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β_3 -Adrenoceptor Is the Predominant β -Adrenoceptor Subtype in Human Myometrium and Its Expression Is Up-Regulated in Pregnancy

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To assess whether pregnancy might influence the functionality and expression of human myometrial β_2 - and β_3 -adrenoceptors (β_2 - and β_3 -AR), we performed functional, binding, Western blot, and molecular biology experiments in human nonpregnant and near-term pregnant myometrium. Inhibition of spontaneous contractions induced by a β_3 -AR agonist, SR 59119A, was significantly greater in pregnant, compared with nonpregnant, myometrial strips ($E'_{max} = 61 \pm 5\%$ vs. $44 \pm 5\%$ for pregnant and nonpregnant myometrium, respectively), whereas salbutamol, a β_2 -AR agonist, was significantly less efficient in pregnant, compared with nonpregnant, myometrium ($E_{max} = 29 \pm 4$ vs. $54 \pm 8\%$). Although two populations of binding sites corresponding to β_2 - and β_3 -AR were identified

in both nonpregnant and pregnant myometrium, we found a clear predominance of the β_3 -AR subtype. Moreover, β_3 -AR binding sites were up-regulated 2-fold in myometrium at the end of pregnancy. Both β_2 - and β_3 -AR mRNA were expressed in human nonpregnant and pregnant myometrium. Contrary to β_2 -AR, the expression of the β_3 -AR transcripts and immunoreactive proteins was increased in pregnant, compared with nonpregnant, myometrium. Such compelling data suggest a predominant role for β_3 -AR in the regulation of human myometrium contractility, especially at the end of pregnancy, which might have important consequences for the clinical management of preterm labor. (*J Clin Endocrinol Metab* 90: 1644–1650, 2005)

THE INCIDENCE OF premature birth has risen over the past 15 yr, mainly because of medically induced births, yet spontaneous preterm delivery still remains stable and relatively high in developed countries despite preventative measures (1, 2). Approximately 6–12% of all pregnancies end up prematurely in Western countries, and deaths of premature babies represent approximately 85% of all perinatal mortality. Several stimulatory and inhibitory pathways regulate the balance of uterine quiescence and contractile activity during pregnancy. The mechanisms that govern the switch between these opposing functional states are still poorly understood. Worldwide, β_2 -adrenoceptor (AR) agonists are the most commonly used tocolytic agents. However, besides their maternal and fetal side effects, their efficacy remains controversial (3, 4). β_2 -ARs may vary according to hormonal status in human (5) or rat (6). The different proportion of each β -AR in pregnant and nonpregnant myometrium in goat and

cow was suggested over 20 yr ago by Larsen (7). We previously demonstrated that β_2 -ARs were predominant (~65%) over β_1 -AR in the human pregnant myometrium and that the number of β -ARs was diminished at the end of pregnancy (8). An additional β -AR subtype, the β_3 -AR, has since been characterized (9), and its functionality has been recently shown in the human near-term myometrium (10). Because adrenergic responsiveness is dependent on hormonal regulation (11, 12), we studied the status of the β_3 -AR in comparison with the β_2 -AR in the myometrium of near-term and nonpregnant women. More specifically, we investigated the functional contractile inhibition of specific β_2 - and β_3 -AR agonists, salbutamol and SR 59119A, respectively, concomitantly with the binding properties and the expression of the transcripts and the immunoreactive proteins of these receptors.

Materials and Methods

Biological samples

Myometrial biopsies were obtained from nonpregnant cycling women undergoing hysterectomy for benign gynecological indications or from pregnant women delivered by elective cesarean section before the onset of labor (38–40 wk of pregnancy) because of a diagnosed cephalopelvic disproportion in an otherwise uncomplicated normal pregnancy. Myometrial strips were excised from an immediately subserosal site in which the majority of the fibers are in a longitudinal orientation at an antipalental site, as previously described (13). This study was approved by the Comité Consultatif de Protection des Per-

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Abbreviations: ADU, Arbitrary densitometric unit; AR, adrenoceptor; B_{max} , maximal binding capacity; CHO, Chinese hamster ovary; CRC, concentration-response curve; E_{max} , maximal effect; ICYP, (-)-[¹²⁵I]iodocyanopindolol; K_D , dissociation constant; K_i , inhibition constant; TBST, Tris-buffered saline/Tween 20.

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sonnes pour la Recherche Biomédicale (Paris-Cochin, France), and written informed consent was obtained from all donors.

