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Progesterone receptor profile in the decidua and fetal membrane

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1. ABSTRACT

Whereas in most mammals the onset of labor is preceded by a rapid fall in the maternal progesterone levels, in humans and in higher primates, maternal, fetal and amniotic fluid concentrations of progesterone are sustained before the onset of labor. Therefore, the mechanism for parturition, which has been proposed for humans, is 'functional' progesterone withdrawal. This review is focused on the expression profile, activity and interaction of the progesterone receptor (PR) isoforms in the decidua and the fetal membrane during the initiation of labor. Binding of progesterone to PR induces a significant conformational change on the receptor proteins. These changes result in dimerization, increased receptor phosphorylation and binding of receptor dimers to specific hormone responsive DNA elements in the promoter of target genes. Interaction with specific co-activator proteins and general transcription factors are responsible for the formation of a productive transcription initiation complex.

The PR also mediates the activation of cytoplasmic signaling pathways, participating in the induction of signal transduction pathway in the cytoplasm. Balanced expression of the two major progesterone receptors isoforms is crucial for progesterone function as uterine muscle inhibitor. Change in PR isoforms profile seems to be responsible for decidual activation. Decidua without contractions shows consistent profile with PR-B being the dominant isoform. PR-A, PR-C and two additional truncated isoforms are also detected but in significantly smaller concentration. After initiation of contractions, a sharp decline in PR-B shifts the PR-A/PR-B ratio toward PR-A dominance. This shift in the decidua towards increase expression of progesterone receptor isoform A and decrease in PR isoform B is having a pivotal role in decidual activation and initiation of labor.

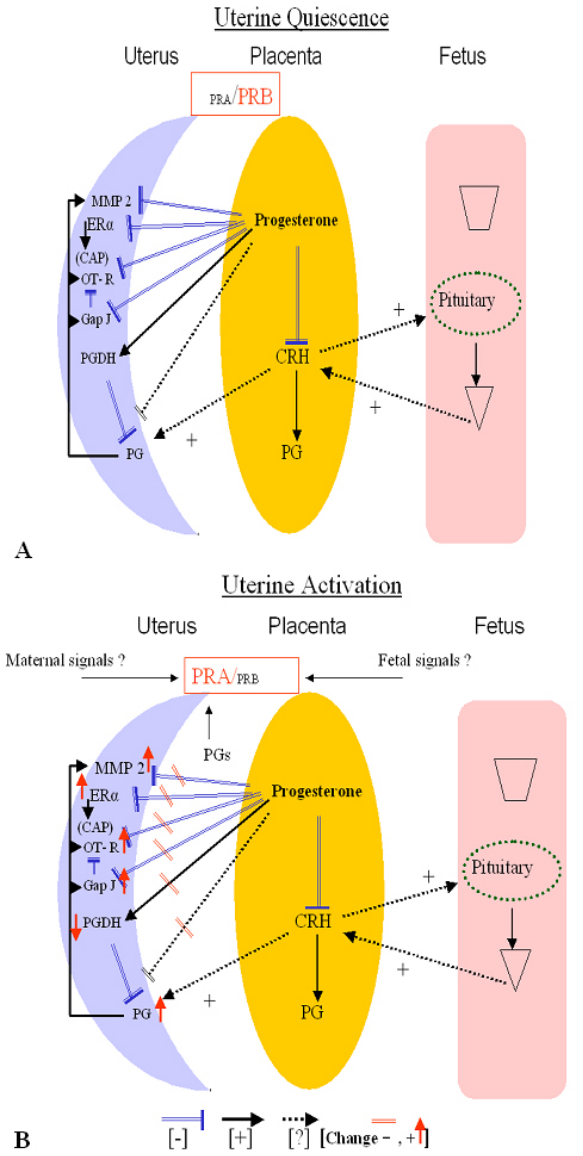

Figure 1. Progesterone is promoting relaxing effects during uterine quiescence (A) by the inhibition of CAP, which are responsible for the number of gap junctions and OtR availability, inhibition of MMP 2 and ERά expression and activation of catalytic enzyme, hydroxyprostaglandin dehydrogenase (PGDH), responsible for degradation of the PGs. Decidual and uterine activation (B) involves a shift in PRA/PRB ratio with dominance of PRA. Genes inhibited by progesterone, such as cyclo-oxygenase-2, which induce PG production, MMP 2, and ERα increases their expression level. Activation of PGDH is inhibited and the net PG is significantly increased. PGs that reduces PR-B levels are shifting further the PR-A/PR-B ratio toward PR-A and further increases the expression of MMP2. OtR expression is also up regulated due to increased expression of ER-alpha.

2. INTRODUCTION

Hierarchical associations, which start before the active process of labor is appreciated, characterize normal labor at term. During pregnancy, the uterus is maintained in a quiescent state owing to the action of several putative inhibitors. The process of labor begins as uterine release phenomena from these myometrial inhibitors (1). Progesterone, which is synthesized from pregnenolone by placental syncytiotrophoblast and chorionic trophoblasts, is known to have a relaxing effect on myometrial smooth muscle. It has been already shown that progesterone blocks the action of contraction-associated proteins (CAP) (Figure 1A), which includes connexin 43, a key component of gap junctions, agonist receptors prostaglandins (PGs) and oxytocin receptor (OtR), as well as proteins encoding ion channels (2-4). In high primates and in humans, synthetic antiprogestins were found to stimulate myometrial contractions, whereas inhibitors of 3beta-hydroxysteroid dehydrogenase (3beta-HSD), which lower systemic progesterone levels, induce labor and delivery (5). Furthermore, in a recent clinical study, weekly injections of 17 alpha-hydroxyprogesterone caproate reduced the rate of recurrent preterm delivery in high-risk women (6). In most mammals the onset of labor is preceded by a rapid fall in maternal progesterone levels (7) and progesterone withdrawal has been suggested in these animals to play pivotal role in the initiation of labor. However, in the humans and in high primates, maternal, fetal and amniotic fluid concentrations of progesterone are sustained before the onset of labor. Therefore, the mechanism for parturition, which has been proposed for humans, is "functional" progesterone withdrawal (8-10).

