Effect of progesterone on intracellular Ca\(^{2+}\) homeostasis in human myometrial smooth muscle cells

VICTOR P. FOMIN,1 BLAIR E. COX,2 AND R. ANN WORD1

Departments of 1Obstetrics and Gynecology and 2Pediatrics,
University of Texas Southwestern Medical Center, Dallas, Texas 75235

Fomin, Victor P., Blair E. Cox, and R. Ann Word. Effect of progesterone on intracellular Ca\(^{2+}\) homeostasis in human myometrial smooth muscle cells. Am. J. Physiol. 276 (Cell Physiol. 45): C379–C385, 1999.—Although it is well known that progesterone alters uterine contractility and plays an important role in maintenance of pregnancy, the biochemical mechanisms by which progesterone alters uterine contractility in human gestation are less clear. In this investigation we sought to identify progesterone-induced adaptations in human myometrial smooth muscle cells that may alter Ca\(^{2+}\) signaling in response to contractile agents. Cells were treated with vehicle or the progesterone analog medroxyprogesterone acetate (MPA) for 5 days, and intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]) was quantified after treatment with oxytocin (OX) or endothelin (ET)-1. OX and ET-1-induced increases in [Ca\(^{2+}\)] were significantly attenuated in cells pretreated with MPA in a dose-dependent manner. Progesterone receptor antagonists prevented the attenuated Ca\(^{2+}\) transients induced by MPA. ET\(_A\) and ET\(_B\) receptor subtypes were expressed in myometrial cells, and treatment with MPA resulted in significant downregulation of ET\(_A\) and ET\(_B\) receptor binding. MPA did not alter ionomycin-stimulated increases in [Ca\(^{2+}\)], and had no effect on inositol trisphosphate-dependent or -independent release of Ca\(^{2+}\) from internal Ca\(^{2+}\) stores. We conclude that adaptations of Ca\(^{2+}\) homeostasis in myometrial cells during pregnancy may include progesterone-induced modification of receptor-mediated increases in [Ca\(^{2+}\)].

Progesterone receptor; endothelin receptors; uterus; endothelin; oxytocin; antiprogestin; medroxyprogesterone acetate

In most species at full term, progesterone withdrawal, together with increasing levels of estrogen, leads to increased expression of a number of genes believed to be important in the onset of parturition (6, 21, 42). It has also been suggested that the contractile phenotype of uterine smooth muscle is increased by estradiol treatment and decreased by progesterone (8, 39). Although it is well known that progesterone alters uterine contractility and plays an important role in maintenance of pregnancy in most species (5), the biochemical mechanisms by which progesterone alters uterine contractility in human gestation are less clear.

The effects of progesterone on uterine smooth muscle appear to be multiple. Progesterone may promote uterine relaxation by nongenomic and genomic mechanisms. Nongenomic effects of acute progesterone exposure include inhibition of transmembrane Ca\(^{2+}\) entry, release of Ca\(^{2+}\) from intracellular stores (19), and membrane hyperpolarization with subsequent activation of K\(^{+}\) channels (27). Recently, the list of nongenomic effects of progesterone has been expanded to include direct inhibition of oxytocin (OX) binding to the rat, but not the human, OX receptor (11). Most effects of progesterone, however, are mediated through its specific binding to nuclear hormone receptors with subsequent changes in expression of target genes. Specifically, progesterone inhibits expression of connexin43 (10, 21), modulates OX receptor density (16, 34), decreases estradiol-induced increases in cGMP-dependent protein kinase (39), and decreases the expression of interleukin-8 in myometrial and cervical stromal cells (15). The effects of progesterone on intracellular Ca\(^{2+}\) homeostasis in intact myometrial cells have not been systematically evaluated.