Functional contractile study

Segments of myometrium (8–12 \times 2–3 mm) were suspended isometrically under a resting tension of 2 g in a 10-ml organ bath containing Krebs solution (composition, millimoles: NaCl, 118; KCl, 5.4; CaCl₂, 2.5; KH₂PO₄, 0.6; MgSO₄, 1.2; NaHCO₃, 25; glucose, 11.7; ascorbic acid, 0.1) maintained at 37 C and continuously exposed to a mixture of 95% oxygen–5% carbon dioxide (pH 7.4). The 2-g initial tension was chosen according to previous reports including our own experiments (10, 14). One end of each strip was connected to a force-displacement transducer, and tension changes were measured using Pioden strain gauges (UF1) that were amplified (EMKA, Paris, France) and recorded on a pen-writing oscillograph (Linseis, L65514, Munich, Germany). After 1 h, during which the myometrial strips were washed every 15 min and the resting tension readjusted to 2 g, the strips were then allowed to equilibrate for an additional hour until they showed regular spontaneous rhythmic contractile activity. Once contractions became regular in both amplitude and frequency, cumulative concentration-response curves (CRCs) (from 0.01 μ M to 30 μ M) were determined to characterize the effects of exposure to either SR 59119A (β_3 -AR agonist, Sanofi-Aventis, Milan, Italy) or salbutamol (β_2 -AR agonist, Sigma, St. Quentin Fallavier, France). The exposure time for each additional concentration was around 20–40 min, time necessary to reach a plateau of contractions. Because 3–4 h were necessary for the construction of a cumulative CRC, only one complete curve was obtained for each strip. Because we previously described that there was no time-related loss of contractions during our experiments (10, 13), we did not use time-matched control in this study. For salbutamol, because saturation of CRCs was reached, the maximal effect (E_{max}) was determined as a percentage of inhibition of initial amplitude of spontaneous contractions.

Potency was expressed as pIC_{50} , which is the $-\log IC_{50}$, where IC_{50} is the concentration of agonist producing half of its maximal response. Because saturation of CRC was not reached for SR 59119A, E'_{max} indicates the maximal response obtained at the maximal concentration tested (30 μ M), and potency was not calculated. Data were analyzed by nonlinear regression sigmoidal dose-response curve using the Prism 4.01 software (GraphPad Software, San Diego, CA). The equation curve is $Y = Y_{min} + (Y_{max} - Y_{min})/[1 + 10^{-(\log IC_{50} - X)}]$ where X is the logarithm of concentration, Y is the response, Y_{min} is the minimal inhibitory effect, and Y_{max} the maximal inhibitory effect.

Binding studies

Crude membranes were prepared from different biopsy specimens of near-term pregnant or nonpregnant myometrial tissues as reported previously (8, 15). Membranes were diluted at a protein concentration of 1–3 mg·ml⁻¹, in a cold 50 mM Tris-HCl (pH 7.4) buffer. Protein concentration was determined by the method of Bradford (16) using BSA as standard. Binding studies were performed at 30 C for 60 min in a 0.25-ml crude membrane preparation (12 μ g protein per sample) that contained 50 mM Tris-HCl (pH 7.4), 154 mM NaCl, 0.1% BSA, and (–)-[¹²⁵I]iodocyanopindolol (ICYP) from 2 to 1000 pM (saturation analysis). The ICYP (specific activity 2200 Ci/mmol) was obtained from PerkinElmer Life Sciences (Boston, MA). Reactions were terminated by dilution with 5 ml of ice-cold 50 mM Tris-HCl (pH 7.4), 0.01% Triton X-100, and filtration over glass-fiber filters (Whatman GF/C, Maidston, UK) presoaked in 0.1% BSA in 50 mM Tris-HCl (pH 7.4). The filters were washed with an additional 15 ml of 50 mM Tris-HCl (pH 7.4) and 0.01% X-100 Triton, dried, and counted on a Packard γ -counter with 85% efficiency.

In previous studies examining β_2 -AR (8), specific binding was defined as the difference between the amount of ICYP bound in the absence (total binding) and presence (nonspecific binding) of 500 μ M unlabeled (–)-isoproterenol (Sigma). For β_3 -AR binding studies, nonspecific binding was defined by the amount of ICYP bound in the presence of unlabeled (\pm)-alprenolol (500 μ M, Sigma). Competition experiments were carried out at a fixed concentration of ICYP (400 pM) with increasing concentrations of competing agents and in the presence of 0.1 μ M (–)-propranolol to block β_1 - and β_2 -AR (17). At ligand concentrations near the ICYP dissociation constant (K_D), nonspecific binding was less than 15 and 30% of total binding for β_2 - and β_3 -AR, respectively. The inhibition

constants values (K_i) were calculated by the equation of Cheng and Prusoff (18): $K_i = IC_{50}/(1 + [L]/K_D)$, where IC_{50} is the concentration of competing agent producing 50% of a maximal response, [L] is the concentration of ICYP used in the assay, and the appropriate K_D value is the dissociation constant of ICYP as determined by nonlinear regression. Radioligand binding data were analyzed by nonlinear regression using the GraphPad Prism 4.01 software. Results are expressed as SEM.