Many studies have focused on local mechanisms that, despite maintenance of high circulating progesterone levels, might diminish or uncouple progesterone action in the myometrium or at the level of the decidua and fetal membranes. Recent data indicate a close association between progesterone withdrawal and the expression of MMPs, the production of prostaglandins and pro-inflammatory cytokines in intrauterine tissues. Although there is strong evidence that progesterone is a potent repressor of these molecules, both in vivo and in vitro, there is continuing debate regarding the importance of decidual or amnion activation as the initiating event in parturition (11,12). Because the enzymes for steroid metabolism and prostaglandins (PGs) production, cytokines and matrix metalloproteinases (MMPs), coexist within decidua and fetal membranes, it is tempting to postulate that changes in steroid action are linked, in a paracrine fashion, to the release of these important mediators of parturition. In recent study we have shown a different PR profile between decidua and amnion, which in both compartments decline after contractions begin and following PGs administration (13). In this review we will discuss and will try to present new insight on the mechanisms and role of the progesterone receptor, including its co-relations with other proteins, in activation of the decidua.

3. PROGESTERONE RECEPTOR

The steroid hormone, progesterone, is a key modulator of normal reproductive functions. Progesterone

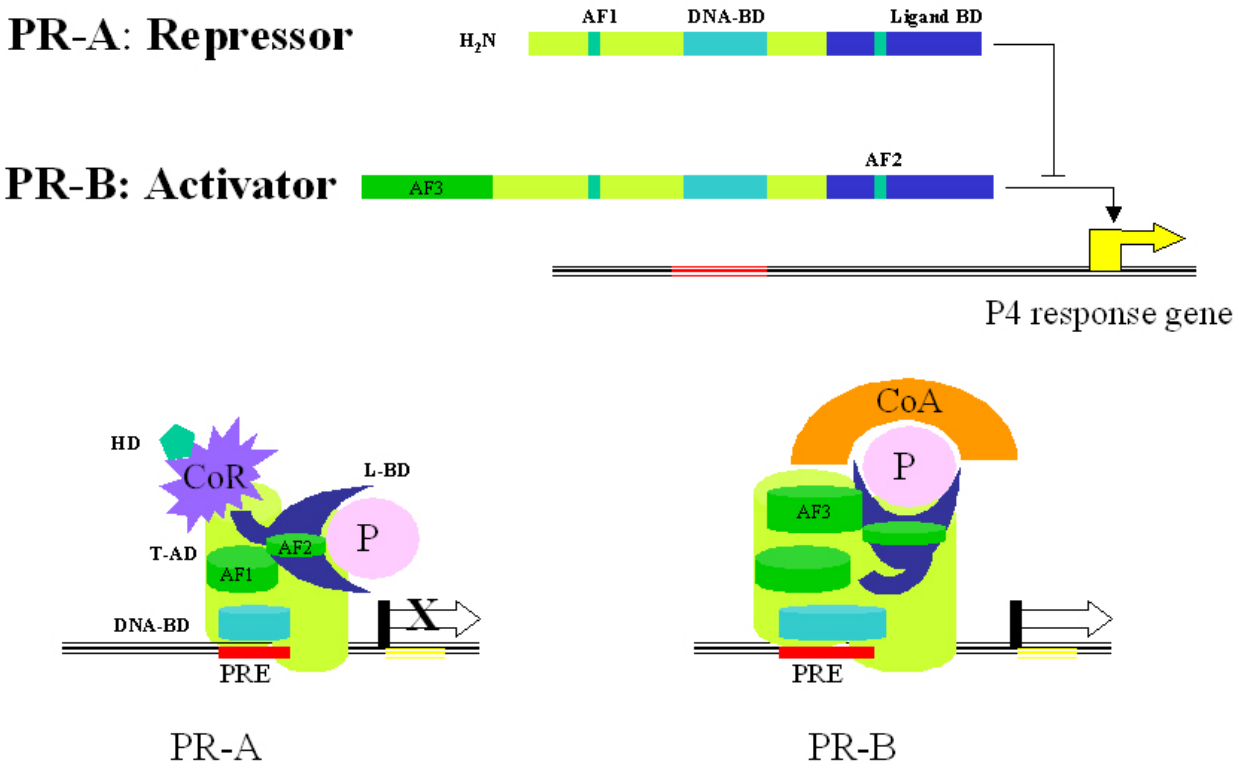

Figure 2. Progesterone receptor (PR) major subtypes. PRB binned through its DNA binding domain (DBD) to the progesterone response element (PRE) on the promoter, and function as activator of progesterone response gene. PRA is truncated form lacking the first 164 N-terminal, and is transcriptional inactive. PRA, which also differ in its conformation, is lacking a third transactivation domains (AF3) located in the truncated area, is known to repress transcriptional activity mediated by PRB and some other steroids.

receptor (PR) exists in human reproductive tract tissues in at least three functional isoforms: PR-A, PR-B and PR-C (14-16). The expression of the two forms of PR, i.e. the classical nuclear (PR-A and PR-B) and nonclassical forms (PR-C and other truncated forms), has been demonstrated in several reproductive tissues i.e. ovary; sperm; decidua and fetal membranes (13, 17-19). The physiological effects of progesterone are usually mediated by interaction of the hormone with the two classical intracellular progesterone receptor isoforms (14-16). Progesterone receptor isoforms are members of a larger family of structurally related gene products known as the nuclear receptor (NR) super-family of transcription factors. The PR is a member of a larger family of ligand-activated nuclear transcription regulators, which are characterized by organization into specific functional domains and are conserved, to differing degrees, between species and family members. The PR is made up of a central DNA binding domain and a carboxyl-terminal ligand-binding domain (Figure 2) (20-23). Studies on human PR, indicates that two PR isoforms, namely PR-A and PR-B, mediate the effects of progesterone. These isoforms are transcribed from a single gene using distinct estrogen-inducible promoters with translation initiation at two alternative AUG initiation codons and differ only by the presence of 164 amino acids in the amino- terminal (20-23). Detailed structure/function studies on these PR isoforms indicate that PR-B in all cellular contexts in vitro, functions as a ligand-dependent transactivator of progesterone-responsive genes in contrast to PR-A, which in some contexts, acts as a ligand-dependent transcriptional repressor of PR-B as well as of other steroid hormone receptors (20-23).

