Published in final edited form as:

Am J Obstet Gynecol. 2007 December ; 197(6): 599.e1–599.e7.

Comparison of progesterone and glucocorticoid receptor binding and stimulation of gene expression by progesterone, 17-alpha hydroxyprogesterone caproate (17-OHPC), and related progestins

Barbara J. Attardi, PhD¹, Anthony Zeleznik, PhD², Hyagriv Simhan, MD³, Jye Ping Chiao, MS³, Donald R Mattison, MD⁴, and Steve N Caritis, MD³

¹Molecular Endocrinology Laboratory, Bioqual, Inc, Rockville, MD, USA

²Cell Biology & Physiology, University of Pittsburgh, Pittsburgh, PA, USA

³Obstetrics, Gynecology & Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA

⁴Center for Research for Mothers and Children, NICHD, Bethesda, MD, USA for the Obstetric-Fetal Pharmacology Research Units (OPRU) Network, NICHD, Bethesda, MD

Abstract

Condensation—17-hydroxyprogesterone caproate is not better than progesterone in binding to progesterone or glucocorticoid receptors or eliciting gene expression in progesterone responsive genes.

Comparison of progesterone and glucocorticoid receptor binding and stimulation of gene expression by progesterone, 17-alpha hydroxyprogesterone caproate (17-OHPC), and related progestins.

Objective— To determine whether the reduction in premature birth attributable to 17-OHPC occurs because of a greater affinity for progesterone (PR) or glucocorticoid (GR) receptors or by enhanced stimulation of progestogen responsive genes when compared with progesterone.

Study Design— We performed competitive steroid hormone receptor binding assays using cytosols expressing either recombinant human PR-A (rhPR-A) or B (rhPR-B) or rabbit uterine or thymic cytosols. We used four different carcinoma cell lines to assess transactivation of reporter genes or induction of alkaline phosphatase.

Results— Relative binding affinity of 17-OHPC for rhPR-B, rhPR-A and rabbit PR was 26–30% that of progesterone. Binding of progesterone to rabbit thymic GR was weak. 17-OHPC was comparable to progesterone in eliciting gene expression in all cell lines studied.

Conclusions— Binding to PR, GR or expression of progesterone-responsive genes is no greater with 17-OHPC than with progesterone. Other mechanisms must account for the beneficial effect of 17-OHPC on preterm birth rates.

Keywords

Preterm birth; 17-hydroxyprogesterone caproate; progesterone receptors; glucocorticoid receptors; transactivation

Presented at the Annual Meeting for the Society for Gynecologic Investigation March 22-25, 2006 Toronto, Ontario, Canada

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

In a recent large multicenter study from the NICHD-sponsored Maternal-Fetal-Medicine Network, weekly intramuscular injections of 17-OHPC reduced the rate of preterm birth by 33% in high risk women¹. This study was prompted by smaller studies and a meta-analysis suggesting efficacy of this treatment². A role for progesterone in regulating parturition was championed by Csapo³, and the mechanism of that regulation was demonstrated in sheep by the landmark studies of Liggins and colleagues⁴. In this species and others, labor is preceded by a fetal mediated decrease in plasma progesterone concentrations⁴⁻⁵ and a rise in estrogen concentrations⁵⁻⁷. Unlike sheep, however, in humans or non-human primates, neither preterm nor term labor is associated with a reduction in plasma progesterone concentrations⁶⁻⁸. The value of supplemental progestogens as a preventative for preterm birth, therefore, seems to lack biological plausibility. Furthermore, plasma progesterone concentrations are far greater than required to occupy the progesterone receptor (concentrations of progesterone in pregnant women are in the μM range, while progesterone receptors are typically 50% occupied in the nM range)⁹. With such an abundance of progesterone in the maternal circulation and the lack of any evidence of progesterone withdrawal prior to labor onset, the mechanism by which 17-OHPC reduces preterm birth is enigmatic. Data from humans and animals indicate that 17-OHPC has a more potent progestational effect on endometrium and is longer lasting than progesterone¹⁰⁻¹². Thus, a possible mechanism of action of 17-OHPC is that it binds more avidly to progesterone receptors (PR) than does progesterone resulting in increased expression of progestin responsive genes. Another potential explanation for the beneficial effect of 17-OHPC on rates of preterm birth is that the hormone binds more avidly to placental glucocorticoid receptors (GR). Progesterone competes with glucocorticoids at the placental GR and may prevent the increase in placental corticotropin releasing hormone (CRH) that is associated with the onset of term and preterm labor¹³⁻¹⁴. Furthermore, if 17-OHPC binds more avidly than progesterone to the placental GR, the endocrine signal for parturition may be delayed. The purpose of this study was to compare binding of 17-OHPC, progesterone, and related progestins in various PR and GR containing cytosols and the consequences of this binding in terms of regulation of gene expression in several cell systems.

