

Nuclear progesterone receptor isoforms and their functions in the female reproductive tract

R. Rękawiecki, M.K. Kowalik, J. Kotwica

Institute of Animal Reproduction and Food Research of Polish Academy of Science,
Tuwima 10, 10-747 Olsztyn, Poland

Abstract

Progesterone (P4), which is produced by the corpus luteum (CL), creates proper conditions for the embryo implantation, its development, and ensures proper conditions for the duration of pregnancy. Besides the non-genomic activity of P4 on target cells, its main physiological effect is caused through genomic action by the progesterone nuclear receptor (PGR). This nuclear progesterone receptor occurs in two specific isoforms, PGRA and PGRB. PGRA isoform acts as an inhibitor of transcriptional action of PGRB. The inactive receptor is connected with chaperone proteins and attachment of P4 causes disconnection of chaperones and unveiling of DNA binding domain (DBD). After receptor dimerization in the cells' nucleus and interaction with hormone response element (HRE), the receptor coactivators are connected and transcription is initiated. The ratio of these isoforms changes during the estrous cycle and reflects the different levels of P4 effect on the reproductive system. Both isoforms, PGRA and PGRB, also show a different response to the P4 receptor antagonist activity. Connection of the antagonist to PGRA can block PGRB, but acting through the PGRB isoform, P4 receptor antagonist may undergo conversion to a strongly receptor agonist. A third isoform, PGRC, has also been revealed. This isoform is the shortest and does not have transcriptional activity. Alternative splicing and insertion of additional exons may lead to the formation of different PGR isoforms. This paper summarizes the available data on the progesterone receptor isoforms and its regulatory action within the female reproductive system.

Key words: nuclear receptors, progesterone receptor isoforms, PGRA, PGRB.

Introduction

The corpus luteum (CL), is an endocrine gland which is formed from the cells of the ovarian follicle after its ovulation. The gland, as a source of progesterone (P4) is the main regulator of the estrous cycle duration and maintenance of pregnancy in female farm animals, including cattle (Davis and Rueda 2002). CL is composed of two main types of ster-

oidogenic cells: small and large luteal cells forming the main part of the secretory CL. During the functional dominance of CL, proper conditions are created in the uterus for blastocyst implantation and fetal development. One of the main conditions for successful implantation of the embryo and the further development of pregnancy is the reduction of uterine contractility. Uterine and oviductal contractions determine appropriate fertilization, by the movement

of sperm and oocyte in the female genital tract. However, pregnancy is a process that can occur only under conditions of complete abolition of motor activity of the uterus (atony). These conditions are provided by the action of the P4 which is produced by the luteal cells. P4 connects to a specific nuclear receptor, activates the appropriate signal transduction pathways, and initiates a specific response of the cell. This paper presents the latest data on the structure and function of progesterone receptor isoforms. It should also be noted that P4 may also affect the cell through a non-genomic way, without the action of their nuclear receptors. The effect of the P4 activity is revealed after a few seconds or minutes of its administration and is not delayed by inhibitors of transcription and translation (Simoncini and Genazzani 2003, Wehling and Losel 2006). Non-genomic activity of P4 has been demonstrated in the female reproductive tract of different species (Grazzini et al. 1998, Simoncini and Genazzani 2003, Peluso 2006, Bishop and Stormshak 2008), including cattle (Bogacki et al. 2002, Bramley 2003, Duras et al. 2005, Kowalik et al. 2008, 2009, Rękawiecki et al. 2008); however, the mechanism of this effect is not fully understood. It is suggested that: (a) P4 directly modulates membrane receptors, or impairs the binding of these receptors with their ligands, which may reduce the impact of steroids on the target cells. This has been demonstrated for the OT receptor (Grazzini et al. 1998, Bogacki et al. 2002, Bishop and Stormshak 2008). (b) P4 as a lipophilic substance can modify cell membrane fluidity and as follows alters the affinity of other membrane receptors to connect to their ligands (Gimpl and Fahrenholz 2002). (c) P4 binds to specific membrane receptors, activates the appropriate pathways of signal transduction and initiates a specific response of the target cells (Rae et al 1998a, b, Bramley 2003, Peluso 2006, Wehling and Losel 2006). The physiological significance of this type of P4 action is not fully understood, but it is considered that it plays an important role in the development of pathological conditions.