There is increasing evidence to date that PR-A and PR-B are functionally different, and that differences between the receptor forms in normal tissues are yet to be fully understood (24-26). The amino terminal region of PR is the most hyper-variable region in terms of both size and amino acid sequence among members of the super-family. This region contains transactivation domains (AF-1 and AF-3) that recruit co-activator proteins to the receptor to modulate the level and promoter specificity of target gene activation as well an inhibitory domain responsible for recruitment of transcriptional inhibitory co-repressor proteins. The most conserved region is the DNA binding domain (DBD) that, in the case of PR, is centrally located. This domain consists of approximately 66–68 amino acids and is composed of two type II zinc finger structures that facilitate binding of the receptor to specific cis-acting DNA sequences and are the hallmark of the nuclear receptor superfamily. A highly conserved ligand-binding domain is located on the carboxy terminal side of the DBD (14,26). In addition to its progesterone binding function, this region contains an additional transactivation domain (AF-2)

required for hormone-dependent co-activator recruitment, sequences important for interaction of inactive receptors with heat shock proteins and for receptor dimerization (14-17, 20-23).

When expressed in equimolar ratios in cells, the PR-A and PR-B proteins can dimerize and bind DNA as three distinct species: A:A or B:B homo-dimers or A:B heterodimers. The differential transactivation properties contributed to these complexes by the presence or absence of the PR-B-specific AF-3 domain is likely to contribute to the complete repertoire of physiological responses to progesterone (27-28). Analysis of poly(A)+ RNA derived from T47Dv human breast cancer cells using a variety of 5'-specific probes has identified three separate structural classes of human PR transcripts, indicating extensive 5'-termini heterogeneity. The third PR isoform, identified by Horwitz et al (29), at approximately 50-60 kD, has been termed PR-C. This receptor is hypothesized to originate from an in-frame translation start site, and in vitro transcription at this start site with subsequent expression does result in an appropriately sized protein (30). Yet the naturally occurring transcript for this protein has not been identified, and it has not been proven that the 60-kD protein is indeed encoded by this hypothesized start site.

4. PROGESTERONE RECEPTOR ACTIVITY-TRANSCRIPTIONAL REGULATION

Newly transcribed cytoplasmic PR isoforms are assembled in an inactive multiprotein chaperone complex that dissociates on ligand binding and receptor activation. Binding of progesterone to PR induces a significant conformational change on the receptor proteins that results in dimerization of two ligand receptor complexes, increased receptor phosphorylation, binding of receptor dimers to specific hormone responsive DNA elements located in the promoter regions of target genes, and interaction of the receptor complex with specific co-activator proteins and general transcription factors to form a productive transcription initiation complex on specific target gene promoters (31-36). PR-B exhibits hormone-dependent transactivation in all cell types examined irrespective of the complexity of the response elements, whereas the transcriptional activity of PR-A is cell specific and reporter specific. With reporter constructs containing a single palindromic PRE, PR-A displays similar transactivation activity to PR-B (37-38). This activity of PR-A is reduced when more complex response elements, such as the mouse mammary tumor virus long terminal repeat and PRE2TATAtk constructs are used. It was suggested that PR-A acts as a transdominant inhibitor of PR-B in situations where PR-A has little or no transactivational activity (37-39). Moreover, PR-A can regulate the transcriptional activity of other nuclear receptors such as glucocorticoid, mineralocorticoid, androgen and estrogen, suggesting that PR-A may play a central role in regulation of activity of a number of nuclear receptors in addition to PR-B. It was reported that PR-A but not PR-B, in the presence of either progesterone or anti-progestins, lessened the ability of estrogen to induce an estrogen-responsive reporter when the two constructs were transfected into CV-1 or HS578T cells, but not into HepG2 cells. PR-A had similar anti-estrogenic effects on endogenous estrogen receptor activation of a minimal estrogen-responsive reporter in MCF-7 breast cancer cells in the presence of RU 38486 (39-43). Overexpression of PR-A resulted in a decrease in corticotrophin-releasing hormone promoter activity following progesterone treatment, whereas an increase in promoter activity was observed with overexpressed PR-B (44). In endometrial cancer, cells that express only PR-B were found to be the most proliferative and migrative, while cells that express only PR-A, or no PR at all, showed minimal growth and spread (45).

Leung et al, documented a differential role of PR-A and -B isoforms in transcription regulation of the human GnRH receptor gene (46). The exact mechanism of each receptor on the transcriptional mechanism is not fully understood. A number of possible scenarios have been proposed. The physical differences at the N-terminal end of the two receptors are clearly responsible for some transcriptional differences. In addition to the fact that AF3 is unique to PR-B, the PR-B-specific region has a distinct conformation in solution and is likely to mask an inhibitory domain that is active in the N-terminus of the PR-A protein (38-39). This could act to enhance the transcriptional activity of PR-B, as well as preventing it from acting as an inhibitor of other receptors. The unique AF in PR-B, may confer a difference in affinities of the two PR isoforms for co-regulators. Several studies have shown that two PR forms bind to distinct subgroups of peptides. This suggests that co-activators may bind differently to the two isoforms or that the two isoforms binds to different subgroups of co-activators. Motifs contained in AF-3, with the same sequence as the NR boxes of co-activators, have been shown to be necessary for the transcriptional activity of the PR-B-unique AF and may form contacts between the receptor and a unique set of cofactors, or within the PR dimmer itself (47-48).

Given that the PR acts in combination with multiple other transcription factors to affect transcription, it is possible that variability of the tissue-specific expression of the components of this multiprotein complex may result in different PR-A and PR-B activities in the same cell. It was demonstrated, by an in vitro interaction assay of bacterially expressed proteins, that C/EBPbeta binds PR-B (49). This interaction was not altered by the presence or absence of progesterone. We suggest that proper transcriptional regulation by PR needs the co-expression of both isoforms at similar levels, which is common in normal progesterone target cells. Changes in relative PR-A and PR-B levels, are responsible for physiological changes under progestin induction.