Materials and Methods

Chemicals

17 α -hydroxyprogesterone caproate (hexanoate, 17-OHPC), 17 α -hydroxyprogesterone acetate (17-OHPA), and mifepristone were purchased from Sigma (St. Louis, MO). Mifepristone was 99% pure based on HPLC analysis. 17 α -hydroxyprogesterone (17-OHP) was obtained from Dr. Wayne Bardin. Progesterone and dexamethasone were purchased from Steraloids (Newport, RI). The antiprogestins CDB-4124 (17 α -acetoxy-21-methoxy-11 β -[4-N,N-dimethylaminophenyl]-19-norpregna-4,9-diene-3,20-dione) and CDB-2914 (17 α -acetoxy-11 β -[4-N,N-dimethylaminophenyl]-19-norpregna-4,9-diene-3,20-dione) were synthesized in the laboratory of Dr. P. N. Rao (Southwest Foundation for Biomedical Research, San Antonio, TX) under contract NO1-HD-6-3255. These antiprogestins were 98.8% and 98.1% pure, respectively, based on analysis by HPLC. Most other chemicals were purchased from Sigma.

Binding assays

Competitive binding assays for steroid hormone receptors were performed using cytosolic preparations from tissues or cells as described previously¹⁵. Cytosols containing PR or GR were prepared from uterus or thymus, respectively, of estradiol-primed immature rabbits. Recombinant human PR-A or PR-B (rhPR-A, rhPR-B) were assayed in cytosolic extracts from Sf9 insect cells infected with recombinant baculovirus expressing either rhPR-A or rhPR-B

(provided by Dr. Dean Edwards, Baylor University, Houston, TX¹⁶). For binding to rabbit uterine PR, cytosol was prepared in TEGMD buffer (10 mM Tris, pH 7.2, 1.5 mM EDTA, 0.2 mM sodium molybdate, 10% glycerol, 1 mM DTT) and incubated with 6 nM 1,2- [³H] progesterone (Perkin Elmer Life Sciences, Boston, MA; 52 Ci/mmol); competitors were added at concentrations from 2 to 100 nM. For binding to rhPR-A or rhPR-B, cytosol from Sf9 cells (prepared in TEGMD buffer containing the following protease inhibitors: bacitracin at 100 µg/ml, aprotinin at 2 µg/ml, leupeptin at 94 µg/ml, pepstatin A at 200 µg/ml) was incubated with 6.8 nM 1,2,6,7,16,17- [³H]progesterone (81 Ci/mmol); competitors were added at concentrations from 1 to 100 nM. For binding to rabbit thymic GR, cytosol was prepared in TEGMD buffer and incubated with 6 nM 6,7- [³H]dexamethasone (35 or 40 Ci/mmol); competitors were added at concentrations from 2 to 100 nM. After overnight incubation at 4°C, bound and unbound [³H]-steroids were separated by addition of dextran-coated charcoal and centrifugation at 2,100 x g for 15 min at 4°C. Supernatants from GR assays were decanted and counted in a Beckman LS-1800 liquid scintillation counter. Supernatants containing PR were pipetted into 24-well microplates and counted in a Packard TopCount liquid scintillation counter. Binding data were entered into Packard's RiaSmart™ for calculation of EC₅₀s using a 4-parameter logistic curve fit. Progesterone and dexamethasone were the standards for the PR and GR assays, respectively.

