β-Adrenoceptor Changes in Mononuclear Leukocytes During Pregnancy (43890)

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Abstract. **Leukocyte β_2-adrenoceptors mirror similar receptors in the uterus. This study examined changes in β_2-adrenoceptors and in cAMP production during pregnancy using peripheral mononuclear leukocytes isolated from 17 pregnant women and 5 nonpregnant controls. β_2-Adrenoceptor density was determined by ^{125}I-pindolol binding. cAMP production under basal and stimulated (10 μM isoproterenol) conditions was determined by radioimmunoassay. Groups were compared by unpaired *t* test. There was a nonsignificant decrease in the density of β-adrenoceptors during pregnancy. While basal cAMP production was unchanged during pregnancy, stimulated cAMP production was decreased (44% of control, $P < 0.001$; 95% CI 33%–56%). Stimulated cAMP production per receptor was lower in leukocytes from pregnant women and was not a constant relationship, but was increased markedly in leukocytes having fewer than 400 β-adrenoceptor sites per cell. There are significant changes in the coupling of β-adrenoceptors to cAMP production during pregnancy without changes in β-receptor density, affinity, or basal cAMP production.**

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β_2-Adrenoceptors constitute 80% of β-adrenoceptors in the human myometrial cell membrane and mediate uterine relaxation (1). β_2-Adrenoceptors are coupled through a guanine nucleotide regulatory protein to adenylyl cyclase. Adrenergic stimulation results in an increase in production of second messenger cyclic-AMP and a phosphorylation by protein kinase A of myosin light chain kinase, making it less sensitive to Ca^{++}-calmodulin (2) and producing uterine relaxation.

During pregnancy, the uterus must remain quiescent; therefore, one might reasonably expect an increase in β-adrenoceptor activity in the pregnant uterus. However, studies during pregnancy suggest that the density of β-adrenoceptors decreases during normal pregnancy (3, 4). Indirect measurements of receptor activity have implied that β-adrenoceptor coupling to cAMP production is also decreased during pregnancy (3, 5).

While uterine β_2-adrenoceptors are of profound interest and importance, they are largely inaccessible for study, particularly during normal pregnancy. Mononuclear leukocytes contain a similar population of β_2-adrenoceptors coupled to cAMP production. Receptor regulation following tocolytic therapy is similar to that in the myometrium (1), and cAMP production in mononuclear leukocytes strongly correlates with that of uterine tissue obtained at cesarean section (6). Mononuclear leukocytes are therefore a model of similar β_2-adrenoceptors in the uterus.

The aim of our study was to examine β-adrenoceptor function in women at varying times during pregnancy using the leukocyte model. We determined the density of β-adrenoceptors and their affinity for iodopindolol, as well as the ability of receptors to stimulate cAMP production. We found that coupling between β-adrenoceptor stimulation and cAMP production is decreased during pregnancy, even though there are no significant changes in β-adrenoceptor density, affinity, or basal cAMP production.

Materials and Methods

Leukocyte Isolation. This study was approved by Institutional Review Boards of East Tennessee

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State University and the Johnson City Medical Center Hospital. All chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted. Women included in this study were all HIV-negative nonsmokers. The study population included women from our high-risk pregnancy clinic with current or previous history of fetal malformation, significant medical disease, or poor obstetrical outcome. Following written informed consent, whole blood (≈8 ml) was obtained in heparinized tubes during normal prenatal visits; controls were nonpregnant women not using oral contraceptives. Mononuclear leukocytes were isolated by a modification of the method of Böyum (7), but were maintained at 4°C during isolation to inhibit alterations in lymphocyte β-adrenoceptors. Blood was diluted to 15 ml with DPBS and layered onto 8 ml of Histopaque-1077. After separation on this medium, the layer containing mononuclear cells was removed and DPBS added to a volume of 50 ml. Mononuclear leukocytes were pelleted by centrifugation at 400*g* for 10 min. Cells were resuspended in 15 ml and recentrifuged three times. Cells were finally resuspended in Ca^{++}, Mg^{++}-free DPBS at a concentration of 4×10^6 cells/mL. When assessed by trypan blue exclusion cell viability was greater than 90%.