5. PROGESTERONE RECEPTOR ACTIVITY – NON-GENOMIC REGULATION

Sex steroid hormones are known to act through intracellular receptors and their cognate hormone response elements, located in the promoters of hormone-regulated genes (31-36). However, this classical mechanism of action cannot account for a variety of rapid effects of steroids

(within seconds or minutes). The study of signal transducing pathways has recently become of tremendous interest for the understanding of progesterone action like proliferation of target cells, anti-apoptotic activity of tumor cells and cell differentiation (50-54).

It is now clear that transcriptional and signaling stimulatory activities of progesterone receptors are independent. Progesterone receptors are known to shuttle between nuclear and extra-nuclear compartments, and only a tiny amount of these receptors is required to stimulate the signaling (50-51). It is not clear, however, whether, in addition to the shuttling, other mechanisms enhance the interaction of receptors with signaling proteins acting at level of cell membrane. There is conflicting data on the possible existence and nature of the membrane progesterone receptor, and the signaling pathways used in different types of cell (50-54). Several publications have reported on the expression of PR isoforms of low molecular weight that differ in the structure and mechanisms of action from the classical nuclear receptor. A non-classical form of PR involved in the opening of calcium channels in the mitochondrial and cellular membranes has been characterized in the cytoplasmic membranes of human sperm (19). It was suggested that membrane PR caused $Ca2^{+}$ mobilization from the endoplasmic reticulum through the activation of phospholipase C (50-55). Ca2+ is a known regulator of many enzymes, including PKC (40,54). Activated PKC, in turn, serves as an interacting signal in the regulation of gene expression. Studies of rapid, membrane-mediated events in several types of cell have identified a wide range of changes, including Ca2+ mobilization (influx or mobilization from intracellular stores), opening of Na+ and Cl− channels, and the activation of phospholipase C (18), leading to increased intracellular IP3 and diacylglycerol generation and PKC, tyrosine kinase and MAPK pathways (50-57). Expression of the non-classical forms of PR has been demonstrated also in the ovary, particularly in granulosa cells. Expression of an unusual PR isoform has been described in porcine granulosa cells.

The PR can also mediate the activation of cytoplasmic signaling pathways. Intracellular PR isoforms has been described to be recruited to the inner side of plasma membrane by interaction with Src kinase. PR directly activates Src kinase signal transduction pathway via phosphorylation and de-phosphorylation processes. PR contains a total of 14 known phosphorylation sites indicating that the receptor can participate in the induction of signal transduction pathway in the cytoplasm. Serines at positions 102, 294, and 345 in the PR are hormone-induced sites that are maximally phosphorylated 1–2 h after progesterone treatment. Serines at positions 81, 162, 190, and 400 in the PR are defined as "basal" sites, constitutively phosphorylated in the absence of progesterone. Specific kinases responsible for phosphorylation of selected sites have been identified, whereas others remain unknown. For example, the serines at positions 81 and 294 have been demonstrated to be phosphorylated by casein kinase II and MAPK, respectively. Progesterone can also stimulate serine 294 phosphorylation independently of MAPKs by activation of an unknown kinase. Progesterone activates the Src, MEK, Erk pathway as well as the PI3, PKC, MAP, AKT pathway (50-57). Activation of the two pathways is simultaneous and each of them is required to trigger cell signaling. Why different signaling pathways are activated by a single hormone is still an open question. Purified liganded PR-A and PR-B activates, in vitro, the Src-related protein kinase. Mutation of the proline-rich motif sequence in PR abolished the ability of progesterone to bind c-Src and activate both c-Src and p42/p44 MAPKs (50-57).

Recently it was found that another mechanism for non-genomic signaling exists between the PR and estrogen receptors (ER). Cross-talks between steroid receptors are regulated by association between receptors PR-B and ER-alpha. and their interaction with important effectors of the signaling pathways. The hormone-triggered assembly of these macromolecular complexes has multiple effects and implications. First, it reinforces the hormone action, generating a much stronger activation of the signaling pathways. Furthermore, it offers the opportunity of specifically interfering at level of protein–protein interaction with the signaling pathway activation, without affecting the transcriptional activity of the receptors (34, 50-57).

6. PROGESTERONE RECEPTOR IN THE DECIDUA

Although progesterone receptor is essential for the establishment of pregnancy in humans, its expression during pregnancy was not intensively investigated. There is limited data concerning this issue. Progesterone receptors were studied in the non-pregnant state, in early pregnancy and at term using monoclonal antibody enzyme immunoassays. The receptor was found in tissues from non-pregnant patients and patients in early pregnancy. Progesterone receptor was undetectable in chorion, amnion and placenta at term, while present in extremely low concentrations in decidua and myometrium. PR mRNA has been extracted from human fetal membranes, however PR protein has not been clearly visualized by immunohistochemical methods or quantified in binding assays (7, 9, 57, 58). Padayachi and colleagues (59), using ligand binding and enzyme-immunoassay, found higher levels of PR in myometrium and endometrium from non-pregnant women and in decidua during the first trimester than in tissues obtained at term. This is consistent with protein data from others studies that found a decrease in PR level towards term. It was also suggested that a decline in PR co-activator expression and in histone acetylation, in the uterus near term, might impair PR function by causing a functional progesterone withdrawal (60). Furthermore, a 9-fold decrease in the binding of PR to its response element was observed in decidua obtained after the onset of labor. Condon at al (61) suggested that in the uterus near term the decline in PR coactivator expression and in histone acetylation may impair PR function causing a functional progesterone withdrawal.