Structure and physiological properties of the progesterone receptor

Progesterone action in target tissues is mainly carried out by the nuclear receptor (PGR). Protein expression of the PGR has been demonstrated in the human brain (Brinton et al. 2008), pancreas (Doglioni et al. 1990), bones (Bland 2000), testis (Abid et al. 2008), mammary gland (Branchini et al. 2009), ovary (Horie et al. 1992), oviduct (Teilmann et al. 2006), uterus (Thijssen 2005) and urinary tract (Batra and Iosif 1987). Progesterone receptor occurs in the form of two main isoforms, i.e. isoform A (PGRA) and

B (PGRB), which are diversified in terms of their structure. This receptor, together with receptor for estradiol, mineralocorticoids, glucocorticoids and androgens belong to the superfamily of nuclear receptors. Except of PGR, also estradiol receptor (ER α and ER β) and androgen receptor (AR-A and AR-B) are present in two forms (Griekspoor et al. 2007). The action of PGR isoforms has been demonstrated in species other than humans, *i.e.* in monkeys (Duffy et al. 1997), cattle (D'Haeseleer et al. 2007), pigs (Shimada et al. 2004, Durlej et al. 2010), chicken (Conneely et al. 1989, Gonzalez-Moran et al. 2001), mouse (Gava et al. 2004, Shao et al. 2006) and rats (Kariagina et al. 2007). Progesterone receptor gene, in humans, consists of 8 exons and is located in chromosome 11 (Misrahi 1993) (Fig. 1). Both receptor isoforms are transcribed from the same gene, but they are under the influence of two different promoters (Mulac-Jericevic and Conneely 2004). Receptor gene transcription is activated by the interaction of estradiol with estrogen response elements (EREs) located in the promoter of the gene (Savouret et al. 1989). Progesterone receptor isoforms differ in their protein structure. Characteristic element that differentiate PGRB from PGRA, is an additional section located at the N-terminus of the protein. The length of this segment ranges from 128 amino acids in chickens (Conely et al. 1989) to about 164 amino acids in human (Mulac-Jericevic and Conneely 2004). Progesterone receptor is a member of the steroid hormone receptor family which share a similar, modular architecture, consisting of a number of independent functional domains. The receptor molecule consists of a number of different regions, which are responsible for different functions of the receptor. The N-terminus part of the PGR contains two domains: AF-1 and AF-3 (Fig. 1), which bind transcriptional factors responsible for the activation of the appropriate promoter and turn on the transcription of the isoforms. The AF-1 domain is present in both PGRA and PGRB, while AF-3 is present only in PGRB. Over the AF-1 domain, there is inhibitory domain (ID), which consists of about 140 amino acids (Giangrande et al. 1997). This domain is responsible for the connection of receptor antagonists, thereby regulating their activity. Centrally located is the DNA binding domain (DBD) which is located next to the AF-1 domain. This domain consists of approximately 66-68 amino acids and contains two zinc finger structures. It is also responsible for the connection of hormone/receptor complex to hormone response element sequence (HRE), which is located within the target gene promoter (Giangrande et al. 1997). A ligand binding domain (LBD) is located at the carboxy-terminal side of the DBD. Within the LBD domain, an activation domain AF-2 is included, which is responsible for receptor interaction with heat shock proteins (HSP) and