β-Adrenoceptor Binding. Radioligand binding studies were performed using intact leukocytes. Steady-state binding of $(\pm)^{125}$I-pindolol (2200 Ci/mmol;Dupont NEN, Boston, MA) was determined on aliquots of 4×10^5 cells, using a modification of the method of Halper *et al.* (8). Cells incubated in polypropylene tubes at 37°C with varying concentrations of labeled pindolol (1.25 to 1000 p*M*) for 30 min were rinsed (3 × 5 mL) with ice-cold 25 m*M* MOPS (3[N-morpholino]propanesulfonic acid) pH 7.4 and vacuum filtered through Whatman GF/B filters in a Hoefer (San Francisco, CA) binding manifold.

Saturation binding curves were used to determine receptor density (B_{max}), receptor affinity (K_d) for the radioligand, and nonspecific binding.

Binding Analysis. Binding parameters were determined by nonlinear least squares weighted (1/Y) regression using the computerized curve fitting program GraphPAD Inplot (San Diego, CA). The following equation for binding to a single specific receptor site was used:

$$\text{Bound} = \frac{B_{max} \cdot [^{125}\text{I-Pindolol}]}{K_d + [^{125}\text{I-Pindolol}] + K_{nonspecific} \cdot [^{125}\text{I-Pindolol}]}$$

where B_{max} is the maximum specific binding, K_d is the dissociation constant for iodopindolol, and $K_{nonspecific}$ is the nonspecific binding constant. A two-site model of specific binding was rejected, since there was no significant improvement in fit using the sequential *F* test (10).

cAMP Generation. cAMP was generated in 10^6 cells in the presence of 20 μ*M* phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine using a modification of the method of Halper *et al.* (8). In a final volume of 250 μl, cells were incubated in glass tubes for 15 min at 37°C in the absence (basal cAMP) or presence (stimulated cAMP) of 10 μ*M* β-adrenoceptor agonist (−)isoproterenol. After placing tubes in boiling water for 5 min, tubes were centrifuged for 15 min at 2500*g* and the supernatant removed and frozen at −40°C for later analysis of cAMP.

cAMP Radioimmunoassay. cAMP was determined using a radioimmunoassay kit (New England Nuclear, Boston, MA). A 0.1 ml sample from cAMP generation protocol was measured for cAMP content. Acetylation of cAMP, which improves assay sensitivity but increases variability, was not necessary. Cross-reactivity in this assay for cGMP, GMP, ATP, ADP, and AMP are less than 0.02%; cross-reactivity for theophylline is less than 0.0001%.

Calculations. cAMP production per receptor was calculated by dividing cAMP generation per cell by the number of receptors per cell. Values for K_d represent geometric means.

Statistics. Values are expressed as mean ± standard error for the total number of observations. Normal distribution of data was assessed by χ-square analysis. Statistical comparisons of the pregnant and control groups were made by unpaired *t* test with a $P < 0.05$ significance level. Statistical analyses were performed using SAS-PC version 6.04 (9).

Data values for B_{max}, K_d, and cAMP production were analyzed by χ-square analysis and appeared to be normally distributed ($P > 0.6$; data not shown).

Results

Table I presents information on the patient population. The five control patients ranged in age from 23 to 37 years (mean 31.6 ± 2.4; 95% confidence interval 24.8 to 38.4 years), 17 pregnant patients ranged in age from 18 to 42 years (mean 24.0 ± 1.5; 95% confidence interval 20.7 to 27.2 years). Because of the nature of this clinical practice many patients had a history of pregnancy complications. All pregnant patients were taking daily multivitamins. One patient (Patient 7) was receiving ticarcillin/clavulanic acid; results did not appear different from those of other patients not receiving medication and are included in the pregnant group.