Progesterone receptor exists in at least three major forms that are known to have different roles in gene

regulation and signal transduction activation (62). We have studied the expression pattern and expression level of PR in the decidua before and after contractions. Decidua without contractions shows PR isoform profiles with PR-B (116 kDa) as the main isoform, PR-A (82 kDa), PR-C (60 kDa), and two smaller isoforms of 45 and 36 kDa were also detected (13). In decidua obtained from women with contractions, all isoforms of PR sharply decreased or vanished. A significant decrease in the relative mRNA level of PR-B in the decidua after contractions began was found. A decrease in PR-B in the decidua after contractions resulted in a shift from PR-B expression to PR-A expression (13). We also found that progesterone can increase the PR-B level in the decidua obtained from women without contractions, indicating a possible autocrine or paracrine mechanism (13). This could be an additional mechanism by which progesterone may prevent preterm labor (63), where the high progesterone level maintains a low PR-A/PR-B ratio. These results support the hypothesis of a 'functional progesterone withdrawal' in humans (8,13). After initiation of contractions, a sharp decline in PR-B shifts the PR-A/PR-B ratio toward PR-A dominance (13). Since progesterone acts mostly via its PR-B receptor, it is more than likely that genes inhibited by progesterone, such as cyclo-oxygenase-2, which induce PG production, and proteolytic enzymes like the MMPs, will increase their expression level as a result of functional progesterone withdrawal.

In decidua without contractions, an additional two bands were observed, 45 and 36 kDa (13). Although one cannot exclude the possibility that these two forms are degradation products, several publications have reported on the expression of PR isoforms of low molecular weight that differ in the structure and mechanisms of action from the classical nuclear receptor (16, 51, 64-65). A non-classical form of PR involved in the opening of calcium channels in the mitochondrial and cellular membranes has been characterized in the cytoplasmic membranes of human sperm (19). Expression of the non-classical forms of PR has been demonstrated also in the ovary, particularly in granulosa cells (52, 64). Expression of an unusual PR isoform has been described in porcine granulosa cells, which mediates its actions through mobilization of Ca2+ from the endoplasmic reticulum through the activation of phospholipase C. Ca2+ is a known regulator of many enzymes, including PKC (42,56). Activated PKC, in turn, serves as an interacting signal in the regulation of gene expression. The importance of Ca2+ in labor activation is well documented (66-67). PR-C and the additional two truncated low molecular weight PR expressed in the decidua and in the fetal membranes, could be the mediators to the non-genomic effect of progesterone during pregnancy.

7. PROGESTERONE RECEPTOR IN FETAL MEMBRANE

Several PR isoforms are expressed in the amnion. PR-C (60 kDa) and the 36-kDa isoform were dominant (13). After contractions began, all PR isoforms reduced sharply (13). It is possible that the amnion is a critical component in the initiation and propagation of labor. In the amnion, the dominant isoforms were PR-C and 36 kDa. Although the possible role and mechanism of these forms in decidua and fetal membrane is still unclear, the low molecular weight PR isoforms (36 and 45 kDa) could be mediators of rapid progesterone effect via non-genomic signaling pathway (19, 52, 65).

Progesterone increased 36 kDa isoform expressions in the amnion suggesting a different mechanism by which progesterone acts in the amnion. The faint expression of the two nuclear forms (PR-A and PR-B) further support a different role for progesterone in the amnion. The low level of PR-B might suggest that the amnion is less affected by progesterone concentrations and is more vulnerable to changes in PG concentration; this could imply that the amnion plays a role in the initiation of labor.

A major source of PGE2 is the amnion (68), which also contains high concentrations of the prostaglandin precursor, arachidonic acid. Prostaglandin synthesis in the amnion is entirely via inducible type 2 cyclooxygenase (COX-2) (67). Expression of COX-2 increases exponentially with increasing gestational age to term. Expression of COX-2 mRNA increases 6-fold from early in the third trimester (69).

Much attention has been focused on the role of amnion-derived prostaglandins in ripening of the uterine cervix and the lower segment. Several research groups have demonstrated increased COX-2 expression and prostaglandin synthesis in amnion associated with labor. Van Meir et al. (69) found that prostaglandin dehydrogenase activity in fetal membranes decreases in the lower segment of the uterus in association with labor. Progesterone suppressed IL-8 induction by IL-1 before labor (71-72). However, in post labor amnion cells, although IL-1 stimulated IL-8 production, still, this protein production was not suppressed by progesterone. This data further support the finding of PR reduction after contractions began. The rise in the level of PG towards labor will result in the further decrease of PR-B, inducing a cascade of events leading to the propagation of labor. Recent studies have shown that PR are present also in human amnion, although less abundantly than in decidua and myometrium (58, 59, 62). The amnion is a major source of prostaglandins, which have been linked to the initiation of labor. One mechanism that might limit PG secretion, before the onset of labor, is an increase in the expression of PG dehydrogenase. This enzyme expression is progesterone dependent. Thus the relatively low level of PR-B in the amnion might suggest a mechanism enabling PG to overcome progesterone inhibition near labor.

8. PROGESTERONE REGULATION OF PROSTAGLANDINS

The prostaglandins PGE_2 and PGF_2 are believed to have a key role in the initiation of parturition in many species, including humans (73-74). Much of the research on the potential role of PGs in regulating the onset of labor has