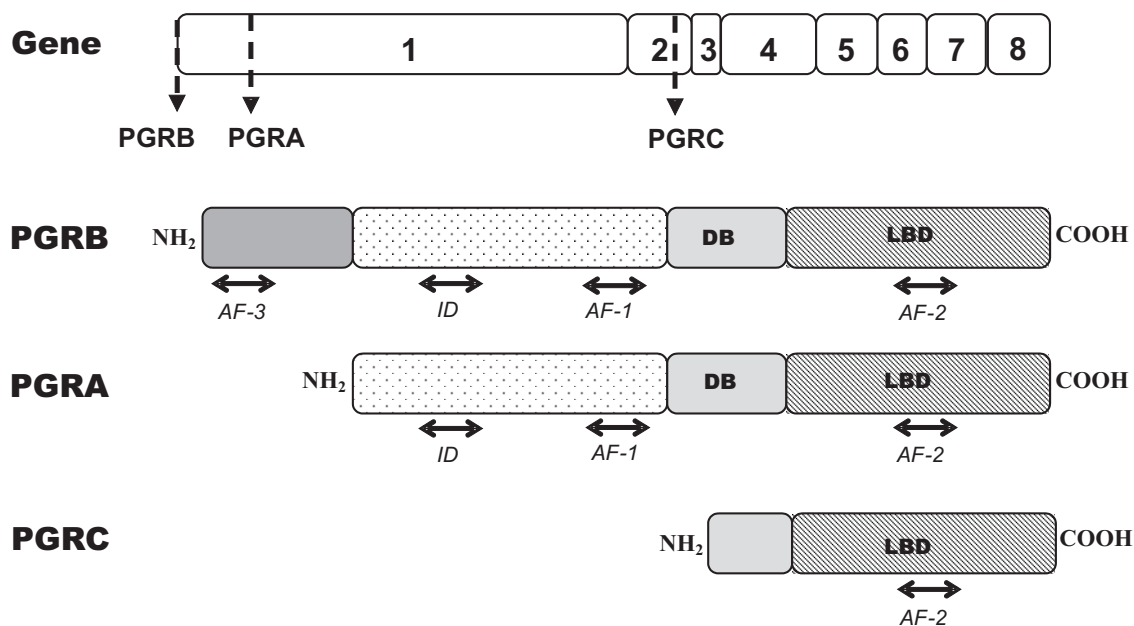


Fig. 1. Schematic representation of human progesterone receptor gene and protein domains organization of progesterone receptor isoform B (PGRB), progesterone receptor isoform A (PGRA) and progesterone receptor isoform C (PGRC). Progesterone receptor gene, in humans, consists of 8 exons. Receptor isoforms are transcribed from the same gene, but they are under the influence of different promoters (arrows). DBD – DNA binding domain, LBD – ligand binding domain, AF1-AF3 activation domains, IF – inhibitory domain.

is responsible for receptor dimerization. This domain also recruits coactivator proteins for receptor dimerization (Mulac-Jericevic and Conneely 2004). Isoforms, PGRA and PGRB, in a different manner affect the target genes. PGRB isoform is a potent activator of progesterone-dependent genes in different cells, whereas PGRB is a weak activator of these genes. When both isoforms of the receptor are activated in the cell, PGRA acts as a potent inhibitor of PGRB action and decreases progesterone effect on target cells (Pieber et al. 2001). Inhibitory domain is located in both receptor isoforms, but its activity in PGRB is limited through the third activation sequence of PGRB. The PGRA isoform could not only inhibit the PGRB isoform but also the other nuclear receptors such as estrogen receptors, glucocorticoids receptors or mineralocorticoids receptors (Kraus et al. 1995).

In human breast cancer cell lines, besides PGRA and PGRB isoforms, a third isoform has also been identified. PGRC isoform shows a lack of one zinc finger in DBD domain, and as a result, loss of their transcriptional activity is observed. The sequence of PGRC contains a complete LBD and the sequence responsible for dimerization and location of PGRC in the cell nucleus. This isoform shows a binding capacity of synthetic progestins and antagonists of PR with the same affinity as the PGRA and PGRB. The action of PGRC is not fully explained yet, but it could form heterodimers with PGRA and PGRB, and in this way

regulates the transcription of proper genes (Wei et al. 1996, Taylor et al. 2009). Screening of human cDNA libraries allowed for the identification of insertion of additional exons in the sequence of PGR gene. Additional exons T and S are located between exons 3 and 4. The process of transcription involving exons T and S allows the formation of isoforms which contain almost the entire region of LBD, but not the N-terminal domain of the receptor (Hirata et al. 2002). Two additional exons, i.e. i45a and i45b, are located between 4 and 5 exons. In this case, the newly created receptor has a short LBD, which leads to the lack of transcriptional activity of this isoform (Yamanaka et al. 2002). Several exon skipped in alternative splicing of mRNA which allows the formation of new variants of PGR which differ in their structure. Nomenclature of these isoforms contains a number of the deleted exon: del.4, del.6, del.5 + 6, del.4 + 6, del.4 + 5 + 6, del.3 + 4 and del.3 + 4 + 5 + 6 (Misao et al. 2000). Some of these PGR variants do not encode a functional receptor because they lose a large number of protein domains required for their proper functioning (Balleine et al. 1999). An example might be del.4 + 6 variant which lacks the nuclear localization sequence and LBD, consequently it is present more frequently in malignant than healthy human breast tissue (Hirata et al. 2002). Functions of these isoforms are not fully explained yet. These variants can compete with the correct form of the PGR receptor in binding to coregulators and as a result blocking proper activity