Leukocytes were sampled during pregnancy (Table II) from 6.4 to 39.7 weeks of gestation; three patients (Patients 1, 5, and 9) were sampled twice. We noted no striking relationship between leukocyte measurements and birth outcome (not shown) or between

Table I. Patient Population

Patient number	Age (years)	Gravida[a]	History[b]	Sampling gestational age (weeks)
1	25	4	N	32.4
				36.6
2	23	4	Y	33.5
3	22	4	N	13.2
4	22	1	N	35.0
5	18	3	Y	28.3
				36.3
6	26	2	Y	30.1
7	22	3	N	35.5
8	34	3	Y	30.4
9	19	2	N	35.2
				38.0
10	42	8	N	11.2
11	20	2	N	6.4
12	26	6	Y	7.2
13	29	2	Y	26.3
14	19	2	N	22.5
15	20	4	Y	16.3
16	18	1	N	28.5
17	23	2	Y	10.5
18	33	2	—	Control
19	30	2	—	Control
20	35	3	—	Control
21	23	0	—	Control
22	37	3	—	Control

[a] Pregnancy number.
[b] History of pregnancy complications.

leukocyte measurements and gestational age (not shown) in our study.

[^{125}I]-pindolol binding was saturable and displayed properties of a single binding site (Fig. 1). There was no indication of additional sites of iodopindolol binding in any of our patients. Iodopindolol dissociation constants for leukocytes from both nonpregnant and pregnant women were similar (Table III). While mean B_{max} was 23% less in leukocytes from pregnant women, the decrease was not significant because of the larger variation in individual B_{max} values in the pregnant group.

cAMP production in leukocytes from nonpregnant and pregnant women are shown in Figure 2. While there is little difference in cAMP production under basal conditions, 3.0 ± 0.6 vs 2.4 ± 0.5 pmol/10^6 cells/15 min for control and pregnant, respectively, cAMP production stimulated by 10 μM isoproterenol was significantly depressed (16.5 ± 3.1 vs 7.3 ± 2.4) to 44% (confidence interval 33% to 56%) of nonpregnant controls ($P < 0.001$).

In order to determine the relationship between β-adrenoceptor density and cAMP production in human mononuclear leukocytes, we plotted β-adrenoceptor density against cAMP production per receptor as shown in Figure 3. If cAMP production were proportional to the number of β-adrenoceptors on each cell, the values would be distributed along a horizontal line. Instead, we found that cAMP production per receptor site is constant at higher receptor densities, but appears to be increased when receptor densities are less than 400 sites per cell.

Discussion

The β-adrenoceptor system linked to cAMP production is one of the most well understood receptor systems (12). β-Adrenoceptors, when stimulated, catalyze the release of GDP from the guanine nucleotide regulatory protein G_s, which then binds the more readily available GTP and disperses into subunits. The α-subunit stimulates the enzyme adenylyl cyclase to produce second messenger cAMP from ATP. Intracellular cAMP activates the enzyme protein kinase A (PKA) which phosphorylates proteins and produces cellular effects (13); phosphorylation of myosin light chain kinase by PKA decreases sensitivity to Ca^{++}-calmodulin and results in uterine relaxation. Because of the complexity of the β-adrenoceptor-cAMP system, the ability of receptors to stimulate cAMP production is dependent upon the function of receptors, G_s, and adenylyl cyclase.

Alterations in β-adrenoceptor response to stimulation may result from a number of cellular changes, including changes in receptor sensitivity, changes in receptor density, and/or changes in coupling of receptors to adenylyl cyclase and cAMP formation. Short-term exposure to drugs which activate receptors results in desensitization due to receptor phosphorylation (12), and receptor internalization as part of the resensitization process (14). Long-term exposure to agonists results in receptor downregulation with a decrease in the density of receptors.