Cell culture and gene expression

Cell culture reagents were obtained from GIBCO Invitrogen Corp. (Carlsbad, CA) unless otherwise specified. We utilized three T47D human mammary carcinoma cell lines and the HepG2 hepatocellular carcinoma cell line to assess gene expression. The T47D (A1-2) cell line constitutively expresses comparable levels of PR and GR. These cells possess a stably integrated mouse mammary tumor virus-luciferase (MMTV-LUC) reporter gene. The MMTV promoter is recognized by both receptors, but the luciferase gene is highly inducible by glucocorticoids and nearly refractory to progestins¹⁷. The T47D-2963.1 cell line stably expresses both PR-A and PR-B and non-functional GR. The cells are stably transfected with MMTV attached to the bacterial chloramphenicol acetyltransferase (CAT) gene¹⁸ (both lines courtesy of Dr. T. Archer). We also used the T47Dco cell line which expresses equimolar concentrations of PR-A and PR-B (courtesy of Dr. K. Horwitz)¹⁹. All cells were grown routinely in monolayer culture in phenol red-free DMEM supplemented with 10% fetal bovine serum (FBS), 10 U/ml penicillin G, and 10 µg/ml streptomycin sulfate (pen/strep). In the T47Dco cell line, the endogenous alkaline phosphatase gene is induced by progestins. T47Dco cells were transiently transfected with the PRE₂-tk-LUC reporter plasmid, containing two copies of a progestin/glucocorticoid/androgen response element upstream of the thymidine kinase (tk) promoter and the firefly luciferase reporter gene (kindly provided by Dr. Dean Edwards). HepG2 human hepatoblastoma cells were obtained from ATCC (Manassas, VA). They were grown in monolayer culture in phenol red-free MEMα supplemented with 10% FBS and pen/strep. HepG2 cells were used to assess glucocorticoid agonist or antagonist activity. These cells were cotransfected with PRE₂-tk-LUC and a rat GR expression plasmid (6RGR from Drs. Roger Miesfeld and Keith Yamamoto, UCSF, San Francisco, CA).

For the T47D (A1-2) and the T47D-2963.1 cells, we performed 4 dose-response experiments in duplicate for each cell line, incubating 50,000 cells/well (A1-2) or 100,000 cells/well (2963.1) in 48 well microplates for 48 h with various concentrations of progesterone, 17-OHP, or 17-OHPC. Each hormone was dissolved in ethanol, and the final concentration of ethanol in the medium was 0.1% (v/v). Following stimulation, cell lysates were prepared and analyzed for luciferase activity (Promega, Madison, WI) with a luminometer (A1-2) or for CAT activity using CAT ELISA kits (Roche Applied Sciences, Indianapolis, IN) with spectrophotometric measurement at 490 and 405 nm. T47Dco cells, plated in 6-well dishes (500,000 cells/well), were transiently transfected with PRE₂-tk-LUC using FuGENE 6 transfection reagent (Roche)

according to the manufacturer's instructions (ratio of FuGENE 6 to DNA: 6 to 1) and incubated with various concentrations of progesterone, 17-OHP, 17-OHPA, or 17-OHPC for 20 h as described previously²⁰. For assessing antagonist activity, cells were incubated with 10^{-8} M 17-OHPC in the presence of increasing concentrations of the antiprogestins, mifepristone, CDB-2914, or CDB-4124. Cell lysates were prepared in Passive Lysis Buffer (Promega) and analyzed for protein content using the Bio-Rad Bradford assay (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. Luciferase activity (LAC) was determined in 20 μ l aliquots of the lysates using Promega's Luciferase Assay System. Light emission was measured in a microplate luminometer (Fluoroskan Ascent FL, Labsystems, Franklin, MA) and expressed as relative light units (RLU). Luciferase data were normalized for differences in protein content per well. Induction or inhibition of endogenous alkaline phosphatase activity in T47DCO cells was assessed as described previously using the procedure of Markiewicz and Gurside²¹. Briefly, cells in 96-well plates (50,000 cells/well) were incubated for 72 h with various progestins. After cell lysis, alkaline phosphatase assay mix (Pierce, Rockford, IL) was added, and plates were incubated for 2–4 h for color development. The absorbance at OD at 450 nm was determined.

Statistical analysis

Data are expressed as the mean $\pm$ SE ($n \geq 3$) or the mean $\pm$ SD ($n = 2$). GraphPad PRISM, version 4.0 (GraphPad Software, San Diego, CA), was used for graphics and determination of IC_{50} 's and EC_{50} 's for inhibition or stimulation, respectively, of transactivation or alkaline phosphatase activity by antagonists or agonists of PR or GR. In all steroid receptor binding assays, cpm were entered into RiaSmartTM for calculation of IC_{50} 's using a 4-parameter logistic curve fit. Relative binding affinities (RBA, %) for each compound were calculated as follows: IC_{50} of standard/ IC_{50} of competitor $\times 100$. One-way ANOVA followed by a suitable post hoc test was used for comparison of EC_{50} 's or IC_{50} 's for the various progestins using SigmaStat, version 3.0 (Jandel Scientific, San Rafael, CA). $P < 0.05$ was considered statistically significant.