Previously, we reported that treatment of human myometrial smooth muscle cells in culture with endothelin (ET)-1 resulted in marked increases in intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]), and the extent of myosin light chain phosphorylation (41). ET-1 is a member of a family of sarafotoxin-like peptides and appears to be an important endogenous modulator of uterine contractility (26, 40, 41). The agonist and its receptors have been identified in the human uterus (30). ET-1 and OX increase [Ca\(^{2+}\)], by at least two mechanisms. Both agonists bind to plasma membrane receptors, resulting in influx of extracellular Ca\(^{2+}\) and inositol trisphosphate (IP\(_3\))-mediated release of Ca\(^{2+}\) from intracellular stores (9, 24). In myocytes, this transient elevation of [Ca\(^{2+}\)] is quickly reversed by high-efficiency plasma membrane and sarcoplasmic reticulum Ca\(^{2+}\) pumps and an Na\(^+\)/Ca\(^{2+}\) exchange mechanism (19).

In this investigation we sought to identify progesterone-induced adaptations in OX- and ET-1-mediated Ca\(^{2+}\) signaling in myometrial smooth muscle cells. Treatment of human myometrial smooth muscle cells in culture with the progesterone analog medroxyprogesterone acetate (MPA) resulted in significant decreases in ET-1- and OX-mediated increases in [Ca\(^{2+}\)]. This effect was reversed by the specific progesterone receptor antagonist ZK-98299. Treatment with MPA also resulted in a significant decrease in ET receptor binding. Progesterin treatment had no effect on IP\(_3\)-dependent or -independent release of Ca\(^{2+}\) from the sarcoplasmic reticulum. These results suggest that human myometrial smooth muscle cells in primary culture are responsive to progesterone with decreases in OX and ET receptor density. Thus one mechanism by which progesterone promotes uterine relaxation during pregnancy may be to decrease binding of contractile agents with resulting decreases in [Ca\(^{2+}\)].
MATERIALS AND METHODS

Human myometrial smooth muscle cells. Human uterine tissue was obtained from nonpregnant women at the time of hysterectomy for reasons other than endometrial or myometrial disease. Consent for the use of tissue was obtained from the women undergoing surgery according to a protocol approved by the Institutional Review Board. The tissue was transported to the laboratory in Hanks' balanced salt solution that contained 25 mM HEPES and 0.1875% sodium bicarbonate, pH 7.4. Fresh tissues were minced and incubated for 16 h at 34°C in a solution that contained collagenase B (1 mg/ml) and DNase (0.15 mg/ml) to disperse the smooth muscle cells. Thereafter, the smooth muscle cell fraction was purified by a discontinuous Percoll density gradient (1.03/1.06) for 20 min at 2,000 g. Smooth muscle cells were collected and diluted with DMEM buffered with 25 mM HEPES and 0.1875% sodium bicarbonate, pH 7.4. The medium was supplemented with fetal bovine serum (10% by volume), penicillin (200 U/ml), streptomycin (200 µg/ml), and sodium pyruvate (1 mM) and centrifuged, and the final pellet was resuspended in DMEM that contained 10% fetal bovine serum. Myocyte viability was >90% as determined by trypan blue staining. The cells were plated at 5 × 10⁵ cells/cm² with the culture medium changed every other day until confluency (2–4 days). The cells were treated with MPA for 5 days, including serum-free medium 24 h before experimental protocols. For comparison, smooth muscle cells from rabbit aorta were prepared in an identical fashion, except elastase (1 mg/ml) was added to the tissue digest medium, and cells were dispersed within 4 h.