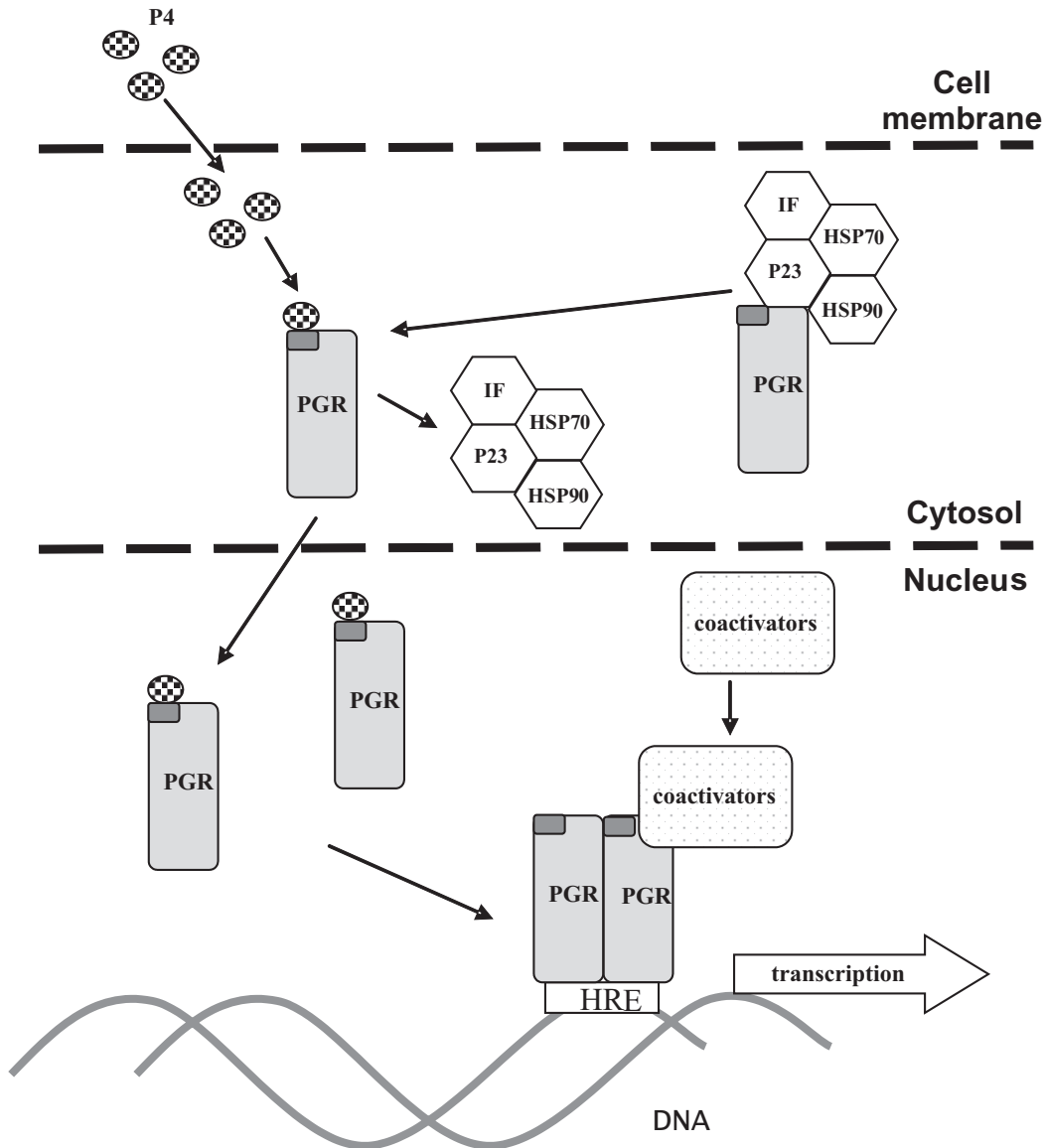


Fig. 2. Schematic representation of the PGR receptor action. The inactive form of the receptor is located in the cytoplasm and is associated with a complex of chaperone proteins. Progesterone penetrates the cell membrane and connects to LBD of the receptor. Aggregation of the hormone causes disconnection of associated chaperone protein complex and dislocation of the receptor to the nucleus where it undergoes dimerization. Receptor dimer connects to the hormone response element (HRE) located within the specific gene promoter. After activation of the receptor dimer by the receptor coactivators, the transcription process begins.

of PGRA and PGRB isoforms. The activity of alternative spliced isoforms appears in breast cancer tissues which may be associated with reduced activity of DNA repair systems in that kind of tissues (Cork et al. 2008).

Activation of receptor PGR

The inactive form of the receptor is associated with a complex of chaperone proteins including heat shock proteins HSP 90, HSP 70, p23 and im-

munophilins (Cheung and Smith 2000). Formation of this intermediate complex requires energy released from ATP breakdown. The combination of receptor chaperone protein provides specific conformation that allows proper ligand binding. Association of P4 to LBD initiates the conformational change in the receptor, and disconnection of the chaperone proteins and nuclear translocation (Fig 2). This process also requires energy from ATP breakdown (Smith 2000). Inside the nucleus, receptors bind (as a dimer), to HRE, which is located in regulatory regions of target gene. Progesterone receptor isoforms, PGRA and PGRB,

can bind as a homodimer A: A, B: B and heterodimer A: B. Receptor dimerization consequently modulates the transcriptional activities of PGR and determines the diversity of physiological responses associated with the P4 action (Mulac-Jericevic and Conneely 2004). The next step is connection of coactivators to the receptor dimer, and then the transcription process of a target gene is initiated (Griekspoor et al. 2007).

Regulation of receptor of PGR transcriptional activity