Results

Receptor Binding

Table 1 compares the relative binding affinity (RBA) of progesterone, 17-OHP, 17-OHPC, and 17-OHPA (another ester of 17-OHP), for rhPR-B, rhPR-A, and rabbit uterine PR. Additionally, the binding of these progestins to rabbit thymic GR is compared to that of dexamethasone. The IC_{50} (the concentration of unlabeled hormone that reduces binding of the radioligand by half) as well as the RBA of the various hormones (IC_{50} progesterone or dexamethasone/ IC_{50} test hormone $\times 100$) for the specified receptors are indicated in Table 1. The results demonstrate that progesterone binds more avidly to PR than the other progestins except for binding of 17-OHPA to rabbit uterine PR. Binding of 17-OHPC to the various PR was only 26–30% that of progesterone, and binding of 17-OHP to PR was 1% that of progesterone. Binding to the rabbit thymic GR by the test progestins was dramatically less than that of dexamethasone (RBA of 4% for 17-OHPC).

Gene Expression

Tables 2 and 3 summarize the EC_{50} 's for gene expression in each of the three cell lines as assessed by transactivation of reporter genes and induction of endogenous alkaline phosphatase activity. In each cell line, reporter gene expression elicited by 17-OHPC was no more efficient than expression elicited by progesterone. In the T47D (A1-2) cell line with a relatively refractory PR, neither progesterone nor 17-OHPC elicited very robust gene expression (i.e. EC_{50} 's 10^{-7} to 10^{-6} M). In both the T47D-2963.1 (Table 2) and T47Dco (Table 3) cell lines, the EC_{50} for transactivation by progesterone and 17-OHPC was far more robust (i.e. 10^{-9} to

10^{-8} M range), yet the EC_{50} 's for progesterone and 17-OHPC were similar. The data for the latter cell lines also demonstrate that 17-OHP was very inefficient in eliciting gene expression ($EC_{50} \sim 10^{-6}$ M), whereas 17-OHPA was more potent than progesterone (EC_{50} in T47Dco = $6.9 \pm 5.2 \times 10^{-10}$ M). Figs. 1 and 2 depict, respectively, the CAT response of the T47D-2963.1 cell line and the luciferase activity in the T47D (A1-2) cell line after stimulation by various progestins. In the T47D-2963.1 cell line, which expresses both PR-A and PR-B, progesterone and 17-OHPC were comparably effective. In the T47D (A1-2) cell line, which expresses GR and a relatively unresponsive PR, none of the progestins elicited a response except at high concentrations. The qualitative response between progesterone and 17-OHPC was similar. In T47Dco cells, the EC_{50} 's for transactivation of PRE₂-tk-LUC and stimulation of endogenous alkaline phosphatase activity were comparable for each progestin (Table 3). It is noteworthy that the acetate ester of 17-OHP was the most potent of the progestins tested ($p < 0.05$ Table 3) whereas progesterone and 17-OHPC showed equivalent activity ($p > 0.05$) in both the transactivation and alkaline phosphatase assays. Several antiprogestins which bind to PR with high affinity, mifepristone and CDB-4124 (Fig. 3) as well as CDB-2914 (not shown), were potent inhibitors of 17-OHPC-stimulated transcription or alkaline phosphatase activity (IC_{50} 's 10^{-10} to 10^{-9} M).

In HepG2 cells in which transactivation is mediated by GR, all the progestins exhibited partial agonist activity: EC_{50} 's were in the range of 10^{-8} M to 10^{-6} M, and maximal induction was about 30–50% that of dexamethasone, a strong synthetic glucocorticoid. Transactivation by both progesterone and 17-OHPC was biphasic: at concentrations $> 10^{-6}$ M, transcription was inhibited suggesting cytotoxicity in this cell line at high concentrations (data not shown).

Comment

We have demonstrated that 17-OHPC is not superior to progesterone in eliciting a hormonal response via the classic steroid receptor-mediated pathway. The first step in this pathway is binding of the hormone (agonist) to its receptor followed by translocation of the hormone-receptor complex to the nucleus. Subsequently, this complex binds to the hormone response element (HRE) of progesterone-responsive genes and elicits transcription of mRNA which encodes a specific protein²². We evaluated two steps in this pathway, receptor binding and expression of both exogenous and endogenous genes. We have shown that binding of 17-OHPC to PR, either human recombinant or rabbit uterine, is lower than that of progesterone. Furthermore, the EC_{50} 's and extent of transactivation or induction of alkaline phosphatase activity by the two progestins in several cell lines were very similar. The biological effects of 17-OHPC are mediated, at least in part, by PR as antiprogestins are potent inhibitors of 17-OHPC-stimulated transactivation and alkaline phosphatase activity (IC_{50} 's 10^{-10} to 10^{-9} M), in close agreement with our previous results demonstrating their inhibition of gene expression induced by the synthetic progestin R5020²⁰. Thus, in seeking a mechanism by which 17-OHPC reduces the rate of preterm birth in high risk women, neither enhanced binding to PR nor enhanced gene expression appears to be a plausible explanation. We also demonstrated that 17-OHPC is poorly bound to GR and is only weakly effective in causing gene expression in a cell line with a GR linked to a luciferase reporter or in cells with a cotransfected GR. The binding of 17-OHPC to GR is so limited that it is not likely to effectively compete with glucocorticoids for binding to placental GR.