Determination of [Ca²⁺]i in myometrial cells. Smooth muscle cells were plated on glass coverslips (9 × 35 mm) as described above. Cells were loaded with fura 2 by incubation in culture medium that contained 5 µM fura 2-AM for 30 min. Coverslips were washed three times and incubated for an additional 15 min in buffer containing (in mM) 4.8 KCl, 130 NaCl, 1.5 CaCl₂, 2.0 NaHPO₄, 15 glucose, and 10 HEPES (pH 7.4) supplemented with 0.1% (wt/vol) human serum albumin. Experiments were completed within 1 h of loading. Fura 2-containing cells were rinsed with albumin-free buffer, and coverslips were mounted in a cuvette equipped with an electronically controlled, mini-motorized Teflon rotor (Instech Laboratories, Horsham, PA). Fura 2 fluorescence was recorded with a fluorescence spectrophotometer (model 650-105, Perkin-Elmer, Norwalk, CT) at excitation wavelength of 340 nm, and emission was monitored at 510 nm. Measurements were corrected for autofluorescence and extracellular fluorescence. [Ca²⁺]i was calculated according to the formula: [Ca²⁺]i = K_d(F - F_min)/(F_max - F), where F is the experimentally determined fluorescence, F_max the maximum fluorescence in the presence of 50 µM ionomycin, and F_min the minimum fluorescence in the presence of 12 mM EGTA plus 20 mM Tris base (22). The Ca²⁺ dissociation constant (K_d) for fura 2 is 244 nM (12).

Ca²⁺ fluxes in permeabilized myocytes. IP₃-induced Ca²⁺ release from internal stores was quantified as described previously (31) with modification. Briefly, confluent primary cultures of myometrial cells in 35-mm dishes were rinsed three times with Hanks' balanced salt solution and permeabilized for 10 min in the medium containing 120 mM KCl, 2 mM MgCl₂, 1 mM ATP, 1 µM EGTA, 30 mM imidazole-HCl (pH 6.8), and saponin (20 µg/ml). Cells were washed with buffer without saponin for 40 min. Thereafter, intracellular Ca²⁺ stores were loaded for 30 min at 25°C in buffer containing (in mM) 120 KCl, 10 NaCl, 5 MgCl₂, 5 ATP, 0.44 EGTA, 0.08 ⁴⁵–⁴⁰CaCl₂, and 30 imidazole-HCl (pH 6.8). Loading of intracellular Ca²⁺ stores was terminated by replacing loading medium with efflux medium containing (in mM) 120 KCl, 5 NaCl, 2 MgCl₂, 1 ATP, 1 EGTA, and 30 imidazole (pH 6.8). Concentration of free Ca²⁺ in the efflux medium was brought to 4 × 10⁻⁸ M. The cells were rinsed for 3 min with 1-mi1 aliquots of efflux medium. The first two aliquots were discarded, and 50 µM IP₃ not taken up by the stores. Subsequent aliquots were placed in scintillation vials, and radioactivity was determined by scintillation counting. Permeabilized cells were treated with IP₃ and ionomycin at specified time points. Non-specific ⁴⁰Ca²⁺ efflux before treatment was subtracted from the sum of aliquots obtained during 5 min after treatment with test agents to quantify Ca²⁺ release from intracellular stores as nanomoles of Ca²⁺ per milligram of protein per 5 min. Cells were then removed from the dishes, and protein concentration was determined by the Lowry method.

Radioligand binding studies and ET receptor subtype determination. ET receptor binding characteristics and subtype expression were determined in confluent myocytes treated identically to those used for analysis of [Ca²⁺]i. Confluent myocytes in short-term primary culture were treated for 4 days with vehicle (0.1% ethanol) or 10⁻⁷ M MPA in 12- or 24-well plates. Control and MPA-treated cells were placed in serum-free medium 24 h before radioligand binding assays. Thereafter, cells were washed twice with prewarmed serum-free, steroid-free DMEM supplemented with 0.3% BSA (binding buffer). ET receptor binding and subtype assays were performed in a total volume of 1 ml of binding buffer. Specific binding of ¹²⁵I-labeled ET-1 reached equilibrium by 60 min; therefore, 60-min incubations were used in all binding studies. Incubations were conducted at 37°C and terminated by four rapid 1-ml washes of ice-cold binding buffer. The cells were dislodged and dissolved by incubation with 0.1 M NaOH at 20 min at 25°C. The protein concentration for cells in each well was determined by a modification of the methods of Lowry. The effluent was transferred to scintillation vials, and the radioactivity was determined with a scintillation counter (Packard Instruments, Downers Grove, IL) with an efficiency of 83% for ¹²⁵I counting.