RT and PCR analysis

As described previously (15, 19), extraction of total RNA from myometrial tissues using the Trizol reagent method, reverse transcription using Muloney murine leukemia virus reverse transcriptase, and PCR using *Taq* DNA polymerase were performed under the conditions recommended by the manufacturer (Life Technologies, Cergy Pontoise, France). The primers were designed according to Roberts *et al.* (20). The amplification profile for β_2 - and β_3 -AR consisted in denaturation at 94 C for 1 min, annealing at 64 C for 1 min, extension at 72 C for 1 min with a final extension at 72 C for 10 min. An aliquot from the PCR mixture was resolved by electrophoresis on a 2% agarose gel (Life Technologies) and visualized by ethidium bromide staining under UV light. To check the size of PCR products, a DNA molecular mass standard ladder (123-bp DNA ladder, Life Technologies) was concomitantly subjected to electrophoresis. An additional control of validity was carried out using Southern blot analysis of the PCR products with specific internal oligonucleotides (data not shown). Amplification of an endogenous marker, the human β_2 -microglobulin cDNA (21), was performed as internal control. Absence of DNA contamination was confirmed by conducting PCR control reactions containing mRNA without reverse transcriptase. The intensities of the bands on Polaroid pictures of the ethidium bromide staining gel were analyzed densitometrically using the NIH Image 1.62 program (National Institutes of Health, Bethesda, MD). Results are expressed as relative levels [arbitrary densitometric units (ADUs)] of specific mRNA normalized to β_2 -microglobulin mRNA in each sample.

Western blotting analysis

Snap-frozen myometrial tissues obtained from nonpregnant and near-term pregnant women were homogenized with Ultra-Turrax in homogenization buffer [10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 40 mg·ml⁻¹ leupeptine, 2 mM Pefabloc]. After an initial centrifugation at 500 \times g for 15 min at 4 C, the supernatant was removed and centrifuged at 48,000 \times g for 20 min at 4 C. The resulting pellet was suspended in solubilization buffer (300 mM NaCl, 50 mM Na₂HPO₄, 1% Triton X-100) overnight at 4 C. A further centrifugation was performed at 48,000 \times g for 20 min at 4 C. Total protein content was determined by the Bradford method with BSA as standard. Samples (40 μ g protein by lane) were dissolved (vol/vol) in 2 \times Laemmli buffer (22) and boiled for 5 min before electrophoresis on a 10% SDS-PAGE. Proteins were transferred to a nitrocellulose membrane (Hybond-P, Amersham Biosciences, Freiburg, Germany). To block nonspecific antibody binding, blots were incubated for 1 h in 10% nonfat dried milk powder in Tris-buffered saline/Tween 20 (TBST) [10 mM Tris, 150 mM NaCl, and 0.1% Tween 20 (pH 7.8)] at room temperature. Blocked membranes were washed three times with TBST. The blots were then incubated overnight at 4 C with a 1:500 dilution of primary β_2 -AR polyclonal antibody (H-20, sc-569, Santa Cruz Biotechnology, Santa Cruz, CA) or a 1:500 dilution of primary β_3 -AR polyclonal antibody (AB5122, Chemicon International, Temecula, CA) in 1% nonfat dried milk powder in TBST. After three washes with TBST, the blots were incubated for 45 min with horseradish peroxidase-conjugated antirabbit IgG whole antibody (NA 934, Amersham) at a dilution of 1:5000 at room temperature and washed five times with TBST. Immunoreactive proteins were detected by chemiluminescence (ECL detection reagents, RPN2105, Amersham Pharmacia Biotech, Little Chalfont, UK). The intensities of the bands were analyzed densitometrically using the NIH Image 1.62 program. Results are expressed as the mean \pm SEM in ADUs. The specificity of each immunoreactive band was assessed by specific blocking in the presence of the respective antigenic peptide against which the antibodies have been raised (sc-569P from Santa Cruz Biotechnology for β_2 -AR and AG388 from Chemicon International for β_3 -AR).

For preadsorption, a mixture of each primary antibody with its re-

spective antigen (1:5) was incubated under mild agitation in a small volume of PBS buffer for 2 h at room temperature (for β_2 -AR) or 24 h at 4 C (for β_3 -AR). In addition, homogenate of Chinese hamster ovary (CHO) cells transfected with the human β_3 -AR (graciously provided by Dr. P. Marini, Sanofi-Aventis Research Center, Milan, Italy) was used as positive control.

Drug and solution

SR 59119A (*N*-[(7-methoxy-1,2,3,4-tetrahydronaphtalen-(2R)-2-yl)-methyl]-(2R)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride) was a gift from Sanofi-Aventis, Sanofi-Aventis Research Center. SR 59119A was dissolved in a mixture of absolute ethanol 30%, dimethylsulfoxide 2%, and distilled water for the 30- μ M solution and thereafter diluted in distilled water. Salbutamol sulfate was dissolved in distilled water. In the functional studies, drug concentrations were given as final bath concentrations.

Statistical analysis

In the functional experiments, differences among groups were determined by ANOVA and expressed with a *t* test using the Bonferroni correction. Differences in potencies and maximal effects, as well as differences among groups in binding experiments, were determined by Student's *t* test for unpaired data or ANOVA as appropriated. All differences were considered significant when $P < 0.05$.