It is well established that human peripheral mononuclear leukocytes contain a population of β_2-adrenoceptors similar to those found on human uterus. Berg *et al.* (6) found a high correlation between β-adrenoceptor-stimulated cAMP production in myometrial tissue and leukocytes, supporting the use of leukocytes as an indicator of β-adrenoceptor function in the myometrium. Michel *et al.* (1) found a similar high correlation for β-adrenoceptor binding in myometrium and leukocytes. In women treated with the β_2-agonist terbutaline, the density of β-receptors and their stimulation of cAMP production are decreased in both leukocytes (6) and myometrium (11).

As in the studies cited above, our study utilized a population of mononuclear leukocytes which includes lymphocytes and monocytes. It has not been established which subpopulation(s) of leukocytes change(s) during pregnancy. Experiments performed on a mixed population of cells are more likely to detect changes in leukocytes, since a subpopulation chosen at random

Table II. Patient Population

Patient number	Gestational age (weeks)	Basal cAMP[a]	Stimulated cAMP[a]	Basal cAMP per β-adrenoceptor[b]	Stimulated cAMP per β-adrenoceptor[b]
1	32.4	1.22	6.89	35	198
1[c]	36.6	2.04	9.91	72	351
2	33.5	1.26	3.69	98	287
3	13.2	2.66	11.97	492	2215
4	35.0	2.73	10.35	85	321
5	28.3	1.34	6.07	27	123
5[c]	36.3	1.50	2.41	138	221
6	30.1	2.23	5.33	86	206
7	35.5	1.84	10.88	269	1589
8	30.4	1.61	9.86	116	708
9	35.2	1.22	5.98	265	1298
9[c]	38.0	0.66	2.53	79	301
10	11.2	3.78	12.72	755	2541
11	6.4	1.49	8.87	66	388
12	7.2	2.00	6.12	105	321
13	26.3	2.71	9.46	123	428
14	22.5	3.87	5.99	169	261
15	16.3	1.93	3.65	148	281
16	28.5	1.03	6.74	20	134
17	10.5	1.37	7.13	39	201
18	CTRL[d]	3.89	23.23	111	662
19	CTRL[d]	4.08	15.95	149	582
20	CTRL[d]	3.23	13.22	139	567
21	CTRL[d]	0.87	6.78	39	304
22	CTRL[d]	3.02	23.30	101	776

[a] pmol/15 min/10^6 cells.
[b] Molecules of cAMP/min/β-adrenoceptor site.
[c] Second sample on same patient.
[d] Control patient.

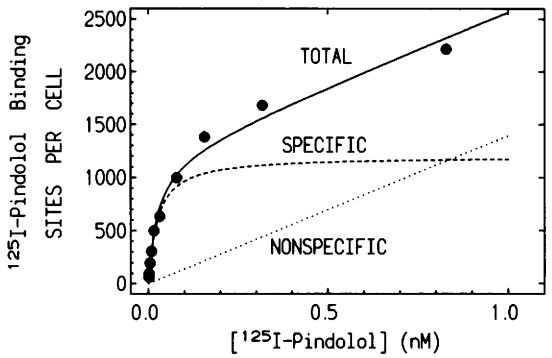

Figure 1. Representative binding curve for [^{125}I]-pindolol. Leukocytes were incubated with concentrations of iodopindolol ranging from 1 to 1000 p*M* for 30 min at 37°C. Actual data (●) are resolved by curve fitting (solid trace) into specific binding to β-adrenoceptors (dashed trace) and nonspecific binding (dotted trace). There was no indication of two specific sites of binding in our experiments.

might not be the one which changes during pregnancy. However, it is also possible that results presented herein represent changes occurring in discrete subpopulations of leukocytes; for example, a 10% decrease in receptors could reflect a total loss of receptors in 10% of the leukocytes or a modest 10% decrease in receptors on all subpopulations of leukocytes.