Competitive radioligand binding studies, 30 pM ¹²⁵I-labeled ET-1 (2,200 Ci/mmol; NEN Research Products, Boston, MA) was used. Unlabeled ET-1 was added in increasing concentrations (10⁻¹¹–10⁻⁸ M), and the binding characteristics (receptor density and affinity) were determined from analysis of the displacement of the labeled ligand. Experiments were performed in duplicate at each concentration tested. Receptor-specific antagonists [FR-139317 (ETA antagonist) and IRL-1038 (ETB antagonist)] were utilized to determine ET receptor subtype binding. Specific binding was calculated as the difference between total ¹²⁵I-labeled ET-1 binding and non-specific binding measured in the presence of unlabeled 10⁻⁶ M ET-1. The relative distribution of two receptor subtypes was extrapolated from the percent inhibition observed when the specific displacement curves were compared. The percent concentration of receptor subtypes was also determined by subtraction of binding in the presence of 10⁻⁶ M ET-1 from the respective subtype-selective antagonist-specific binding (4). Displacement curves for each ET receptor antagonist, their respective IC₅₀ values, and the percent ET receptor subtype were calculated from the specific binding data with use of a modification of the computer program LIGAND adapted for microcomputers by McPherson (Elsevier BIOSOFT, Cambridge, UK).

Chemicals. Fura 2-AM was obtained from Molecular Probes (Eugene, OR), ionomycin and IP₃ from Calbiochem (Alexan-
Effect of progesterone on [Ca\textsuperscript{2+}] in myometrial cells.

To study the effect of progesterone on intracellular Ca\textsuperscript{2+} homeostasis in human uterine smooth muscle, myometrial smooth muscle cells in short-term primary culture were used as a model system. Cells were treated with 10\textsuperscript{-7} M MPA for 5 days, and intracellular Ca\textsuperscript{2+} transients in response to contractile agents and Ca\textsuperscript{2+} ionophore were quantified with fura 2. The synthetic derivative of progesterone, MPA, was utilized in these studies, because these cells metabolize progesterone to 3α-hydroxy-5α-pregnan-20-one, 3β-hydroxy-5α-pregnan-20-one, and 5α-pregnane-3,20-dione (38; unpublished observations). Relatively long preincubation times were utilized to recapitulate results obtained in studies utilizing steroid hormones to modulate uterine contractility in vivo (15, 16, 20, 23). Myometrial cells responded to 20 nM OX and 10\textsuperscript{-7} M ET-1 with marked increases in cytoplasmic Ca\textsuperscript{2+} (Fig. 1). As expected, progestin pretreatment resulted in significant attenuation of OX-induced Ca\textsuperscript{2+} transients. These results confirmed that these cells were responsive to OX and progesterone in vitro in a manner analogous to those in vivo. Short-term incubations (<1 h) with 10\textsuperscript{-7} M progesterone or 10\textsuperscript{-7} M MPA or simple inclusion of the steroid in the buffer did not alter [Ca\textsuperscript{2+}] responses in these cells.

In addition to OX, responses to ET-1 were also significantly attenuated in progestin-treated cells (Figs. 1 and 2). Maximal inhibition of ET-1-induced Ca\textsuperscript{2+} transients was observed with 10\textsuperscript{-7} M MPA and an EC\textsubscript{50} of ~3 nM (Fig. 2). Progesterone attenuated ET-1-induced increases in [Ca\textsuperscript{2+}] at 3 × 10\textsuperscript{-10}–1 × 10\textsuperscript{-7} M ET-1 (Fig. 3). Whereas progesterone inhibited receptor-mediated increases in [Ca\textsuperscript{2+}], maximal increases in [Ca\textsuperscript{2+}] in response to the Ca\textsuperscript{2+} ionophore ionomycin (10\textsuperscript{-6}–10\textsuperscript{-4} M) were not altered in progestin-treated cells: 393 ± 28 (control, n = 13) compared with 368 ± 46 nM (MPA, n = 9). Furthermore, the rate of decline of [Ca\textsuperscript{2+}], with ionomycin was not altered significantly by MPA.