Binding of PR to the HRE is followed by the recruitment of coregulators. These molecules participate in the regulation of the transcription machinery, leading to an increase or decrease in target gene transcription. Coregulators work by interacting with the protein-receptor complex within the AF-2 domain of the receptor and do not bind to the DNA sequence (Glass and Rosenfeld 2000). Coregulators are divided into two groups: coactivators, proteins which support activation of transcription; and corepressors, proteins which inhibit the process of transcription. The first group includes proteins of the family SRC/p160 (SRC-1, SRC-2, SRC3, NCoA-1, NCoA-2, GRIP1, TIF-2, ACTR, AIB-1, TRAM-1, RAC3) (Han et al 2005, 2006). Included in the second group is the protein family, CBP/p300 (CBP, p300) (Chakravarti et al. 1996). Outside these groups are included unassigned coactivators (L7/SPA, RIP140, TIF1, ARA70, HMG-1/2E6-AP, RPF-1) (Rowan and O'Mailey 2000). Interaction of these coactivators with the PGR occurs through highly conservative "NR box" motif which consists of three leucine amino acids and two unspecified amino acids (LXXLL) (Heery et al. 1997, McKenna et al. 1999). Some of the coactivators show the ability of histone acetylation resulting in rearrangements of the chromatin. This effect involves changes in the chromatin structure under the influence of histone acetyltransferase (HAT) and result in a loosening of chromatin and consequently to the greater availability of transcription factors to the appropriate sequence (Tyler and Kadonaga 1999).

Corepressors group includes two main proteins: Nuclear Receptor CoRepressor (N-CoR) and mediator of retinoid and thyroid receptor (SMRT) (McKenna et al. 1999). They also have a conservative sequence, with structure the same as in coactivators (CoRNR box) which is responsible for interaction of the corepressor with the PGR receptor (Hu and Lazar 1999). Corepressor proteins cooperate with histone deacetylases (HDAC). Disconnection of histone acetyl group maintains chromatin condensation and transcription of the target genes is not initialized (Lazar 2003).