Results

Functional contractile study

Application to muscle strips of SR 59119A ranging from 0.1 to 30 μ M caused a concentration-dependent inhibition of the spontaneous contractile activity of myometrium obtained from nonpregnant or near-term women (Fig. 1). A comparison of the CRC for both β_2 - and β_3 -AR agonists in pregnant and nonpregnant myometrium is shown in Fig. 2, A and B. Application to muscle strips of salbutamol or SR 59119A, ranging from 0.01 to 30 μ M, caused a concentration-dependent inhibition of the spontaneous contractile activity of myometrial strips obtained from nonpregnant or near-term

pregnant women. In the near-term myometrium, salbutamol effect was weaker and the CRC was downward shifted, compared with that obtained in the nonpregnant myometrium (Fig. 2A, ANOVA, $P < 0.001$). The E_{\max} value of salbutamol was significantly ($P < 0.05$) lower in the pregnant than in the nonpregnant tissue ($E_{\max} = 29 \pm 4$ and $54 \pm 8\%$ for pregnant and nonpregnant myometrium, respectively), whereas no significant difference was observed between the pIC_{50} values (Table 1). SR 59119A was responsible for a strong inhibition of spontaneous contractions in both the pregnant and nonpregnant myometrium (Fig. 2B). In contrast to salbutamol, the CRC of SR 59119A obtained in the near-term myometrium was shifted to the left, compared with that obtained in the nonpregnant myometrium (Fig. 2B, ANOVA, $P < 0.01$). This was associated with an increase, although not statistically significant, in E'_{\max} values (61 ± 5 vs. $44 \pm 5\%$ for pregnant and nonpregnant myometrium, respectively) (Table 1). In pregnant myometrium, the CRC of salbutamol was shifted to the right, compared with that of SR 59119A (ANOVA, $P < 0.01$), and the effect obtained at the maximal dose tested (30 μ M) was lower for salbutamol than SR 59119A (29 ± 4 vs. $61 \pm 5\%$ for salbutamol and SR 59119A, respectively, $P < 0.001$) (Table 1).

Characterization of β_2 - and β_3 -AR binding sites

ICYP bound to membrane preparations of nonpregnant and pregnant myometrium in a concentration-dependent manner. Scatchard representation of saturation curve is shown in Fig. 3A. In pregnant myometrium, nonlinear analysis of the saturation curve demonstrated the appearance of a two-site plot, indicating both a low-affinity site [$K_D = 771 \pm 109$ pM; maximal binding capacity (B_{\max}) = 92.9 ± 13.1 fmol \cdot mg $^{-1}$ protein] and a high-affinity site ($K_D = 39 \pm 4$ pM; $B_{\max} = 4.9 \pm 0.6$ fmol \cdot mg $^{-1}$ of protein) (Table 2). Consistent

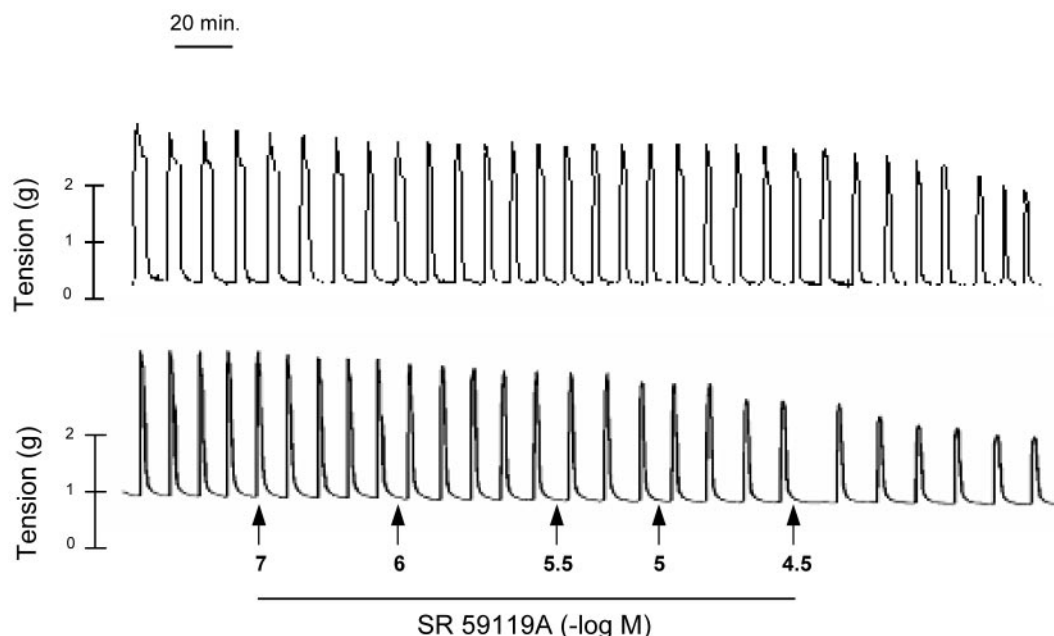


FIG. 1. Representative recording of the effect of SR 59119A on spontaneous contractions of human nonpregnant (*upper trace*) and near-term (*lower trace*) myometrial strips.

