Br J Clin Pharmacol 1996; **41**: 414–416

Effects of exogenous female sex-steroid hormones on lymphocyte β_2-adrenoceptors in normal females

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We have previously shown that lymphocyte β_2-adrenoceptors (AR) are under cyclical control of sex-steroid hormones with greater receptor density during the luteal phase of the menstrual cycle. It has also been postulated that abnormal cyclical regulation of β_2-AR might be a possible mechanism for premenstrual asthma. The effects of exogenous female sex-steroid hormones on lymphocyte β_2-AR function were studied in eight normal healthy females. They were evaluated at two successive menstrual cycles, during the follicular phase (day 1–6). They were randomized to receive single oral doses of either ethinyloestradiol 50 μg or medroxyprogesterone 10 mg in a cross-over study. Lymphocyte β_2-AR parameters were evaluated at baseline (t_0), 24 h (t_{24}) and 72 h (t_{72}) after ingestion. Baseline levels of progesterone and oestradiol were comparable on both cycles. Receptor density (B_{max}) increased significantly ($P < 0.01$) from t_0 after progesterone but not oestradiol at t_{24}: a 1.39-fold geometric mean difference (95% CI 0.96–2.00) between t_{24} *vs* t_0. Receptor affinity (K_d) and maximal cAMP response to isoprenaline (E_{max}) were not altered by either treatment. These results show that exogenous progesterone but not oestradiol, given during the follicular phase, significantly increased β_2-AR. This, therefore, suggests that endogenous progesterone is probably responsible for previously observed increase in B_{max} during the luteal phase of the female menstrual cycle. These findings may suggest possible therapeutic strategies for modulation of β_2-AR in premenstrual asthma.

Keywords oestrogen progesterone β_2-adrenoceptors lymphocyte menstrual cycle

Introduction

Female sex-steroid hormones have a role in the regulation of β_2-AR function. This has been demonstrated in a previous study where lymphocyte β_2-AR density was significantly greater during the luteal phase as compared with the follicular phase, in association with raised premenstrual levels of progesterone and oestradiol [1].

There is also evidence from *in vitro* studies which show that female sex-steroid hormones potentiate the bronchorelaxant and vasorelaxant effect of catecholamines [2, 3]. A further putative role of female sex-steroid hormones in β_2-AR regulation during the luteal phase is suggested by the observation that a proportion of female asthmatic patients suffer a premenstrual exacerbation of their condition [4, 5, 6]. Indeed, it has been postulated that abnormal premenstrual β_2-AR regulation may be a possible mechanism for this phenomenon.

The aim of this present study was to determine which of the female hormones, progesterone or oestradiol, is responsible for the previously observed change in lymphocyte β_2-AR density during the menstrual cycle. This was investigated by administering exogenous high doses of oestradiol and progesterone during the follicular phase when endogenous levels of both hormones are low.

Methods

Eight healthy female volunteers participated in this study, mean ($\pm$s.e.mean) age was 25 ± 3 years. All subjects had a normal full blood count and biochemical profile prior to entry.

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None of the subjects had any significant past medical history, nor were they taking any regular medication. All but one (para 3) were nulliparous, with a normal menstrual history and regular cycles and none had recently (at least 3 months) taken oral contraceptives. All volunteers provided written informed consent prior to entry to the study which was approved by the Tayside Committee on Medical Ethics.

Volunteers attended the laboratory during the follicular phase (day 1–6) on two successive menstrual cycles, day 1 being the first day of the menstrual cycle. Baseline levels of serum progesterone and oestradiol were measured at each cycle. They were randomized to receive either progesterone (medroxyprogesterone 10 mg, Upjohn Ltd) or oestrogen (ethinyloestradiol 50 μg, Evans Medical Ltd) as single oral doses in cross-over fashion for the two menstrual cycles. Peripheral blood (40 ml) was withdrawn, prior to the ingestion of the tablet, at baseline (t_0), and then at 24 h (t_{24}) and 72 h (t_{72}) after ingestion, for lymphocyte β_2-AR assay on each of the two cycles.