In measuring PR binding, we assessed both recombinant human PR-A and PR-B because one possible explanation for labor onset is functional progesterone withdrawal. PR-A has been shown to increase while PR-B decreases prior to labor onset in rhesus monkeys²³. Thus, a differential effect of 17-OHPC on PR-A or PR-B could provide a mechanism for the action of 17-OHPC. Clearly, this mechanism is not responsible for the beneficial effects of 17-OHPC given similar binding of the hormone to both receptor subtypes. This is not surprising as PR-

A is an N-terminally truncated variant of PR-B, and both possess the same ligand binding domain²⁴. A third PR, termed PR-C, was described by Wei and Miner²⁵ in T47D human breast cancer cells. PR-C is an N-terminally truncated variant of PR-B which is transcribed from the same gene using an alternative promoter and/or translated using an alternative translation initiation site. PR-C has a molecular weight of approximately 60,000 daltons and contains only one of the zinc fingers of the DNA binding domain and a complete hormone binding domain. In two T47D clonal cell lines containing an integrated MMTV-CAT reporter gene, PR-C enhanced the transcriptional activity of endogenous PR by about 2-fold²⁶. In contrast, in human myometrial cells, PR-C inhibited PR-B transactivation. As PR-C is up-regulated in laboring myometrium by activation of NF- κ B, it has been proposed to play a role in the loss of uterine quiescence and the onset of labor²⁷. We are currently investigating the effect of PR-C on PR-A- and PR-B-mediated transcription in several cell systems.

We also evaluated binding of 17-OHPC and related progestins to GR because of the role of progesterone in competing with glucocorticoids at the placental GR¹³. Binding of glucocorticoids to placental GR increases placental CRH production. Increasing CRH is closely correlated with labor onset, both term and preterm. Progesterone, by competing with glucocorticoids at the placental GR, could reduce placental CRH. Since both 17-OHPC and progesterone bind weakly to GR, it is unlikely that the beneficial effect of 17-OHPC on preventing preterm birth is mediated by a differential effect on GR.

Thus, how 17-OHPC reduces the risk of preterm birth remains a mystery. The hormone clearly does not bind to PR with higher affinity than progesterone and elicit a more robust progesterone or glucocorticoid gene expression response nor does it increase progesterone concentrations^{28,29}. The caproate ester is not cleaved from 17-OHPC (unpublished observations), so it is not converted to 17-OHP (a very weak progestin), to progesterone, or to free caproic acid. Caproic acid and other short chain fatty acids such as acetic and valproic acids may disinhibit methylation-directed gene silencing and could therefore impact on progesterone signaling pathways³⁰. The limited metabolism of 17-OHPC at the caproic acid ester site (C-17) suggests that this is not a likely mechanism. Another progesterone ester, 17-OHPA, produced a robust response on gene expression in vitro. Despite binding to PR that was less than that of progesterone, transactivation and induction of alkaline phosphatase were enhanced four-fold. This raises the question of whether other progesterone esters such as 17-OHPA might be as effective or even more effective in preventing preterm labor than 17-OHPC. In this regard, a recent paper describing a mouse model for preterm labor caused by intrauterine inflammation, suggested that medroxyprogesterone acetate treatment was more effective than 17-OHPC treatment in the prevention of preterm birth and resulted in live pups at term³¹.

There is ample evidence in humans, primates, and mice that 17-OHPC elicits a more sustained and robust progestational effect on endometrium than progesterone^{10–12}. In fact, the hormone was used in the 1960's as a treatment for endometrial cancer^{32,33}. This enhanced progestational effect is not due to impaired metabolism of 17-OHPC because metabolism of 17-OHPC in human hepatocytes is enhanced via a PXR-mediated increase in the CYP3A4 enzyme (unpublished observations). The data in this study on genomic effects of 17-OHPC raises the question of whether those endometrial progestational effects noted above occurred through classical steroid-receptor pathways – the same question we posit with prevention of preterm birth. In the absence of a steroid-receptor pathway to explain the effect of 17-OHPC, alternative pathways may be considered. Progesterone affects estrogen-mediated gene expression in decidua and antagonizes the estrogen stimulatory effect on connexin 43 and oxytocin receptor expression in myometrium^{34–35}. Progesterone also has anti-inflammatory effects, and parturition is thought to be mediated in part by inflammation³⁶. Either of these mechanisms could play a role in reducing preterm birth rates and merit additional investigation.