Factors which negatively regulate receptor interaction with HRE are also progesterone receptor antagonists. These compounds impair or prevent proper binding of agonist to receptor and thus their proper activation. One of the most popular PGR antagonists is mifepristone, otherwise known as RU486 (Cadepond et al. 1997). This compound has a greater affinity for the receptor than P4 alone and competes with P4 for binding to the LBD, but each of them connects differently to this domain. This is supported by the fact that the removal of 42 amino acids from the C-terminus of the receptor abolishes P4 binding to the LBD domain, but has no effect on antagonist binding (Vegeto et al. 1992). Whereas, single substitution of Gly-Cys amino acids at position 722 of the LBD, inhibits the binding of the antagonist to this domain and does not affect the binding of P4 to LBD (Benhamou et al. 1992, Leonhardt and Edwards 2002). The mechanism of inhibition of PGR may occur in different ways. Antagonist, changes the conformation of C-terminus segment of the receptor in a different way than P4, thus blocking connection of coactivators to the AF-2 domain resulting in no receptor activation (Onate et al. 1995). Full activity of PGR requires interaction between -C and -N terminus parts of the receptor. RU486 causes conformational changes that inhibit the above contact and, as a result, there is no proper binding of the coactivators to the receptor (Tetel et al. 1999). Receptor antagonists may work not only by inhibition the attachment of the receptor to HRE, but also through an indirect way, by the interaction of PGR receptor with another transcriptional factor. This happens when HRE of the receptor is partially overlapping with the transcription factor binding site. For example, RU486 induced inhibition of NF- κ B activity (nuclear factor kappa-light-chain-enhancer of activated B cells) associated with blocking PGR receptor activity. The NF- κ B receptors are present in the endometrium and they are mediators of immunosuppressive effect of P4 during pregnancy (Kalkhoven et al. 1996, Leonhardt and Edwards 2002). In addition to the inhibitory effect of RU486 on PGR, it can also display partial agonist activity. After connection of RU486 to PGRA, the receptor becomes inactive and does not affect the target gene. Otherwise, RU486 after connection to PGRB isoform, using energy from cAMP breakdown may be converted to a highly active agonist of the receptor (Meyer et al. 1990, Rothchild 1996, Conneely and Lydon 2000). Additionally, RU486 action through both PGR isoforms inhibits the activity of estrogen receptor (Kraus et al. 1995). Thus, the ratio of isoforms PGRA/PGRB in the tissue may reflect a physiological effect of the PGR inhibitor action.

Physiological role of PGRA and PGRB isoforms

Transgenic mice are a model to study the function of PGR receptor in the ovary, uterus and mammary gland. These animals had both receptor isoforms inactivated by the “null” mutation (PRKO) which caused: anovulation, uterine dysfunction, impaired sexual behavior and impaired pregnancy-associated mammary gland morphogenesis (Lydon et al. 1995). Progesterone receptors are necessary for the proper activation of ovulation, while the absence of PGR does not result in LH-dependent activation of enzymes ADAMTS-1 (a disintegrin and metalloproteinase with thrombospondin motif) and cathepsin-L (lysosomal protease). These enzymes are involved in the hydrolysis of peptide bonds of the follicle membrane, and consequently in the release of the oocyte (Robker et al. 2000). PGRA isoform knockout mice (PRAKO), in which PGRA is ablated, showed that ovulation is partially impaired but not completely absent. On the other hand, mice with knockout of PGRB isoforms (PRBKO) showed unaffected ovulation (Mulac-Jericevic et al. 2000). Therefore, independent action of PGRB is unable to initiate the normal process of ovulation. It is suggested that PGRA isoform is basically responsible for follicle rupture, and heterodimeric interactions between the PGRA and PGRB proteins are not required for the regulation of essential progestin-responsive target genes associated with ovulation (Connely et al. 2001).