Effect of anti-progestins on [Ca\textsuperscript{2+}] in myometrial cells.

We used progesterone receptor antagonists to test the hypothesis that inhibition of OX and ET-1 responses involved progesterone receptors. Myometrial cells were treated with 2 × 10\textsuperscript{-8} M MPA, progesterone receptor antagonists (RU-486 or ZK-98299, 10\textsuperscript{-6} M), MPA + RU-486, or MPA + ZK-98299 (Table 1, Fig. 4). Intracellular Ca\textsuperscript{2+} transients in response to OX and ET-1 were inhibited by MPA. Although differences between OX and ET-1 responses in control and RU-486-treated cells did not reach statistical significance, these responses tended to be decreased in RU-486-treated cells (Table 1). RU-486 partially prevented MPA-induced inhibition of OX and ET-1 responses (Table 1). It is likely that the mixed agonist-antagonist properties of RU-486 resulted in these complex responses (7, 26, 36). The "pure" progesterone receptor antagonist ZK-98299 alone resulted in small, but significantly enhanced, OX responses, whereas ET-1 responses were unaffected by ZK-98299 (Fig. 4). Similar to RU-486, ZK-98299 com-
progestin treatment had no effect on ET-1-induced increases in [Ca^{2+}] in cells deficient in progestrone receptors, e.g., rabbit aortic smooth muscle cells: 221 ± 10.2 nM (n = 8, control) compared with 238 ± 15 nM (n = 8, MPA). Moreover, the inhibitory effect was specific to progesterone, inasmuch as pretreatment with other steroids up to 10^{-6} M had no effect on ET-1-induced increases in [Ca^{2+}] in control cells (Table 2). IP_3 (0.4 and 4 µM) resulted in 36% and 68% increases in [Ca^{2+}] in response to 10^{-6} M ET-1 were quantified. Values are means ± SE of 3–5 determinations.

The effect of progesterone on ET-1-induced increase in [Ca^{2+}] was specific for myometrial smooth muscle cells. Progestin treatment had no effect on ET-1-induced increases in [Ca^{2+}] in cells deficient in progestone receptors, e.g., rabbit aortic smooth muscle cells: 221 ± 10.2 nM (n = 8, control) compared with 238 ± 15 nM (n = 8, MPA). Moreover, the inhibitory effect was specific to progesterone, inasmuch as pretreatment with other steroids up to 10^{-6} M (17β-estradiol, dexamethasone, testosterone, or 5α-pregnane-3,20-dione) did not alter responsiveness to OX or ET-1 (data not shown).

Effect of progesterone on intracellular Ca^{2+} stores. The finding that progesterone altered receptor-mediated increases in [Ca^{2+}] but not ionophore-mediated responses suggested that release of Ca^{2+} from IP_3-dependent stores may be altered in progestin-treated cells. To test this possibility, Ca^{2+} efflux in response to IP_3 and ionomycin was quantified in MPA-treated and control cells (Table 2). IP_3 (0.4 and 4 µM) resulted in release of Ca^{2+} from intracellular stores in control and MPA-treated cells. Additional intracellular Ca^{2+} was released from IP_3-insensitive stores after treatment with 10 µM ionomycin. IP_3-sensitive and total internal Ca^{2+} stores were similar in control and MPA-treated cells (Table 2). These results indicate that the amount of intracellular Ca^{2+} stores and IP_3-mediated release of intracellular Ca^{2+} are not altered in progestin-treated cells.