Acknowledgements

We would like to thank Dr. Richard Blye of the Contraception and Reproductive Health Branch of NICHD for his input into the design of these studies and for his continued support. We would like to thank Margaret Krol, Trung Pham, and Laurent Pessaint for their excellent technical assistance and Drs. Jerry Reel, Sheri Hild, and Sailaja Koduri for reading of this manuscript. This work was supported in part by NICHD contract NO1-HD-2-3338 awarded to BIOQUAL, Inc and by 1 U10-HD047905-02.

Supported by NICHD contract NO1-HD-63259 and NIH grant HD-047905-2.

References

1. Meis P, Klebanoff M, Thom E, Dombrowski M, Sibai B, Moawad A, Spong C, Hauth J, Miodovnik M, Varner M, et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesteron caproate. *N Engl J Med* 2003;348(24):2379–85. [PubMed: 12802023]
2. Keirse M. Progesterone administration in pregnancy may prevent preterm delivery. *Br J Obstet Gynecol* 1990;97:149–54.
3. Csapo A. The “see-saw” theory of parturition. The fetus and birth. *Ciba Found Dymp* 1977;47:159–210.
4. Liggins GC, Fairclough RJ, Grieves SA, Forster CS, Knox BS. Parturition in the sheep. *Ciba Found Symp* 1977;47:5–30. [PubMed: 205398]
5. Liggins GC, Fairclough RJ, Grieves SA, Kendall JZ, Knox BS. The mechanism of initiation of parturition in the ewe. *Recent Prog Horm Res* 1973;29:111–59. [PubMed: 4356273]
6. Challis JR, Manning FA. Control of parturition in subhuman primates. *Semin Perinatal* 1978 Jul;2(3):247–60.
7. MacDonald PC, Porter JC, Schwartz BE, Johnston JM. Initiation of parturition in the human female. *Semin Perinatal* 1978 Jul;2(3):273–86.
8. Challis J, Matthews S, Gibb W, Lye S. Endocrine and paracrine regulation of birth at term and preterm. *Endocr Rev* 2000;20(21):514–50. [PubMed: 11041447]
9. Adashi, EY.; Rock, A.; Rosenwahr, Z., editors. *Reproductive Endocrinology, Surgery and Technology*. Lippincott-Raven Publishers; Philadelphia, PA: 1996. p. 458
10. Junkmann K. Estrogens with prolonged action. *Exp Pathol Pharmacol* 1953;220 (5):358–64.
11. Kessler W, Borman A. Some biological activities of certain progestogens. *Ann NY Acad Sci* 1953;220 (5):358–64.
12. Reifenshtein, E. Introduction of marked as well as prolonged biologic activity by esterification. 8. IN: *Unique Regional Compound*; 1957.
13. Karalis K, Goodwin G, Majzoub JA. Cortisol blockade of progesterone: a possible molecular mechanism involved in the initiation of human labor. *Nat Med* 1996 May;2(5):556–60. [PubMed: 8616715]
14. Jeschke U, Mylonas T, Richter DU, Hocker T, Briesse V, Markrigiannakis A, Friese K. Regulation of progesterone production in human term trophoblasts in vitro by CRH, ACTH and cortisol (prednisone). *Arch Gynecol Obstet* 2005 June;272(1):7–12. [PubMed: 15834733]
15. Attardi BJ, Burgenson J, Hild SA, Reel JR, Blye RP. CDB-4124 and its putative monodemethylated metabolite, CDB-4453, are potent antiprogestins with reduced antiglucocorticoid activity: in vitro comparison to mifepristone and CDB-2914. *Mol Cell Endocrinol* 2002;188:111–123. [PubMed: 11911951]
16. Christensen K, Estes PA, Onate SA, Beck CA, DeMarzo A, Altmann M, et al. Characterization and functional properties of the A and B forms of human progesterone receptors synthesized in a baculovirus system. *Mol Endocrinol* 1991;5:1755–1770. [PubMed: 1779977]
17. Archer TK, Zaniewski V, Moyer ML, Nordeen SK. The differential capacity of glucocorticoids and progestins to alter chromatin structure and induce gene expression in human breast cancer cells. *Mol Endocrinol* 1994;8(9):1154–1162. [PubMed: 7838148]
18. Mymryk JS, Berard D, Hager GL, Archer TK. Mouse mammary tumor virus chromatin in human breast cancer cells is constitutively hypersensitive and exhibits steroid hormone-independent loading of transcription factors in vivo. *Mol Cell Biol* 1995;15(1):26–34. [PubMed: 7799933]

