoceptor density (B_{max}) and binding affinity (K_d) were assayed by incubating the suspension for 1 h at 37°C with (–) [^{125}I]-iodocyanopindolol (ICYP) (NEN-du Pont Ltd, Stevenage, UK) at concentrations of between 5 and 160 pM. Half of the tubes contained CGP 12177 (Upjohn Pharmaceuticals, Crawley, UK) to prevent ICYP binding to β_2 -receptors. After washing with assay buffer, the suspensions were aspirated onto filter paper using a Brandel Cell Harvester (Semat Technical Ltd, St Albans, UK) and the resultant counts determined by a gamma counter (LKB Wallac, Wallac OY Pharmacia, Turku, Finland). Specific (receptor) binding was calculated from total binding minus non-specific (non-receptor) binding. Receptor density was calculated by extrapolation from the plateau of the specific binding curve plotted for each concentration of ICYP. Receptor affinity was calculated from the concentration of radioligand required to produce 50% of maximal specific binding.

The intra-assay coefficient of variation for analytical imprecision was 5.9% for K_d and 10.3% for B_{max} .

Cyclic AMP stimulation

The suspension containing 5×10^6 cells was centrifuged and the pellet resuspended in PBS containing $100 \mu\text{M}$ theophylline and bovine serum albumin. Cells were stimulated using 10^{-4} M isoprenaline at 37°C , before the reaction was terminated by heating to 95°C . After centrifugation, the supernatant was removed and the cAMP concentration determined by radioimmunoassay (Incstar Ltd, Wokingham, UK). The intra-assay coefficient of variation for analytical imprecision was 2.0%.

Serum oestradiol and progesterone concentrations

Serum concentrations of oestradiol and progesterone were determined by radioimmunoassay (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, California). The lower limits of detection for oestradiol and progesterone were 8 pg ml⁻¹ and 0.03 ng ml⁻¹ respectively. The intra-assay coefficient of variation for analytical imprecision was 7.0% for oestradiol and 6.4% for progesterone.

Statistical analysis

All data were analysed using a 'Statgraphics' software package (STSC Software Publishing Group, Bethesda, USA). Comparisons were made using multifactorial analysis of variance and Bonferroni multiple-range testing to determine where differences were significant. A probability value of $P < 0.05$ was considered to be of statistical significance. Where significant changes occur, results are expressed in the text as differences between the means with the associated 95% confidence interval.

Results

Serum oestradiol and progesterone concentrations (Figure 1)

A significant increase in serum concentration of both oestradiol (3.3-fold) and progesterone (10.6-fold) occurred between visits 1 and 2 in females, whereas in males no changes were seen. In males, serum concentrations of both hormones were equivalent to those of females on visit 1. Mean differences (95% CI) between visits 1 and 2 in females were 62.6 (19.7 to 105.5) pg ml⁻¹ for oestradiol ($P = 0.007$), and 7.03 (3.19 to 10.87) ng ml⁻¹ for progesterone ($P = 0.001$).

Lymphocyte β_2 -adrenoceptor density (Figure 2a)

In males, $B_{\max}$ did not change between the two visits and was not significantly different from that of females on visit 1. However, a significant increase in $B_{\max}$ occurred on visit 2 in female, compared with male subjects. The mean difference (95% CI) between male and female subjects on visit 2 was 1.09 (0.49 to 1.69) fmol/10⁶ cells ($P = 0.001$).

Cyclic AMP generation (Figure 2b)

A similar pattern of response to that of $B_{\max}$ was seen, with there being no difference between the sexes on visit 1. There was an increase in cAMP generation ($E_{\max}$) in females on visit 2 which was significantly greater than that on visit 1, and also significantly different from that of males on both visits. The mean difference (95% CI) between males and females at visit 2 was 3.42 (0.80–6.04) pmol/10⁶ cells ($P = 0.02$); and between visits 1 and 2 in females was 2.57 (0.32 to 4.82) pmol/10⁶ cells ($P = 0.03$).