Effects of progesterone on ET receptor binding characteristics and subtype expression. The effect of progesterone on specific binding of ET-1 in intact myometrial smooth muscle cells was determined. LIGAND analysis of the displacement of [^{125}I]-labeled ET-1 by unlabeled ET-1 demonstrated a one-site model for receptors in primary cultured myometrial smooth muscle cells (P < 0.05, r > 0.93). Representative Scatchard plots of specific binding data for myometrial smooth muscle cells from nonpregnant women cultured in the absence or presence of MPA are illustrated in Fig. 5A. The slopes of the two treatment groups did not differ, reflecting similar K_d values: 0.13 ± 0.02 (control) compared with 0.14 ± 0.03 nM (MPA). Specific binding at K_d for ET receptors in myometrial cells exceeded 81%. Hill coefficients ranged from 0.77 to 0.99 and from 0.87 to 0.96 in control and progestin-treated cells, respectively, indicating that cooperativity was not involved in the [^{125}I]-labeled ET-1 binding to these cells.

Table 1. Effect of RU-486 on OX- and ET-1-induced increases in [Ca^{2+}], in human myometrial smooth muscle cells

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<th>OX</th>
<th>ET-1</th>
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<tr>
<td>Control</td>
<td>234 ± 15</td>
<td>532 ± 47</td>
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<tr>
<td>MPA (2 × 10^{-6} M)</td>
<td>151 ± 14*</td>
<td>271 ± 54*</td>
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<tr>
<td>RU-486 (1 µM)</td>
<td>172 ± 3</td>
<td>419 ± 99</td>
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<tr>
<td>RU-486 + MPA</td>
<td>176 ± 5†</td>
<td>418 ± 49†</td>
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Values are means ± SE of 3–6 determinations from 2 cell cultures. Human myometrial smooth muscle cells in primary culture were pretreated with vehicle, medroxyprogesterone acetate (MPA), RU-486, or RU-486 + MPA for 5 days. Thereafter, maximal increases in intracellular free Ca^{2+} concentration ([Ca^{2+}]_i) in response to 2 × 10^{-7} M oxytocin (OX) or 10^{-6} M endothelin-1 (ET-1) were quantified. *P < 0.05 compared with control; †P < 0.05 compared with MPA. Kruskal-Wallis analysis followed by χ^2 approximation.
revealed that $\text{ET}_A$ and $\text{ET}_B$ receptors constituted ~80 and 20–30% of total ET-1 binding, respectively. $\text{ET}_A$ and $\text{ET}_B$ receptor densities were decreased significantly in progestin-treated cells, with proportionate reductions of both receptors (Fig. 5B). Whereas the $\text{ET}_A$ receptor subtype is predominant in myometrial smooth muscle cells in primary culture, human endometrial stromal cells expressed $\text{ET}_A$ and $\text{ET}_B$ receptors in approximately equal proportions (data not shown). These results indicate that distribution of ET receptor subtypes is cell-type specific, and in myometrial cells the $\text{ET}_A$ receptor subtype is predominant in the presence or absence of MPA.

**DISCUSSION**

Progesterone-induced myometrial quiescence is essential to the maintenance of most, if not all, mammalian pregnancies (5). In a number of systems the effects of progesterone are antagonistic to those of estrogen (9, 10, 14, 18, 28). Progesterone inhibition of estrogen action is complex, involving downregulation of estrogen receptors, induction of estrogen metabolism (35), or transcriptional repression of estradiol-responsive genes (37). Progesterone, however, does not inhibit estrogen responses universally. For example, uterine OX gene expression is synergistically regulated by estradiol and progesterone (20). In this investigation we evaluated the effect of progesterone on $\text{Ca}^{2+}$ homeostasis in myometrial smooth muscle cells in short-term primary culture.