19. Horwitz KB. The antiprogesterin RU38 486: receptor-mediated progestin versus antiprogesterin actions screened in estrogen-insensitive T47Dco human breast cancer cells. *Endocrinology* 1985;116(6): 2236–2245. [PubMed: 4039656]
20. Attardi BJ, Burgenson J, Hild SA, Reel JR. In vitro antiprogestational/antiglucocorticoid activity and progestin and glucocorticoid receptor binding of the putative metabolites and synthetic derivatives of CDB-2914, CDB-4124, and mifepristone. *J Steroid Biochem Mol Biol* 2004;88(3):277–288. [PubMed: 15120421]
21. Markiewicz L, Gursipide E. Estrogenic and progestagenic activities coexisting in steroidal drugs: quantitative evaluation by in vitro bioassays with human cells. *J Steroid Biochem* 1993;48:89–94.
22. Leonhardt SA, Boonyarantanakaornkit V, Edwards DP. Progesterone receptor transcription and non-transcription signaling mechanisms. *Steroids* 2003;68:761–770. [PubMed: 14667966]
23. Haluska G, Wells B, Hirst J, Brenner R, Sadowsky R, Novy M. Progesterone receptor localization and isoforms in myometrium, decidua and fetal membranes from rhesus macaques: evidence of functional progesterone withdrawal at parturition. *J Soc Gynecol Invest* 2002:125–36.
24. Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW, McDonnell DP. Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. *Mol Endocrinol* 1993;7:1244–1255. [PubMed: 8264658]
25. Wei LL, Miner R. Evidence for the existence of a third progesterone receptor protein in human breast cancer cell line T47D. *Cancer Res* 1994;54:340–343. [PubMed: 8275464]
26. Wei LL, Hawkins P, Baker C, Norris B, Sheridan PL, Quinn PG. An amino-terminal truncated progesterone receptor isoform, PRc, enhances progestin-induced transcriptional activity. *Mol Endocrinol* 1996;10:1379–1387. [PubMed: 8923464]
27. Condon JC, Hardy DB, Kovacic K, Mendelson CR. Upregulation of the progesterone receptor (PR)-C isoform in laboring myometrium by activation of NF- κ B may contribute to the onset of labor through inhibition of PR function. *Mol Endocrinol* 2006;20:764–775. [PubMed: 16339279]
28. Johnson JWC, Lee PA, Zachary AS, Calhoun S, Migeon CJ. High-risk prematurity-progestin treatment and steroid studies. *Obstet Gynecol* 1979;54(4):412–418. [PubMed: 492618]
29. Hartikainen-Sorri A, Kauppila A, Tuimala R. Inefficacy of 17 α -hydroxyprogesterone caproate in the prevention of prematurity in twin pregnancy. *Obstet Gynecol* 1980;6:692–695. [PubMed: 7443111]
30. Benjamin D, Jost JP. Reversal of methylation-mediated repression with short-chain fatty acids: evidence for an additional mechanism to histone deacetylation. *Nucleic Acids Research* 2001;29(17): 3603–3610. [PubMed: 11522830]
31. Elovitz MA, Mrinalini C. The use of progestational agents for preterm birth: lessons from a mouse model. *Am J Obstet Gynecol* 2006;195:1004–1010. [PubMed: 17000233]
32. Kistner RW, Baginsky W. Observations on the use of 17- α -hydroxyprogesterone caproate on primary metastatic endometrial carcinoma. *Surg Forum* 1961;12:424–6. [PubMed: 14456488]
33. Varga A, Henrisken E. Clinical and histopathologic evaluation of the effect of 17- α -hydroxyprogesterone-17-n-caproate on endometrial carcinoma. *Obstet Gynecol* 1961 Dec;18:658–72. [PubMed: 13924838]
34. Petrocelli T, Lye SJ. Regulation of transcripts encoding the myometrial gap junction protein, connexin-43, by estrogen and progesterone. *Endocrinology* 1993 Jul 1;133(1):284–90. [PubMed: 8391423]
35. Grazzini E, Guillon G, Mouillac B, Zingg H. Inhibition of oxytocin receptor function by direct binding of progesterone. *Nature* 1988 Apr 2;392(6675):509–12. [PubMed: 9548257]
36. Sargent IL, Borzychowski AM, Redman CW. NK cells and human pregnancy—an inflammatory view. *Trends Immunol* 2006 Sep;27(9):399–404. [PubMed: 16843067]

Fig. 1. Chloramphenicol acetyltransferase (CAT) concentrations in T47D-2963.1 cells after incubation with various progestins. Four experiments were performed in duplicate with 100,000 cells/well (see Materials & Methods section). Values represent means $\pm$ SEM.