Changes in β-adrenoceptor density in myometrium and leukocytes have been studied during normal

Table III. Characteristics of Iodopindolol Binding to β-Adrenoceptors in Human Leukocytes

	Nonpregnant	Pregnant
K_d[a]	27.5 p*M* (25.1–30.1)	23.9 p*M* (16.2–35.3)
B_{max}[b]	1108 cell^{-1} ± 209	851 cell^{-1} ± 560

[a] Geometric mean (95% confidence interval).
[b] Sites per cell ± SEM.

pregnancy and several pregnancy-related abnormalities. Dattel *et al.* (5) found no difference in β-adrenoceptor density in myometrium from women during labor or before labor between 28 and 34 weeks gestation, although this study was complicated by multiple medications and pregnancy outcomes. Nisell (3) found significantly fewer β-adrenoceptors (compared with nonpregnant controls) on lymphocytes from healthy pregnant women; women with pregnancy-induced hypertension had β-receptor densities intermediate between control and healthy pregnant women. Santala *et al.* (4) found no change in the density of lymphocyte β-adrenoceptors during pregnancy, although a decrease in receptor density was noted at delivery and at 4 days in puerperium. In our study, we noted a nonsignificant decrease in β-adrenoceptor density (from 1108 ± 209 to 851 ± 560 sites per cell) during preg-

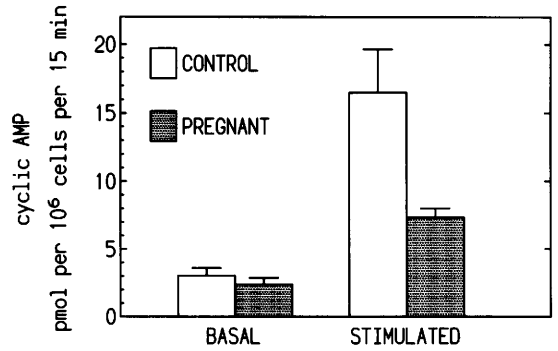

Figure 2. Differences in cAMP production in leukocytes from nonpregnant and pregnant women. While there was no difference in cAMP under basal conditions, stimulated (10 μM isoproterenol) cAMP production in leukocytes from pregnant women was significantly depressed to 44% of nonpregnant controls ($P < 0.001$). There were 20 observations for the pregnant group and five for the control group.

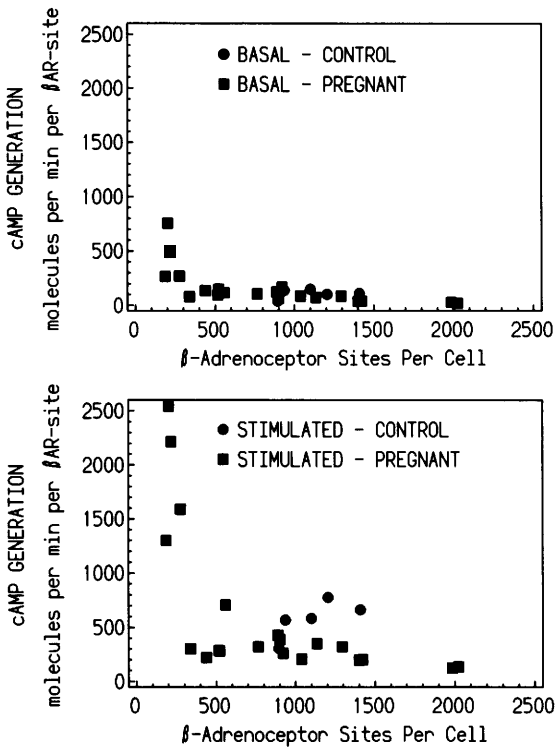

Figure 3. Relationship between receptor density and cAMP production in basal (A) and stimulated (10 μM (±)isoproterenol) (B) human mononuclear leukocytes. cAMP production per receptor site is constant (i.e., horizontal) at higher receptor densities, indicating that cAMP production is directly proportional to the receptor density. However, as receptor density decreases below 400 sites per cell, there is an increase in cAMP production per receptor, suggesting that coupling of receptors to adenylyl cyclase stimulation is more efficient.

nancy, with large variations between individual pregnant patients. This suggests that a common within-patient control measurement (perhaps prepregnancy or 6 weeks postpartum) would provide a better means of determining if changes in β-adrenoceptor density occur during pregnancy.