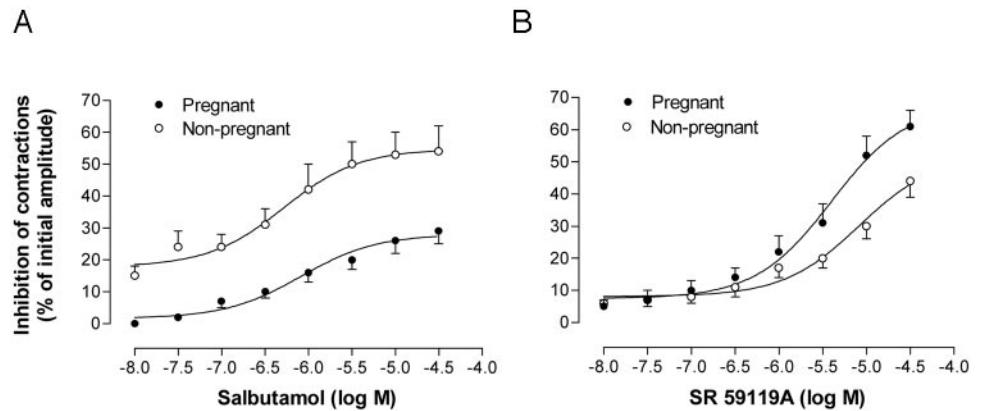


FIG. 2. Effect of salbutamol (β_2 -AR agonist, A) or SR 59119A (β_3 -AR agonist, B) on spontaneous contractions of myometrium from nonpregnant (white circles) or pregnant (black circles) women. Results are expressed as mean \pm SEM (n = 11–31).

with our previous results and those from other groups working on human pregnant myometrium (8, 23), the high-affinity binding site found here resembled that of a β_2 -AR profile. The identity of the low-affinity binding site was confirmed by competition binding experiments with the selective β_3 -AR agonist. SR 59119A competed at the propranolol-resistant ICYP binding site (Fig. 3B) with a K_i value of 276 ± 76 nM. Competition binding data were similar ($K_i = 154 \pm 82$ nM) in nonpregnant myometrium (data not shown). It should be noted that the slope value for these curves was low (Hill coefficient $n_H = 0.71$ and 0.65 in nonpregnant and pregnant myometrium, respectively), indicating that two sites may be present but cannot be significantly differentiated by computer analysis. Nonlinear analysis of saturation curves revealed that the K_D values of β_3 -AR in the nonpregnant and pregnant myometrium were significantly ($P < 0.005$) different (Fig. 3A and Table 2). In addition, the B_{max} of β_3 -AR was significantly ($P < 0.01$) greater in pregnant than nonpregnant myometrium (Fig. 3A and Table 2). For β_2 -AR, the K_D values between nonpregnant and pregnant myometrium were not significantly different, just as were the B_{max} values (Table 2).

Expression of β_2 - and β_3 -AR transcripts

As illustrated in Fig. 4A, electrophoresis on agarose gel stained with ethidium bromide revealed PCR products of the correct predicted sizes, 417 bp for β_2 -AR and 368 bp for β_3 -AR, in both pregnant and nonpregnant myometrial samples. We confirmed successful normalization of RNA amounts by obtaining equivalent intensity for the β_2 -microglobulin band in pregnant and nonpregnant tissues. Densitometric analysis of the gels indicated a slight decrease in the signal for β_2 -AR mRNA (-27.4%) in near-term myometrium

TABLE 1. Maximal effect (E_{max} and E'_{max}) and potency (pIC_{50}) values for salbutamol and SR 59119A on spontaneous contractile activity of myometrium from nonpregnant and near-term pregnant women

	Salbutamol (β_2 -AR agonist)			SR 59119A (β_3 -AR agonist)		
	n	E_{max}	pIC_{50}	n	E'_{max}	pIC_{50}
Nonpregnant	11	$54 \pm 8\%$	6.74 ± 0.15	12	$44 \pm 5\%$	ND
Pregnant	31	$29 \pm 4\%^a$	6.11 ± 0.11	24	$61 \pm 5\%$	ND

n, Number of experiments; ND, not determined. Each experiment was conducted on tissue from a different patient.

^a $P < 0.05$ vs. nonpregnant.

comparatively with nonpregnant tissue, whereas the signal for β_3 -AR mRNA was increased in pregnant myometrium ($+66.3\%$) (Fig. 4B).

Expression of β_2 - and β_3 -AR immunoreactive proteins

Western blotting of plasma membranes prepared from nonpregnant and pregnant myometrium revealed a 67-kDa band corresponding to β_2 -AR (24) (Fig. 5A). For β_3 -AR, the Western blotting revealed a main 68-kDa band (Fig. 5A), which disappears in the presence of the corresponding blocking peptide (data not shown). As positive control, a 68-kDa band was observed with a homogenate of CHO cells transfected with the human β_3 -AR that was also specifically blocked by the antigenic peptide (data not shown). Densitometric immunoblot analysis indicated no change in the intensity of the signal for the β_2 -AR in the myometrium of near-term, compared with nonpregnant, women. At the opposite, an increase ($+40\%$) in the intensity of the signal for the β_3 -AR protein was observed in pregnant, compared with nonpregnant, myometrium (Fig. 5B).