Figure 1 Serum concentrations of a) oestradiol, and b) progesterone in male and female subjects. Mean values (+ s.e.mean) are shown. * $P < 0.05$ females compared with males for each visit. + $P < 0.05$ visit 2 compared with visit 1 for each sex.

Receptor affinity (Figure 2c)

No significant differences in receptor affinity (K_d) were found either between the sexes or between the two visits.

Discussion

The results of the present study showed that an increase in lymphocyte β_2 -adrenoceptor density and cAMP generation occurred in females during the luteal phase of the menstrual cycle, compared with that seen in the follicular phase, and in comparison with male subjects. These findings suggest that lymphocyte β_2 -adrenoceptors are regulated under the influence of ovarian sex-steroid hormones, and may represent an explanation at the receptor level for the previously observed gender differences in response to β_2 -adrenoceptor agonists [1,2]. Furthermore, the rapid hormone flux and fall in β_2 -adrenoceptor density and cyclic AMP response between luteal and follicular phases may also provide a possible mechanism for the deterioration of asthma control which may occur in the pre-menstrual period and its response to progesterone [3–6].

Figure 2 Lymphocyte β_2 -adrenoceptor a) density, b) cAMP generation, and c) affinity, in male and female subjects. Mean values ($\pm$ s.e. mean) are shown. * $P < 0.05$ females compared with males for each visit. † $P < 0.05$ visit 2 compared with visit 1 for each sex.

Whereas in males no changes in lymphocyte β_2 -adrenoceptor fraction or serum concentrations of sex-steroid hormones were seen, marked differences were apparent in female subjects between the early and late phases of their menstrual cycles. Serum oestradiol and progesterone concentrations were not different between the sexes on visit 1, whereas in the luteal phase of the cycle a significant increase in both hormones occurred in female subjects. These changes were mirrored by increases in the density of lymphocyte β_2 -adrenoceptors and cAMP generation, with receptor affinity being unaltered. On the basis of the above findings, the increase in cAMP response to isoprenaline might conceivably be due at least in part to the overall increase in β_2 -receptor density. However, one cannot completely exclude concomitant changes in G-protein coupling, since there was a disproportionate increase in cAMP generation occurred (108%) compared with the increase in B_{max} (29.7%). The

temporal sequence of events, and the differences observed between male and female subjects would therefore suggest a causal relationship between changes in ovarian sex-steroid hormones and alteration of β_2 -adrenoceptor function.

It is well-recognised that steroid hormones potentiate the stimulating effects of catecholamines on smooth muscle [9, 10], although several explanations exist for the mechanism of this effect. Previous *in vitro* studies using animal tissues have demonstrated that both corticosteroids and the sex-steroids 17β -oestradiol and progesterone, produce inhibition of catechol-*O*-methyltransferase (COMT) and a reduction in the extraneuronal uptake of catecholamines [9, 10, 16]. However, it is of note that this effect was not seen with either fenoterol or other non-catecholamine sympathomimetic compounds, where both inhibition of COMT and blockade of the re-uptake process failed to potentiate the response to these agents.

In the *in vivo* situation one might have expected the increase in sex-steroids to have potentiated endogenous catecholamines in the same manner during the second half of the menstrual cycle. Were this the only mechanism involved, the increased influence of endogenous adrenaline would have produced down-regulation of lymphocyte β_2 -adrenoceptors in these circumstances. Since we have demonstrated an increase rather than a decrease of β_2 -adrenoceptor density at this time, this suggests that the sex-steroids directly modulate β_2 -adrenoceptor numbers and that this effect appears to outweigh the other mechanisms of catecholamine potentiation. Although not previously described with the sex-steroids, one study [11] has demonstrated that prednisolone produces rapid reversal of the down-regulation in lymphocyte β_2 -adrenoceptors which occurs in response to the β_2 -agonist terbutaline. It has also been shown that steroid hormones may modulate the expression of β_2 -adrenoceptors by producing an increase in the rate of gene transcription [17], and this may be the mechanism responsible for the above effects.