Medroxyprogesterone was chosen as it is the least virilizing of the progesterones. The tablets are the highest dose licensed which are recommended for normal clinical practice. Two of the subjects experienced nausea with oestrogen and one had nausea after progesterone, indicating that higher doses would have been poorly tolerated.

Lymphocyte preparation

A total of 40 ml of whole blood was collected into tubes containing ethylenediamine tetra acetic acid (EDTA), diluted to 50 ml with phosphate buffered saline (PBS), and then two equal aliquots were centrifuged with 15 ml of lymphoprep (Nycomed Pharma AS, Oslo, Norway) and the lymphocyte layer subsequently removed. Following two further washes with PBS and centrifugation, the lymphocyte pellet was resuspended in 5 ml of PBS prior to lymphocyte counting, 5×10^6 cells being required for cyclic AMP stimulation. Lymphocyte β_2-adrenoceptor density (B_{max}) and receptor affinity (K_d) were determined using (-) [^{125}I]-iodocyanopindolol (ICYP) (NEN-du Pont (UK) Ltd, Stevenage, UK) at eight concentrations of 5 to 160 pm, with CGP 12177 1 μM (Ciba-Geigy) being added to half the tubes to prevent ICYP binding to receptor sites and allow non-specific (non-receptor) binding to be evaluated. Resultant counts were determined by a gamma counter (LKB Wallac, Wallac OY Pharmacia, Timan, Finland) and specific (receptor) binding was calculated from total binding minus non-specific binding. A radioimmunoassay technique was used to evaluate cyclic AMP levels (E_{max}) following suspension in PBS containing theophylline (100 μM) and bovine serum albumin, and stimulation with isoprenaline (10^{-4} M). The inter-assay coefficient of variation for analytical imprecision was 10.3% for B_{max} and 5.9% for K_d. The inter-assay and intra-assay coefficient of variation for analytical imprecision for E_{max} was 10.2% and 2.7% respectively.

Serum oestradiol and progesterone

Samples for serum oestradiol and progesterone were centrifuged at 4 °C and serum extracted and stored at −20 °C until measured in one batch at the end of the study. Serum oestradiol (Sorin Biomedica, Saluggia, Italy) and serum progesterone (Incstar Ltd, Wokingham, UK) were measured by radioimmunoassay. The within assay coefficients of variation for analytical imprecision were 2.9% and 3.1% for oestradiol and progesterone respectively.

Statistical analysis

The power of the study was 80% to detect a 25% difference in B_{max}. For E_{max} and K_d, comparisons between treatments were made by multifactorial analysis of variance (MANOVA) using subjects, treatments and time as within factors for the analysis. Duncan's multiple range test was then applied to ascertain at what times differences occurred within a given cycle for each treatment. For B_{max}, which is not normally distributed, analysis was performed non-parametrically using Friedman's analysis of ranks. A probability value of less than 0.05 (two-tailed) was considered significant. Data for B_{max} are shown as geometric means and non-parametric 95% CI. All other data are shown as means and 95% CI for within each treatment group.

Results

Baseline levels of oestradiol (pmol l^{-1}) and progesterone (nmol l^{-1}) were comparable for the two consecutive cycles (cycle 1 *vs* cycle 2) mean (95% CI): oestrogen 326.5(129.2–523.8) *vs* 252.1(54.8–449.4) and progesterone 5.7(4.8–6.6) *vs* 6.1(5.7–7.0).