Discussion

A few years ago, we first described the presence of a functional β_3 -AR positively coupled to cAMP production in human near-term myometrium (10). We recently demonstrated that contrary to β_2 -ARs, β_3 -ARs are less prone to sustained agonist-induced desensitization in near-term myometrium (15). The present study strengthens these previous reports by providing the first evidence not only that β_3 -ARs are present but also that they are the predominant subtype, compared with the β_2 -ARs, both in human nonpregnant and pregnant myometrium (10- and 20-fold more numerous, respectively). Furthermore, our results indicate that human β_3 -ARs are up-regulated in pregnancy.

These new findings appear to contradict the conventional belief that in human (8, 23) and other species (25, 26), β_2 - and β_1 -ARs account, respectively, for approximately 65 and 35% of the entire myometrial β -AR population. β_3 -ARs have not previously been detected, probably due to their low affinity for the ICYP ligand commonly used for β -AR binding studies. Complementary approaches combining functional, binding, Western blotting, and molecular biology experiments were carried out to fully characterize the changes that might occur regarding myometrial β -AR status at the end of pregnancy. Our functional

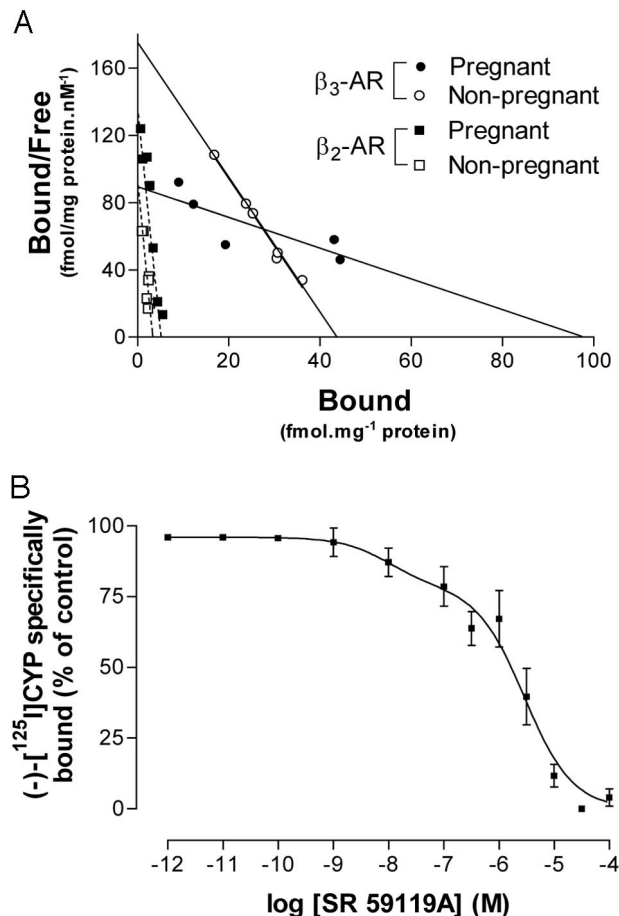


FIG. 3. A, Representative Scatchard saturation plot of total binding of ICYP to human nonpregnant and pregnant myometrial membranes. Although linear Scatchard plots are represented in this figure, a nonlinear regression method was used to determine the K_D and B_{max} values. B, Competition between ICYP and SR 59119A (β_3 -AR agonist) for binding sites in membrane preparations of myometrium from pregnant women. Results are expressed as mean \pm SEM from eight different pregnant women.

experiments demonstrated that the selective β_3 -AR agonist, SR 59119A, was a more efficient inhibitor of spontaneous contractions in pregnant than nonpregnant myometrium. The β_2 -AR agonist salbutamol, in contrast, displayed a weaker inhibition of spontaneous contractions in the near-term, compared with the nonpregnant, myometrium. Few studies have been performed to compare nonpregnant and pregnant myometrial tissues. In 1979, Larsen (7) reported that isoproterenol, a nonse-

TABLE 2. Affinity (K_D) and number of β_2 - and β_3 -AR binding sites (B_{max}) determined after saturation binding experiments in human nonpregnant and pregnant myometrium

	β_2 -AR		β_3 -AR	
	Nonpregnant	Near-term	Nonpregnant	Near-term
n	3	4	6	8
K_D (pM)	78 ± 28	39 ± 4	218 ± 66	771 ± 109^a
B_{max} (fmol.mg ⁻¹ of protein)	4.4 ± 1.2	4.9 ± 0.6	40.0 ± 4.9	92.9 ± 13.1^b

n, Number of patients.

^a $P < 0.005$ vs. nonpregnant myometrium.

^b $P < 0.01$ vs. nonpregnant myometrium.