Previous studies have demonstrated that progesterone decreases OX binding in rabbit, rat, human, and ovine uterine smooth muscle cells (8, 9, 16, 20, 28, 34). Thus we used OX effects as a control for progesterone responsiveness. Although there is no doubt that estrogen and progesterone receptor concentrations varied considerably among the tissues obtained from women in different phases of the menstrual cycle, progesterone responsiveness was remarkably consistent in the cultured cells (plated at high density and used within

![Fig. 4. Effect of ZK-98299 (ZK) on OX (A) and ET-1 (B)-induced increases in $\text{[Ca}^{2+}\text{]}_i$. Primary cultures of myometrial cells were incubated for 5 days in culture medium alone (CTL), $2 \times 10^{-8}$ M MPA, $10^{-6}$ M ZK-98299, or MPA + ZK-98299. Maximal increases in $\text{[Ca}^{2+}\text{]}_i$ in response to $10^{-7}$ M ET-1 were quantified with fura 2 fluorescence. Values are means ± SE of 6–8 determinations conducted in 2 different cell cultures. *$P < 0.05$ compared with control, **$P < 0.01$ compared with control, ***$P < 0.001$ compared with control, by ANOVA.

![Fig. 5. ET binding in human myometrial smooth muscle cells. A: representative Scatchard plots of $^{125}\text{I}$-labeled ET binding in myometrial smooth muscle cells. Human myometrial cells in primary culture were incubated for 5 days in culture medium alone (CTL), $2 \times 10^{-8}$ M MPA, $10^{-6}$ M ZK-98299, or MPA + ZK-98299. Maximal increases in $\text{[Ca}^{2+}\text{]}_i$ in response to $10^{-7}$ M ET-1 were quantified with fura 2 fluorescence. Values are means ± SE of 6–8 determinations conducted in 2 different cell cultures. *$P < 0.05$ compared with control, by ANOVA.

Table 2. Effect of MPA on intracellular $\text{Ca}^{2+}$ stores and IP$_3$ responsiveness in human myometrial cells

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<th>$\text{IP}_3$-induced release</th>
<th>Control</th>
<th>MPA</th>
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<tr>
<td>0.4 µM</td>
<td>0.35 ± 0.09</td>
<td>0.34 ± 0.04</td>
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<td>4.0 µM</td>
<td>0.69 ± 0.08</td>
<td>0.86 ± 0.07</td>
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<th>$\text{IP}_3$-insensitive release</th>
<th>Control</th>
<th>MPA</th>
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<tr>
<td>0.16 ± 0.08</td>
<td>0.19 ± 0.07</td>
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Values are means ± SE of 3 determinations from 6 different cell preparations. Human myometrial smooth muscle cells were treated with control media or media that contained MPA. Cells were permeabilized, intracellular $\text{Ca}^{2+}$ stores were loaded with $^{45}\text{Ca}^{2+}$, and release of $^{45}\text{Ca}^{2+}$ from inositol trisphosphate ($\text{IP}_3$)-sensitive and -insensitive stores was ascertained.

![C383PROGESTERONE EFFECTS ON MYOMETRIAL CELLS](http://ajpcell.physiology.org/ by 10.220.33.4 on April 4, 2017)
7–10 days). During the course of this investigation, 24 primary cultures of human myometrial cells were treated with MPA. With the exception of two cell cultures, all were responsive to progestin treatment with inhibition of OX responsiveness. Cells in passage 1 or >10 days in primary culture were not responsive to progesterone, presumably because of loss of expression of progesterone receptors, as has been described for other uterine cells (33).