focused on the factors determining PG concentrations; specifically, the balance of PGH synthase (PGHS) and PGDH activities in key intrauterine tissues. Expression of 15-hydroxyprostaglandin dehydrogenase (PGDH), the major PG metabolizing enzyme in chorion, is regulated by progesterone and levels correlated with 3beta-HSD in tissue collected adjacent to the placenta, but not in the cervical region (75-81). Most strategies for delaying preterm birth rely on directly reducing uterine contractions. It is well accepted that uterine contractile activity at birth involves uterine activation and increased levels of contractile stimulators. Uterine activation proteins (UAP) include the OtR, $PGF_{2\alpha}$ and PGE_2 and prostaglandin endoperoxide H synthase (cyclooxygenase, PGHS)-2 (75-81). The uterine stimulators include PGs and oxytocin. The PGs have been a target for tocolysis because it has been documented that the myometrium contracts in response to exogenous PGs, *in vivo* and in vitro; PG synthetic enzymes and PG levels in tissues and fluids increase before or at the time of labor; and inhibitors of PG synthesis delays birth and prolongs the pregnancy (75-81). There are five currently recognized G-protein-coupled prostanoid receptor types, encoded by separate genes and named according to the PG that is the most potent agonist: EP (PGE2), DP (PGD2), FP (PGF2), IP (PGI2), and TP (thromboxane) (73-74). In addition, there are at least four subtypes of EP receptor (numbers 1 to 4), encoded by separate genes (73-74). The EP2 and EP4 receptors are positively coupled to adenylate cyclase (AC), the EP1 receptor is coupled to calcium influx, and the EP3 receptor is generally negatively coupled to AC. Consequently, EP2 and EP4 receptor activation inhibits smooth muscle contractility, whereas EP1 and EP3 activation promotes contraction (82). Progesterone inhibits cyclooxygenase (COX-2) mRNA expression and PGE2 synthesis in the lower uterine segment. Progesterone inhibits COX-2 mRNA in rat myometrium and decreases PGE2 output from human first-trimester decidua (83-84). In amnion cells, progesterone also represses COX-2 mRNA expression together with PGE2 release (83). Upon RU-486 treatment on day 19 of pregnancy, rat uterine EP2 receptor mRNA levels were decreased, and FP mRNA levels were increased. Thus, the expression of relaxant EP2 receptors in the rat uterus increases with pregnancy and decreases with labor, and appeared to be progesterone dependent (85). Changes in the expression of isoforms of PG receptors in the rat uterus appear to be related to the changes in ovarian steroid concentration. The expression of EP2 mRNA in the rat uterus was increased during pregnancy when circulating progesterone levels are elevated and decreased during labor, when circulating progesterone levels declined (85). Thus, it is suggested that progesterone modulates uterine functions through at least two prostaglandin receptors: EP2 is involved in relaxation, and FP is involved in myometrial contraction.

We have found (13) that PGF2 reduced PR-B levels shifting PR-A/PR-B ratio toward PR-A. Another support for the capability of PG to regulate PR came from the study of Madsen et al, (63) in which differential control of myometrial PR-A and PR-B expression has been observed by PGE2 and PGF2 and by specific intracellular signaling pathways. The authors conclude that PG acting via the protein kinase C (PKC) pathway, facilitates functional progesterone withdrawal, by increasing the myometrial PR-A/PR-B expression ratio. PG is known to be involved in the cascade of events leading to cervical softening, contractions and labor. Prostaglandins increase towards the end of gestation and have been directly correlated with the onset of labor. Thus, we may speculate that the increase in the expression of PG reduces PR expression leading to an increase in tissue sensitivity to contractile stimulus.

Progestins, produced intracellular from pregnenolone conversion to progesterone by 3beta-HSD, or from the maternal circulation, stimulate PGDH acting to maintain prostaglandin concentrations throughout pregnancy (81). Prostaglandins do not only regulate the contraction cascade but are most probably involved in membrane rupture. In the decidua, fetal membranes and amniotic fluid, the level of some matrix metalloproteinases (MMP), have been demonstrated, to increase dramatically after contractions begin (86-91). The exposure of the decidua and fetal membranes to PGF2α increased dramatically the production of MMP-9 and MMP-2, whereas tissue inhibitor of metalloproteinases (TIMP) -1 was decreased (86).

9. PROGESTERONE REGULATION OF ESTROGEN RECEPTOR

The human Estrogen receptor (ER) exists as two major subtypes, ER-alpha and ER-beta, derived from separate genes, each having different ligand binding affinities and tissue distributions (92). The selective actions of estrogens and various estrogen agonists and antagonists are thought to be due to differential expression of ER-alpha and ER-beta. The roles of ER-alpha and ER-beta expression in functional estrogen activation are unclear. In term human myometrium, the onset of labor is associated with increased expression of ER-alpha whereas ER-beta expression is very low and is not affected by labor status (93-95). PR-A/PR-B correlated with ER-alpha mRNA only in non-laboring myometrium, suggesting PR role in ER expression (96). High PR-A/PR-B expression ratio could be required to block progesterone suppression of ER-alpha. ER mRNA correlates positively with cyclooxygenase (COX) -2 and oxytocin receptor (Otr) mRNA in non-laboring myometrium. A positive association between ER and OtR indicates that estrogen responsiveness is related to ER levels, because OtR expression is known to be up regulated by estrogen. The strong positive association between ER and COX-2 mRNA levels suggests that COX-2 gene expression also is influenced by estrogen responsiveness (97-98). However, estrogen do not up-regulate COX-2 in the pregnant human myometrium, and therefore, the mechanistic basis for the association remains unclear. An inhibitory effect of progesterone on ER-alpha expression is an important mechanism to prevent uterus activation. For most of human pregnancy the myometrium is exposed to very high levels of estrogens (93-95). However, for most of that time the myometrium is refractory to estrogenic actions. This insensitivity is

probably due to progesterone suppression of ER expression. In the pregnant rhesus monkey, the progesterone antagonist RU486 not only induces parturition, but also increases myometrial ER expression (99), suggesting that progesterone decreases myometrial ER expression during pregnancy. A theoretical model was suggested for the role of the myometrial ER and PR systems in the regulation of human pregnancy and parturition. For most of pregnancy, progesterone acting through PR-B inhibits expression of ER. At term, expression of PR-A increases, leading to functional progesterone withdrawal. As a consequence, the expression of ER is coordinately increased (Figure 1B), leading to increased myometrial responsiveness to circulating estrogens, which increases the expression of genes encoding contraction-associated proteins that augment myometrial contractility and excitability. We have found (un-published data) that progesterone reduced ER-alpha in the decidua without contractions. The addition of progesterone reduced ER-alpha./ ER-beta ratio. This data, together with our finding of reduced PR in the decidua and fetal membranes after the initiation of contractions (13) further support the hypothesis that progesterone inhibits estrogenic effect through inhibition of ER-alpha expression level. Reduction in PR-B is most probably the cause for the increase in ER-alpha after contractions.