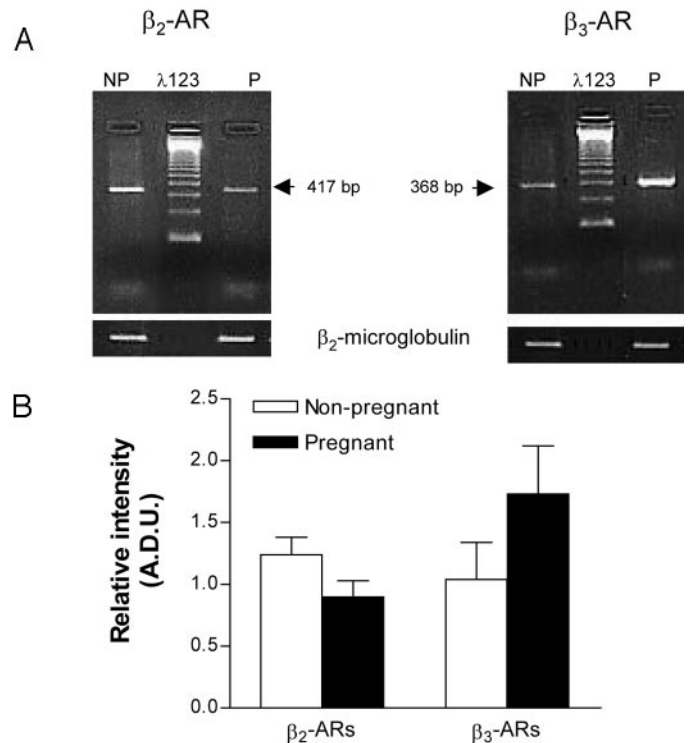
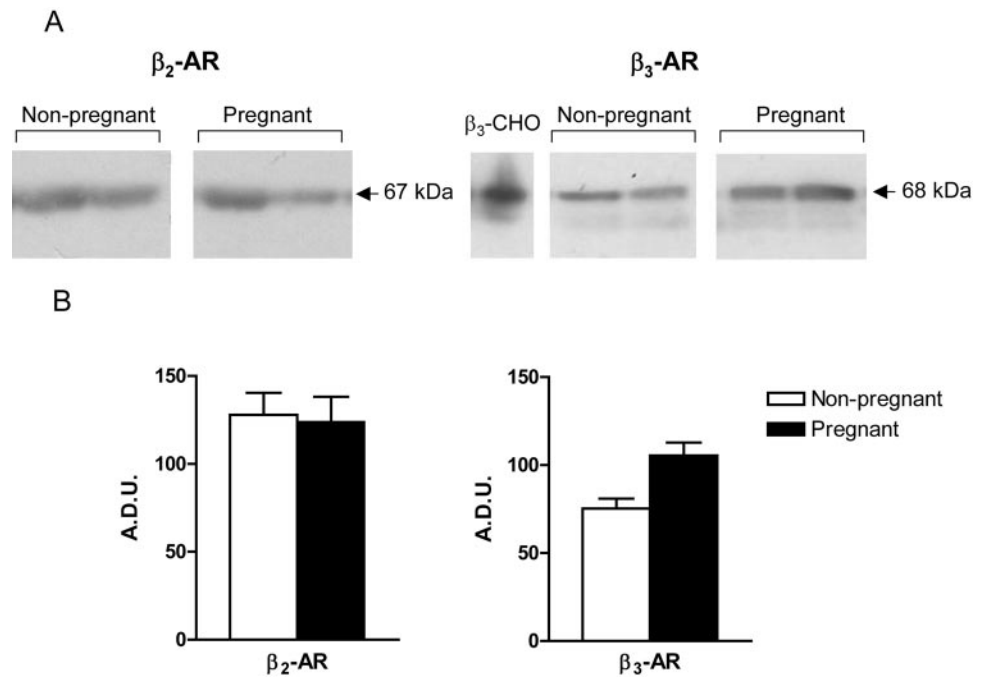


FIG. 4. A, Representative RT-PCR analysis of the mRNA steady-state levels for β_2 - and β_3 -AR in human nonpregnant (NP) and pregnant (P) myometrium. The 123-bp DNA ladder (λ 123) was used to estimate the size of the PCR products, and β_2 -microglobulin expression was used as a standard reference. These experiments were performed on myometrium from six pregnant and six nonpregnant women. B, Densitometric analysis of the relative expression of β_2 - and β_3 -AR transcripts in myometrium of nonpregnant (NP) and pregnant (P) women. A.D.U. represents the ratio between the intensity of the PCR fragments for β_2 - or β_3 -AR and the intensity of the signal for the internal standard β_2 -microglobulin. Each bar represents the mean \pm SEM from six different women.

lective β -AR agonist, and salbutamol and ritodrine, both selective β_2 -AR agonists, caused a concentration-dependent relaxation of pregnant goat and cow myometrium, whereas only isoproterenol was able to reduce the amplitude of spontaneous contractions of nonpregnant myometrium from the same animals.