Progesterone is a key hormone which prepares the uterus for embryo implantation through a temporal decidual transformation of the endometrium during pregnancy. In PRKO knockout mice there is no embryo implantation as a result of inhibition of the decidual transformation in response to P4 (Lydon et al. 1995). Lack of action of P4 causes uncontrolled proliferation of endometrial cells as a result of uncontrolled proliferative effects of estradiol. Progesterone receptors are also present in stromal cells and the myometrium, and dynamically change during the estrous cycle. Analysis of phenotypic changes in PRBKO and PRAKO knockout mice showed that the defect of PGRA or PGRB isoforms, affects the functional properties of the endometrium. In PRAKO mice, the absence of PGRA isoform causes inhibition of decidual transformation of stroma cell, activated by P4 (Mulac-Jericevic et al. 2000). The same effect was observed in PRKO mice with defects of both PGRA and PGRB isoforms, which indicates the significant participation of these isoforms in the decidual transformation of endometrial cells. The role of PGRA isoform is not restricted

to the inhibition of proliferative effects of E2 during the estrous cycle, but also to reduce the P4-induced cell division in the mouse uterus (Connely et al. 2001, Mulac-Jericevic et al. 2003).

Progesterone, estradiol and prolactin (PRL), together with locally-acting growth factors are very important in the development of mammary glands. In humans, estradiol stimulates the ductal elongation and branching, whereas P4 is responsible for increased dichotomous side branching and differentiation of milk-filled alveolar lobules. During pregnancy, P4 and PRL action results in alveolar proliferation, and lobuloalveolar differentiation (Connely et al. 2001, Anderson 2002). Analysis of the phenotype of PRKO mice has demonstrated that the lack of P4 action causes the loss of ductal proliferation and lobuloalveolar differentiation of the mammary epithelium. Thus, P4 action in the mammary gland is opposite to the antiproliferative effect on the development of the uterine endometrial epithelium. In the mammary gland tissue, both progesterone isoforms, PGRA and PGRB, are expressed. PGRA isoform specific knockout mice and mice with both active progesterone receptor isoforms have well developed mammary glands. However, PRBKO mouse lines show abnormal development of mammary glands during pregnancy. It is connected with inhibition of proliferation and increased apoptosis of epithelial cells of ducts and lobules. Therefore, the action of PGRA may reduce effects of excessive proliferation of epithelial cells in the mammary gland by P4 and estradiol (Connely et al. 2001, Mulac-Jericevic et al. 2003).

Relationship between expression of both isoforms is changing during development of CL and depends on the hormonal status of the individual. It is impossible to determine mRNA for PGRA, since its whole sequence is a part of mRNA for PGRB. Therefore, the amount of mRNA for PGRA is depicted as a ratio of mRNA expression for PGRB to the total amount of mRNA for PR described as PGRAB together. The level of PGRB mRNA in human CL was 100-1000-fold lower than PGRAB mRNA, and it was lower in mid-luteal phase than during early and late luteal phase (Ottander et al. 2000). Proportion of PR isoforms mRNA concentrations depends on steroid concentrations. PGR protein concentrations were parallel to their mRNA concentrations. Thus the changes in PGR mRNA isoform concentrations are reflected by their protein concentrations (Misao et al. 1998). This suggests that a high concentration of P4 within luteal cells induce the expression of PGRA mRNA, which represses the transcription of PGRB mRNA, and as a result, PGR function and P4 effect is suppressed. On the other hand, a low P4