Progesterone-induced attenuation of OX responses was prevented by progesterone receptor antagonists. ZK-98299 alone resulted in small, but significant, enhancement of OX responses, whereas ET-1 responses were unaffected. Antiprogestins have been shown to increase uterine sensitivity to OX in a number of in vivo and in vitro studies (2, 3, 6). Increased OX sensitivity is believed to be due to prostaglandin-mediated increases in OX receptors (6, 42), although other mechanisms are clearly possible (3). Thus the enhanced OX sensitivity in myometrial cells treated with ZK-98299 may be consequent to increased prostaglandin production in antiprogestin-treated cells. To our knowledge, expression of ET receptors is not regulated by prostaglandins. Thus the finding that ZK-98299 enhanced sensitivity to oxytocin, but not ET-1, is not surprising. In addition, antiprogestins may alter OX receptor-associated G proteins or other processes associated with G protein coupling that are distinct from those associated with ETA receptors.

Recently, Grazzini et al. (11) reported that progesterone, R-5020, and RU-486 inhibited OX binding to rat OX receptors. Progesterone, R-5020, and RU-486 did not inhibit OX binding to human OX receptors on cell membranes. In the present study we confirmed and extended these findings. Short-term incubations (<1 h) with 10^-7 M progesterone or 10^-7 M MPA did not alter [Ca^{2+}]_i responses in intact human myometrial smooth muscle cells. Inhibition of OX and ET-1 responses, however, was observed after treatment with MPA for 5 days. Thus the results of this study suggest that progesterone, acting through its nuclear hormone receptor, attenuates OX- and ET-1-induced increases in [Ca^{2+}], through downregulation of human OX and ET receptors. The role of progesterone receptors in this process is indicated by the findings that 1) progesterone receptor antagonists precluded the diminished ET-1 response, 2) progesterone did not alter ET-1 responsiveness in cells deficient in progesterone receptors, and 3) other steroid hormones did not alter ET-1 responses.

The data reported here demonstrate that progesterone decreases ETA and ETB receptor density proportionately and decreases the acute response to ET-1 in myometrial smooth muscle cells from nonpregnant women. This effect of progesterone does not appear to be limited to myometrium. Recently, others have reported that progesterone also inhibits ET-mediated luteinizing hormone secretion in cultured rat pituitary cells (28). In vivo, progesterone inhibits estradiol-induced increases in ET receptors in rabbit myometrium (21); however, estradiol did not directly alter ET receptors in myometrial smooth muscle cells in culture (21). Recently, Osada and co-workers (30) reported that ET-1-induced contractile force is increased in human myometrium during pregnancy and that this effect was mediated by increased ET_A receptors. The reported increases in ET_A receptors in myometrium from pregnant women in late gestation may reflect increased estradiol levels during human pregnancy, decline in progesterone responsiveness at full term, or other processes, such as uterine hypertrophy and myometrial stretch, that were not manifest in the current study.

Progesterone has no effect on IP_3-inducible Ca^{2+} release from the sarcoplasmic reticulum or on total intracellular Ca^{2+} stores. Thus fundamental mechanisms of intracellular Ca^{2+} stores are not affected by activation of progesterone receptors. Progesterone attenuates OX- (9), prostaglandin- (25), and ET-receptor binding (present study). Thus one mechanism of progesterone-induced myometrial quiescence during pregnancy may be progesterone-mediated decreases in the binding of contractile stimulants to their G protein-linked transmembrane receptors. The function and cellular distribution (internalization/recycling) of many G protein-linked receptors are regulated by phosphorylation-dephosphorylation processes (for review see Ref. 32). Although the role of progesterone in modulating these events is not known, progesterone may downregulate certain G protein-linked receptors via a common mechanism, e.g., G protein-related kinases.

In summary, these studies provide evidence that intracellular Ca^{2+} homeostasis in human myometrial cells is directly regulated by progesterone. Downregulation of ET and OX receptors by progesterone may result in alterations of many cellular signaling pathways that converge to maintain uterine quiescence during pregnancy. These effects may result in decreased uterine contractility, remodeling of the extracellular matrix (43), and modulation of adenylate cyclase activity (17) during uterine hypertrophy and growth.

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Address for reprint requests: R. A. Word, Dept. of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9032.

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