From a clinical standpoint, the alterations in lymphocyte β_2 -adrenoceptors that we have described during the menstrual cycle might explain the relationship between premenstrual asthma and its response to progesterone. Up to 40% of all female asthmatics are aware of a premenstrual deterioration in their symptoms [3, 4], and in the vast majority this occurs in the five days leading up to their period [3]. Even in asthmatics who have not previously been aware of this phenomenon, it is possible to demonstrate worsening of symptoms and a reduction in peak expiratory flow rates premenstrually [5]. The pathophysiological mechanism of this disorder is presently not clear. It has been shown that airway hyper-reactivity in response to both methacholine [7] and histamine [8] is unchanged in asthmatic subjects during the menstrual cycle, although neither study specifically investigated patients with premenstrual symptoms. It has also been suggested that the rapid fall in serum progesterone which occurs towards the end of the cycle may be important, since progesterone is a smooth muscle relaxant and withdrawal of its bronchodilator action may precipitate asthma in susceptible individu-

als [6]. In keeping with this hypothesis is the observation that in a small number of patients with premenstrual asthma, intramuscular progesterone supplementation eliminated the falls in peak expiratory flow rates which occurred at this time, and allowed patients to be controlled on smaller doses of systemic steroids [6].

Although changes in serum concentrations of progesterone may be important in the pathogenesis of premenstrual asthma by virtue of its direct bronchodilator effects, the results of the present study suggest an alternative explanation for these observations. We postulate that throughout the menstrual cycle alterations in the expression and function of airway β_2 -adrenoceptors may occur, which mirror the changes we have demonstrated on lymphocytes. Since these receptors appear to be directly influenced by sex-steroid hormones, the rapid fall in oestradiol and progesterone in the premenstrual period is associated with an equally rapid decline in β_2 -adrenoceptor density and associated cAMP response. These changes occurring at the level of the β_2 -adrenoceptor would be expected to attenuate both the background effect of endogenous adrenaline, as well as the response to exogenous β_2 -adrenoceptor agonists, and might conceivably precipitate an exacerbation of asthma in certain susceptible subjects. It is likely that rapid fluxes in hormone levels and β_2 -adrenoceptor function are of greater importance than absolute levels, since male and female levels are equivalent during the follicular phase; and during pregnancy, when sex-steroid hormones are greatly increased, the effect on asthma control is very variable [18].

This study has certain limitations which we recognise and accept. We did not measure catecholamine levels, although in this respect there is no conceivable reason why females should be more stressed than males. Indeed, if stress were the explanation for our findings, then one might have expected males to react in the same way and also show a similar pattern

for lymphocyte parameters between the two visits. It is possible that changes in peripheral lymphocyte β_2 -adrenoceptors may not necessarily reflect equivalent changes in the airways. However, whereas lymphocytes are easily obtained and relatively simple to study, to obtain samples of airway smooth muscle is not possible by bronchoscopic biopsy techniques, and would in our view be unethical. We are reassured by data demonstrating that lymphocyte β_2 -adrenoceptors reflect changes in the β_2 -subtype in other tissues [14], and on this basis have used lymphocytes as a surrogate measure of bronchial β_2 -receptors as suggested by Davis *et al.* [15]. However, there is some evidence to suggest that β_2 -adrenoceptors in lung and mononuclear leukocytes may have different propensities to downregulate following exposure to β_2 -adrenoceptor agonists [19]. Although we have demonstrated parallel changes in sex-steroid hormones and lymphocyte β_2 -adrenoceptors, this does not necessarily imply a causal relationship between the two phenomena. This issue may be clarified by further *in vitro* or *in vivo* pharmacological studies to investigate whether changes in parameters of β_2 -adrenoceptor function occur in response to the exogenous administration of sex-steroid hormones. Such studies would also determine the relative contribution of oestradiol and progesterone to this effect. It may also be argued that our data, obtained from normal volunteers, may not be representative of that from asthmatic subjects, although in this respect bronchial smooth muscle relaxant response to isoprenaline is unaltered in asthmatics compared with controls [20]. Furthermore we have previously shown that no differences in parameters of lymphocyte β_2 -adrenoceptor function are seen in asthmatic subjects compared with normal controls [21].

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