Binding competition studies, transcripts, and protein analysis were performed concurrently to determine whether any changes in the mRNA and protein expression and the number of β_2 - and β_3 -AR binding sites might explain the observed change in the contractile response of nonpregnant and pregnant myometrium to β_2 - and β_3 -AR agonists. According to the work of Gsell *et al.* (27), we found no modification in the number of β_2 -AR binding sites in nonpregnant, compared with pregnant, human myometrium. We confirmed these results by Western blotting because, in agreement with Chanrachakul *et al.* (24), we did not find any convincing difference in the level of expression of β_2 -AR immunoreactive protein between human nonpregnant and near-term myometrium. Analysis of the level of transcripts revealed a slight decrease in β_2 -AR mRNA in pregnant, compared with nonpregnant, women. This discrepancy between changes in mRNA expression and the number of binding

FIG. 5. A, Representative Western blot analysis of β_2 - and β_3 -AR expression in plasma membranes from myometrium of nonpregnant and pregnant women. Expected sizes are 67 kDa for β_2 -AR and 68 kDa for β_3 -AR. These experiments were performed on myometrium from five nonpregnant and five pregnant women. Homogenate of CHO cells transfected with the human β_3 -AR (β_3 -CHO) was used as positive control. B, Analysis of the expression of β_2 - and β_3 -AR immunoreactive proteins in myometrium of nonpregnant and pregnant women. A.D.U. represents the intensity of the bands evaluated by densitometry. Each bar represents the mean \pm SEM from five different nonpregnant and five different pregnant women.



sites has already been described (28), and might be explained by an increased stability of mRNA, an enhanced translation rate, or a low receptor turnover (29, 30). The difference in the relaxant effect of salbutamol observed between nonpregnant and pregnant myometrium cannot be completely cleared up by our molecular and biochemical results, and we speculate that changes in coupling between receptor and adenylyl cyclase in both tissues are likely to explain these data.

Contrary to what was observed for β_2 -ARs, the increase in the level of expression of β_3 -AR transcripts in near-term, compared with nonpregnant, myometrium was associated with an increase of the signal for the corresponding immunoreactive protein and accompanied by an increase in the number of β_3 -AR binding sites. Based on the differences in affinity for ICYP, we established that human myometrial β_3 -ARs have a lower affinity than β_2 -ARs. These results are in accordance with the atypically low affinity and potency of β_3 -ARs for conventional β -AR antagonists (31). Such low affinity of β_3 -ARs for ICYP was also found in another smooth muscle, the internal anal sphincter (32), a tissue in which the β_3 -AR is predominant over the β_1/β_2 -AR subtypes. The SR 59119A effect appears to be selective of a β_3 -AR stimulation and not linked to a nonselective binding on β_2 -ARs. Indeed, we have previously shown that SR 59119A-induced inhibition of human colon (33) or near-term myometrium (10) spontaneous contractions was unaffected by the blockade of β_1 - and β_2 -ARs by propranolol (0.1 μ M) but was significantly reduced by the selective β_3 -AR antagonist SR 59230A (1 μ M). Furthermore, functional studies on human isolated bronchi, a tissue that expresses predominantly the β_2 -AR subtype, have shown that SR 59119A was devoid of any β_2 -AR agonist (33) or antagonist (34) properties.

Our study confirms, as previously shown in either human (8) or rat (35) that pregnancy might influence β -AR expression. The influence of sexual hormones on the expression of myometrial β_2 -AR is well documented. Progesterone may increase the density of β_2 -AR in late-pregnant rats (12) by

controlling the transcription rate of the gene (11). In guinea pig, estradiol treatment resulted in a marked up-regulation of β_2 -AR density, whereas progesterone influenced β_2 -AR to a lesser degree (36). By contrast, little is known about modulation of β_3 -ARs by steroid hormones. Pregnancy in the human is associated with a progressive rise in the myometrial 17 β -estradiol to progesterone ratio (37), and it is of interest to note that an increase in the myometrial response to a β_3 -AR agonist was observed near term when this hormonal ratio is the most elevated, compared with the nonpregnant myometrium. Recent results on brown adipocytes demonstrated that estradiol and progesterone are able to modify β_3 -AR affinity and density (38). These data allow us to hypothesize that hormonal changes observed during pregnancy are likely to explain the modulation of β_2 - and β_3 -AR expression, functionality, and affinity observed in pregnant, compared with nonpregnant, myometrium. However, such a hypothesis remains to be tested with further experiments.

One of the major limitations for β_2 -AR agonists' use in clinical practice is their maternal and fetal cardiovascular side effects. The presence and function of β_3 -AR in the cardiovascular system are still a matter of debate (39, 40). However, clinical trials regarding the effect of β_3 -AR agonists on human metabolism did not reveal any change in heart rate, blood pressure, plasma glucose, insulin, or potassium levels (41–43). Another limiting factor for the use of selective β_2 -AR agonists as tocolytic drugs is that their effect, if any, appears to be of relatively short duration (44). This loss of efficacy is due in part to desensitization of their receptors. A distinguishing feature of the β_3 -AR, compared with β_2 -AR, subtype is its relative lack of desensitization either *in vitro* or *in vivo* after activation with agonists (15, 45).

In conclusion, the present study provides compelling evidence for the predominance of the β_3 -AR subtype in the human myometrium and its up-regulation in pregnancy. In light of these findings and our recent data on their resistance

to desensitization (15), we suggest that β_3 -AR agonists may have considerable future pharmaceutical implications in the clinical management of preterm labor.

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