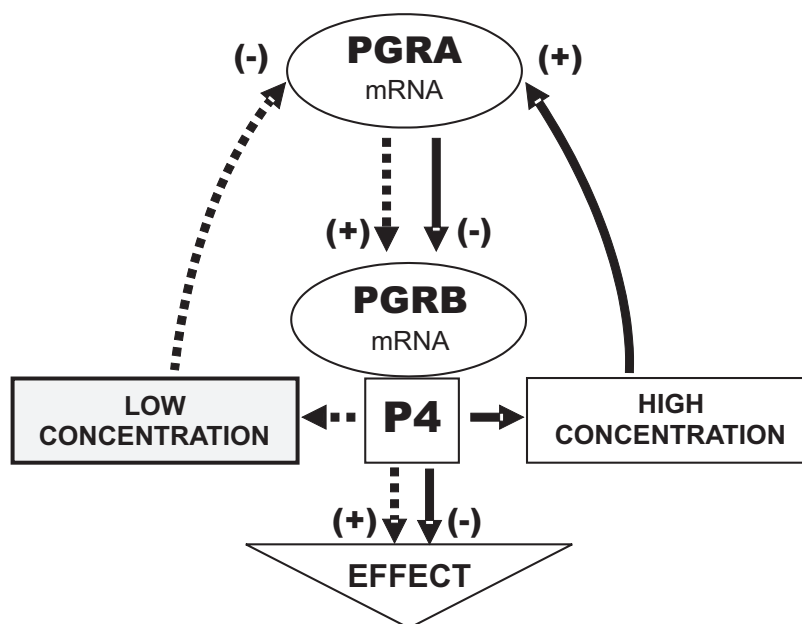


Fig. 3. Schematic representation of the influence of P4 on the mRNA level of PGR isoforms. High impact (black line) and low impact (dotted line) of P4 concentrations. High concentration of P4 induces the expression of mRNA for PGRA, which inhibits mRNA for PGRB transcription followed by the reduction of P4 effect. Low concentrations of P4 suppress the expression of mRNA for PGRA and stimulate transcription of PGRB, which increase the effect by P4.

concentration might suppress the expression of PGRA mRNA followed by the increase in PGRB mRNA transcription. This will induce PGR function and the effect of P4 within the target cell (Fig. 3) (Misao et al. 1998, Rekawiecki et al. 2008). In contrast to the human, there is no data on the expression of mRNA in the monkeys luteal tissue, while the level of PGRB protein expression predominates and remains stable over the level of PGRA protein throughout the duration of the estrous cycle. However, the level of protein expression for PGRA decreases from the highest level in the early phase of the estrous cycle, to the lowest level at the end of the cycle. Changes in the protein level of one of isoforms, increases the ratio of PGRB/PGRA protein expression from early to late stages in the cycle, which suggests the different CL response to P4 action during the estrous cycle (Duffy et al. 1997).

In some animals, the expression of individual isoforms of PGR cannot be distinguished due to the lack of the available sequence of mRNA for PGRB isoforms. The availability of cDNA sequences which contain the common part for both isoforms only allows the determination of the total receptor expression of PGR mRNA. Sakumoto et al. (2010) demonstrated that the total level of PGR mRNA and protein in cattle are the largest at the beginning of the estrous cycle, which next decreases to the lowest level during the late phase of the estrous cycle. This is due to the progressive loss of luteal function of CL because of the increasing effect of prostaglandin F₂ α

which results in functional and structural degradation of CL. When the fertilization of ovum occurs, CL is transformed into the corpus luteum graviditatis in which mRNA for PGR is still expressed. The highest level of PGR was demonstrated in the bovine CL during the first trimester of pregnancy. This is probably due to the effect of P4 that ensures the environment for the fetus development. After the fourth month of pregnancy, CL gradually degrades, resulting in a decline in receptor expression of PGR in the CL graviditatis. The placenta takes over the production of P4 at this time (Tamane et al. 2004).

Abnormal ratio expression of both isoforms results in pathological changes in the reproductive tract. The lower level of mRNA for PGRA and PGRB expression compared with the normal tissue, leads to endometrial cancer. This involves stopping the inhibitory effect of P4 on proliferation effect of E2 followed by uncontrolled proliferation of endometrial cells (Arnett-Mansfield et al. 2001). Breast cancer in humans is associated with impaired PGRA/PGRB ratio, often in favor of PGRA isoform. This results in abnormal response on P4 action and the formation of cancerous tumors (Graham et al. 2005).

In Summary, P4 can affect target cells by both genomic and non-genomic action. This hormone acts mainly through its nuclear receptors which occur in several isoforms, differentiated in their structure. PGRA isoform is shorter than PGRB isoforms by about 164 amino acids and acts as an inhibitor of

transcription of PGRB. As a result of changes in the way of transcription or alternative splicing, new variants of PGR isoforms are formed which regulate the action of PGRA and PGRB isoforms. Changes in the level of mRNA or protein expression of PGR isoforms reflect the influence of P4, which at the moment, is exerted by the hormone to a target tissue. The presence of both isoforms of PGR is therefore dependent on each other, and their correct ratio in normal cells is one of the conditions for the maintenance of the tissue homeostasis.

Acknowledgements

This study was supported by Grants N N311 113638 and N N311 348237 from the Ministry of Science and Higher Education and from Polish Academy of Sciences.

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