Fig. 2.

Luminescence units after incubation of 50,000 cells for 48 hours with various progestins. Four experiments were performed in duplicate. (See Materials & Methods section). Values represent means $\pm$ SEM.

Fig. 3.

Inhibition of 17-OHPC-stimulated transcription or alkaline phosphatase activity in T47Dco cells by the antiprogestins, mifepristone or CDB-4124. T47Dco cells were treated with vehicle, 10⁻⁸ M 17-OHPC, or 17-OHPC in the presence of various concentrations of A, C. mifepristone or B,D. CDB-4124 (10⁻¹¹ to 10⁻⁵ M). After 20 hours (A,B) or 72 hours (C,D), cells were washed and lysed for measurement of luciferase or alkaline phosphatase activity, respectively, as specified in Materials and Methods. The data represent the mean ± SD of duplicate (transcription) or quadruplicate (alkaline phosphatase) values. Experiments were repeated 2–3 times.

Table 1

Binding to Progesterin and Glucocorticoid Receptors

Steroid Hormone	rhPR-B		rhPR-A		Rabbit uterine PR		Rabbit thymic GR	
	IC ₅₀ (nM)	RBA (%) ^a	IC ₅₀ (nM)	RBA (%) ^a	IC ₅₀ (nM)	RBA (%) ^a	IC ₅₀ (nM)	RBA (%) ^b
Progesterone	6.2 ± 0.5	100	5.9 ± 0.6	100	14.7 ± 3.2	100	>1000	<1
17-OHP	723 ± 139	1	944 ± 33	1	693 ± 112	3	719 ± 129	1
17-OHPC	21.7 ± 6.3	30	23.0 ± 1.7	26	43.7 ± 12.4	28	239 ± 54	4
17-OHPA	12.6 ± 1.2	46	16.8 ± 1.0	38	15.6 ± 1.4	115	317 ± 39	3
Dexamethasone							8.4 ± 0.8	100

Values represent the mean ± SE of 3–6 determinations except for binding of progesterone to GR where n=2.

^a RBA=Relative binding affinity=IC₅₀ progesterone/IC₅₀test compound X 100

^b RBA=Relative binding affinity=IC₅₀ dexamethasone/IC₅₀ test compound X 100

Table 2

Stimulation of transcription in T47D (A1-2) and T47D-2963.1 cell lines

Cell line	T47D (A1-2)	T47D-2963.1
	EC ₅₀	EC ₅₀
Steroid hormone		
Progesterone	4.9×10^{-7} M	3.8×10^{-9} M
17-OHPC	4.9×10^{-7} M	5.9×10^{-9} M
17-OHP	5.5×10^{-7} M	1.9×10^{-9} M

EC₅₀s were calculated after averaging dose response values from four experiments, run in duplicate, for each cell line

Table 3
Stimulation of Transcription or Alkaline Phosphatase Activity by Various Progestins in T47Dco Cells

Steroid Hormone	EC ₅₀ for stimulation of PR-mediated transcription [†] (M)	Potency Ratio compared to progesterone [*]	EC ₅₀ for stimulation of alkaline phosphatase activity [†] (M)	Potency Ratio compared to progesterone [*]
Progesterone	$3.1 \pm 1.4 \times 10^{-9a}$	1	$1.9 \pm 0.5 \times 10^{-9a}$	1
17-OHPC	$1.8 \pm 0.4 \times 10^{-9a,c}$	1.7	$2.6 \pm 1.3 \times 10^{-9a}$	0.73
17-OHP	$2.6 \pm 0.8 \times 10^{-6b}$	0.0012	$1.3 \pm 0.05 \times 10^{-6b}$	0.0015
17-OHPA	$6.9 \pm 5.2 \times 10^{-10c}$	4.5	$4.5 \pm 0.5 \times 10^{-10c}$	4.2

[†] Values represent the mean $\pm$ SE of 3–5 determinations.

^{*} EC₅₀ progesterone/EC₅₀ test progestin

^{a,b,c} Means with different letters are significantly different (p<0.05) from each other by one-way ANOVA followed by the Holm-Sidak multiple comparison test. EC₅₀'s were log₁₀ transformed prior to analysis.