10 PROGESTERONE RECEPTOR REGULATION OF CALCIUM INFLUX

The contractile activity of the uterus changes markedly from relative quiescence during pregnancy to the generation of strong coordinated contractions during labor. This transformation has been referred to as 'activation' and is envisaged to involve changes in the identity and number of ion channels, the proteins of the contractile apparatus, second messenger systems and gap junction formation (100-102). Steroids influence (100) several classes of the K+ channels in myometrium. The contractile state of smooth muscle is determined predominantly by the level of phosphorylated myosin, achieved largely via myosin light chain kinase (MLCK) whose activity is regulated by Ca^{2+}-calmodulin. The level of cytoplasmic free Ca^{2+} ($[Ca^{2+}]i$) is influenced by the complement of ion channels, pumps and exchangers in the plasma membrane. As a result of Ca^{2+} release from the endoplasmic reticulum. Uterotonins generally increase intracellular calcium levels, by increased influx of Ca^{2+} through receptor-operated channels, or through the release of calcium from intracellular stores including sarcoplasmic reticulum (103-106). Agents that inhibit myometrial activity do so by increasing intracellular levels of the cyclic nucleotides cAMP or cGMP, which in turn inhibit the release of calcium from intracellular stores (103-106). Once activated, membrane receptors generally initiate very rapid responses.

In the case of progesterone, $[Ca^{2+}]i$ levels are usually altered within seconds of progesterone exposure. However, progesterone does not always increase $[Ca^{2+}]i$. For example, progesterone appears to interact with calcium channels within the membrane of smooth muscle cells to reduce calcium influx, thereby suppressing $[Ca^{2+}]i$. Similarly, progesterone suppresses the increase in $[Ca^{2+}]i$ that is induced by thapsigargin in T lymphocytes. In porcine granulosa cells and human sperm, progesterone acts to rapidly increase $[Ca^{2+}]i$ (107-108).

In Xenopus laevis, progesterone action via a plasma membrane receptor, induces the resumption of meiosis I with germinal vesicle breakdown (109). Progesterone action to increase calcium influx is suggested to be regulated via activation of a phosphatidylinositol diphosphate-specific phospholipase C, and a decrease in cAMP levels (103-106). These findings bring into question a possible non-genomic role of progesterone.

It has been proposed that progesterone mediates its action through a 60-kDa protein that functions as a membrane receptor isoform called PR-C (29). It has been suggested that progesterone acts through this putative membrane receptor to regulate $[Ca^{2+}]i$ levels, via the activation of the MAP kinase pathway (52,110). Although there are insufficient data on the expression of PR-C in the myometrium, it is tempting to assume that this 60 KD progesterone receptor is involved in the regulation of $[Ca^{2+}]i$ in the myometrium. We have studied the expression of PR-C in the decidua and fetal membranes (13). While PR-C was reduced dramatically in the decidua after contractions began, its expression was not changed in the amnion (13). It may suggest that fetal activation needs a shift towards higher PR-C expression in order to regulate $[Ca^{2+}]$ influx in the amnion. The role of PR-C in fetal activation needs further investigation.

11. PROGESTERONE REGULATION OF MATRIX METALLOPROTEINASE

The matrix metalloproteinases (MMP) are part of an expanded family of proteins called the astacin family of zinc metalloproteinases. The human MMP family can be subdivided according to their structural and functional properties into five groups (109): collagenases, gelatinases, stromelysins, membrane type (MT), and a fifth heterogeneous subgroup (111). These five subgroups of extracellular matrix (ECM) – degrading enzymes share common functional domains and activation mechanisms.
They are synthesized and secreted (except the MT-MMPs) as a latent pro-form (zymogens) which requires activation to achieve proteolytic activity. Their active site contains Zn^{2+}, and they need calcium to maintain stability (111). The MMPs are functioning at neutral pH and they are inhibited by specific tissue inhibitors of metalloproteinases (TIMPs).

The gelatinases, MMP-2 and MMP-9, differ from other MMPs in that the catalytic domain is separated from the haemopexin-like domain by a fibronectin-like domain. The matrix metalloproteinases have been implicated in the propagation of labor, at term or earlier (86-91). Specifically, gelatinase A (MMP 2) and gelatinase B (MMP 9) have been studied in this context, owing to the fact that they degrade collagen types 1,4 and 5. It was published that MMPs increase during human parturition and was found in high level in the amniotic fluid obtained from preterm labor (84-89). It is well documented that

both cytokines and prostaglandins increased MMP-2 and MMP-9 expression levels (112-113). Extracellular matrix homeostasis is a key process in the maintenance of the tensile strength of the amnion/chorion membrane. This tensile strength guarantees the role of the membranes as a physical and functional boundary for the fetus during human pregnancy. Pathological rupture of these structures before 37 completed weeks of gestation is known as preterm pre-labor rupture of the membranes (PPROM) and it is a major cause of spontaneous preterm labor and preterm birth (86-91). A mechanism involving the activation of MMP-9, a 92-kDa type IV collagenase, as an essential mediator of tissue damage is under investigation.

The proposed mechanism involves the abnormal expression and activity of MMP-9 in amniotic fluid with subsequent connective tissue degradation taking place at a time that does not synchronize with other events of labor. The local physiological signal by amnion/chorion cells to induce MMP-9 expression is not known, but bacterial products and/or the pro-inflammatory cytokines, IL-1beta and TNF-alpha, as paracrine or autocrine signals may trigger these processes in pregnancies complicated with intra-amniotic infection (90,91, 114-118). The increase in MMP levels lead to increased collagenolytic activity, en route to the tissue disintegration, taking place during labor. This disintegration applies to two main events taking place at the initiation and propagation of labor. One is weakening of the membranes towards rupture and the other is further disintegration of the decidua. We found (86,90,119) that before the onset of contractions, the decidua has the highest production of MMP 2 and 9 and TIMP-1. We further demonstrated that contractions as well as the exposure to PGF2-alpha increased dramatically the production of MMP 9 and 2 whereas TIMP-1 was decreased resulting in a shift of the balance between collagenolysis and its inhibition. In addition to the increase of collagenolytic activity by PGF_{2alpha} we found that upon incubation with Indomethacin, which is an established inhibitor of prostaglandin production and labor, there decreased expression of both MMP 2 and 9 in the two fetal membranes but not in the decidua (86,90). The production of TIMP-1 was at the same time increased in both fetal membranes. Decidual production of MMPs and TIMP was unaffected by incubation with Indomethacin (86). Taken together, these data suggest an overall effect of Indomethacin that by inhibiting PG production and also increasing the production of TIMP-1, protects the membranes from being disintegrated by MMPs, originating in the adjacent decidua.