The changes in lymphocyte β_2-AR parameters following treatment with progesterone and oestradiol are summarized in Table 1. There were no significant differences in any parameter at t_0 when comparing the two treatments. There was a significant increase from baseline (t_0) in receptor density (B_{max}) at t_{24} following treatment with progesterone but not oestradiol ($P < 0.01$ t_{24} *vs* t_0: 1.39-fold geometric mean difference, 95% CI 0.97–2.00). By 72 h, B_{max} had fallen to approximately baseline (t_0) levels. The increase in B_{max} following progesterone is greater than oestradiol at t_{24} ($P < 0.05$, oestrogen *vs* progesterone at t_{24} 1.28-fold geometric mean difference 95% CI 0.95–1.73). There were no significant changes from baseline in K_d and E_{max} following either treatment. Likewise there was no significant difference between the two treatments at each time point; for K_d, at t_0 (95% CI −8.9 to 5.4), at t_{24} (95% CI −10.8 to 7.1) and at t_{72} (95% CI −8.0 to 9.7). For E_{max}, at t_0, (95% CI −0.3 to 2.4), at t_{24} (95% CI −1.3 to 2.3) and at t_{72} (95% CI −2.1 to 2).

Statistical analysis did not show any evidence for order effect.

Table 1 Mean (95% CI) values for K_d and E_{max} following treatment with oestrogen and progesterone at baseline (t_0), 24 h (t_{24}) and 72 h (t_{72}). Values for B_{max} are geometric means (non-parametric 95% CI)

	t_0	t_{24}	t_{72}
$B_{max\ (fmol/10^6}$ cells)			
Oestrogen	1.40 (1.16–1.69)	1.59 (1.32–1.91)	1.43 (1.19–1.72)
Progesterone	1.45 (1.19–1.77)	2.03 (1.66–2.47)*†	1.56 (1.28–1.90)
K_d (pmol l^{-1})			
Oestrogen	10.85 (4.22–17.48)	13.54 (6.91–20.17)	10.65 (4.02–17.28)
Progesterone	9.11 (5.87–12.35)	11.66 (8.42–14.89)	11.50 (8.26–14.73)
E_{max} (pmol/10^6cells)			
Oestrogen	5.53 (4.55–6.51)	5.20 (4.22–6.19)	5.68 (4.70–6.67)
Progesterone	6.60 (5.26–7.93)	5.65 (4.18–7.12)	5.61 (4.28–6.95)

* $P<0.01$ t_{24} *vs* t_0. † $P<0.05$ Oestrogen *vs* progesterone at t_{24}.

Discussion

This study has demonstrated that an exogenous high dose of oral progesterone but not oestradiol, given during the follicular phase, significantly increased lymphocyte β_2-AR density. This would, therefore, suggest that endogenous progesterone is probably responsible for previously observed increase in B_{max} during the luteal phase of the female menstrual cycle [1]. A degree of caution is advisable in extrapolating our results with lymphocytes from normal subjects to changes in asthmatic airways. However, it has recently been shown with positron emission tomography that changes in lymphocyte β_2-AR density closely mirror those on lung β_2-AR, thus making comparisons between airway and lymphocyte β_2-AR parameters seem valid [7]. The lack of difference in E_{max} with progesterone probably reflects the relatively small difference in B_{max} at t_{24} compared with t_0. Whether a higher dose of progesterone would have increased E_{max} as well as B_{max} is unclear.

An alteration in normal cyclical regulation of β_2-AR may be a possible mechanism for premenstrual asthma, as airway hyper-reactivity in response to both methacholine [8] and histamine [9] is unchanged in asthmatic subjects during the menstrual cycle. In keeping with this hypothesis is the observation that in a small number of patients with premenstrual asthma, intramuscular progesterone therapy prevented the falls in peak expiratory flow rates which occurred at this time, and allowed better control of these patients on smaller doses of systemic steroids [10]. The findings of the present study showing that exogenous progesterone up-regulates β_2-AR may suggest a possible mechanism for this therapeutic effect, and provide future therapeutic strategies for modulation of β_2-AR in premenstrual asthma.

This work was supported by the National Asthma Campaign.

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(*Received 2 October 1995, Accepted 3 January 1996*)