Changes in β-adrenoceptor coupling to cAMP formation during pregnancy have been implied by previous studies. The increase in plasma cAMP in response to epinephrine was reduced during normal pregnancy (3). Plasma cAMP is an indirect measurement of receptor-stimulated cAMP production since plasma levels are dependent upon both cAMP formation and cAMP metabolism. Vasodilatation in response to epinephrine infusion, also an indirect measurement of β_2-adrenoceptor coupling to cAMP production, is positively related in nonpregnant controls, but inversely related in normal pregnant women and more so in women with pregnancy induced hypertension (3). The ratio of high- to low-affinity isoproterenol binding, an indirect measurement of receptor efficacy, is unchanged in leukocytes from women before or during labor from 28 to 34 weeks of gestation (5).

In this study, we examined the relationship between the density of β-adrenoceptors and the ability of (−)isoproterenol to stimulate cAMP production. Consistent with previous reports (3), we found no difference in basal cAMP production. We also found a significant decrease in cAMP production stimulated by β-adrenoceptor activation (Fig. 2) implied by previous studies (3, 14).

Because fresh intact leukocytes were used rather than an isolated membrane preparation, we were able to directly measure the ability of β-adrenoceptor density to influence stimulation of adenylyl cyclase. The decrease in cAMP production in response to β-adrenoceptor stimulation (Fig. 2) appears to be due to two factors: a nonsignificant decrease in the number of leukocyte β-adrenoceptors on each cell and a decrease in the amount of cAMP generated per receptor site for leukocytes with greater than 400 sites per cell.

The ability of a cell to produce cAMP is often assumed to be directly proportional to the density of β-adrenoceptors; if this relationship were true, data would be grouped around a horizontal line on Figure 3. Data from control women suggest that this relationship might exist between β-adrenoceptor density and both basal and stimulated cAMP production (Fig. 3). However, in leukocytes from pregnant women the relationship between stimulated cAMP generation and receptor density appeared to be inverse, with higher cAMP generation per β-receptor occurring at lower receptor densities. This result suggests that β-adrenoceptor mediated cAMP generation during pregnancy is controlled by factors other than strictly β-adrenoceptor density. Augmentation of basal cAMP generation in leukocytes with less than 400 sites per cell suggests that changes might be occurring beyond the receptor in the guanine nucleotide regulatory protein, adenylyl cyclase, or in other cellular factors controlling the system. In order to minimize the influence of cAMP phosphodiesterase, the nonselective inhibitor 3-isobutyl-1-methylxanthine was added during cAMP generation;

the dose chosen increases cAMP without increasing histamine release from mast cells (15).

Our results suggest that studies which examine receptor density alone might be incomplete; stimulated cAMP production might also be controlled by other factors, such as intermediate guanine nucleotide regulatory proteins or changes in adenylyl cyclase. In clinical terms, decreased β_2-adrenoceptor density alone does not necessarily prove desensitization or tachyphylaxis.

While we had initially hoped to correlate changes in the β-adrenoceptor system with abnormal pregnancy duration, the low incidence of preterm pregnancy precluded this. Dattel *et al.* (5) found no correlation between β-adrenoceptor density and onset of term or preterm labor. In our limited patient population, there was no striking relationship between pregnancy outcome (not shown) and parameters describing the β-adrenoceptor-cAMP system.

In summary, we studied leukocyte β_2-adrenoceptors during pregnancy. β-Adrenoceptor-stimulated cAMP production was markedly depressed in leukocytes from pregnant women, while there was no significant change in receptor density, affinity, or basal cAMP production between control and pregnant groups. Additionally, coupling of β-adrenoceptor stimulation to cAMP production was not constant, but was greater in leukocytes with fewer than 400 β-adrenoceptors per cell. Our results suggest that β-adrenoceptor coupling to cAMP production (i.e., efficacy) is decreased during pregnancy and that receptor density alone is inadequate to describe the β_2-adrenoceptor system. This represents confirmation of previously implied changes in β-adrenoceptor coupling during pregnancy.

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