The decidua is known to produce phospholipases, which in turn lead to PG production from the arachidonic acid reservoir in the fetal membranes. Our findings, that PG increases the ability of the decidua to degrade its own extracellular matrix by increasing MMP 2 and 9 and reducing TIMP-1, delineates a putative positive feedback mechanism towards further decidual disintegration during labor. In an additional study (90), we documented that MMP-2 and MMP-9 levels significantly increased after contractions began and that in the decidua obtained after the initiation of contractions, MMP-2 was the most active MMP while MMP-9 was the most active MMP in the amnion (90).

Progesterone, which is known as a physiological suppressor of MMP-9 in other species, was found to inhibit MMP secretion from the endometrium and other reproductive tissues (120-121). In humans, progesterone failed to decrease MMP secretion induced by cytokines from myometrium (12). We have found that progesterone inhibits MMP secretion from decidua before contractions begin and failed to do so after contractions (13). The failure to inhibit MMP secretion after contractions could be related with PR-B reduction. It was suggested that PR-B is responsible for MMP inhibition in other tissues (45). A shift towards higher PR-A can be responsible for reduced progesterone's ability to inhibits MMP activation and enable membrane rupture (Figure 1B).

12. PROGESTERONE AND INFLAMMATORY MEDIATORS

Several reports have identified cytokines in amniotic fluid including interleukin 1 (IL-1) and tumor necrosis factor a (TNF-alpha), inhibitory cytokines such as IL-1 receptor antagonist and IL-10 and chemokines such as IL-8. Both decidua and chorion are the sources of several key cytokines in in-vitro systems; cytokines such as IL-1 can induce labor in animal models and it has been suggested that invading neutrophils, which secret MMP-8, digest the collagen of the cervix and thus allow the dilatation necessary for birth (123). Evidence for the involvement of prostaglandins in parturition is strong since inhibitors of prostaglandin synthesis delay labor, prostaglandin concentrations are high in amniotic fluid as labor advances and the administration of prostaglandin can induce labor (68-70,78-82). There are two forms of the enzyme cyclooxygenase (COX) responsible for prostaglandin synthesis: COX-I, a constitutive form, and COX-II, an enzyme that is inhibited by glucocorticoid and induced by cytokines such as IL-1 (123).

In the uterine lower segment, the action of progesterone in maintaining PGDH action was suggested to be overcome near term, by the inhibitory influence of proinflammatory cytokines. Recent studies examining cervical biopsies obtained at Caesarian section have shown that IL-8 concentrations in tissue correlate very well with both MMP-8 (neutrophils collagenase) and MMP-9, which play an important role in cervical ripening and membrane rupture (123). Cytokines production seems to be regulated by progesterone (124,125). Progesterone inhibits, and RU-486, stimulates IL-8 secretion from the chorion-decidual cells in vitro. It is suggested that progesterone also acts as an immunosuppressor. Thus anti progestin can activate a cytokine cascade and neutrophils migration. Inflammatory mediators may also be produced by the trophoblastic villi, which lie in close contact with decidua or maternal blood mRNA for IL-1, IL-2, IL-6 and IL-8 has been demonstrated (125). Research into parturition in women has yielded a confusing picture but one in which many strands of evidence point to important and interconnecting roles for steroids, prostaglandins and cytokines.

13. SUMMARY AND PERSPECTIVE

Progesterone supports pregnancy and prevents parturition mainly by promoting myometrial quiescence. Progesterone withdrawal in human parturition appears to be mediated by a functional desensitization of the decidua, fetal membrane and myometrium to circulating progesterone, in part, through shift in the PR-A/PR-B expression ratio.

Recent in vitro studies have shown that progesterone receptors have differential expression before and after contractions. Moreover, there is a different pattern between the decidua and the amnion. While PR-B was the dominant isoform in the deciduas, PR-C was dominant in the amnion. Expression of all PR isoforms was reduced dramatically after contractions began. PGF_{2alpha} reduces the expression of PR-B in the decidua. The reduction in decidual expression of PR-B induced by $PGF_{2\ alpha}$ results in an altered PR-A/PR-B ratio and diminished progesterone effect. The reduction in PR-B in the decidua after contractions begin suggests that progesterone has a beneficial effect only as preventive modality before the appearance of contractions

Decrease in the expression of ER-alpha as well as progesterone receptor isoforms B characterizes the early phase of first stage of labor. The ability of progesterone to block PG production, ER-alpha expression and cytokine secretion; further supports the concept of preventive treatment of high-risk patients with progesterone.

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Abbreviations: AC: adenylate cyclase;CAP: Contraction associated proteins; C/EBPbeta: CCAAT/enhancer binding protein beta; COX-2: cyclooxygenase; CPEB: cytoplasmic polyadenylation element binding protein; CRH: corticotropin-releasing hormone; DBD: DNA binding domain; DG: diacylglycerol; GnRH: gonadotropin-releasing-hormone; ECM: extracellular matrix; ER: Estrogen receptor; HSD: hydroxysteroid dehydrogenase; IL: interleukins; MEK: MAPK/ERK kinase; MLCK: myosin light chain kinase; MMP: matrix metalloproteinases; MT: membrane type; NR: nuclear receptor; OtR: Oxytocin receptor; PG: prostaglandins; PGDH: hydroxyprostaglandin dehydrogenase; PGHS: PGH synthase; PKC: protein kinase C; PR: Progesterone receptor; PRE**:** progesterone response element; SH3: Src homology 3; TIMP: tissue inhibitor of metalloproteinases; TNF: tumor necrosis factor; UAP: Uterine activation proteins

Key Words: Progesterone Receptor, Decidua, Fetal Membrane, Prostaglandins, Calcium Influx, Matrix Metalloproteinases, Inflammatory Mediators, Review

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