Review article

R. REKAWIECKI, M.K. KOWALIK, D. SLONINA, J. KOTWICA

REGULATION OF PROGESTERONE SYNTHESIS AND ACTION IN BOVINE CORPUS LUTEUM

Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

The main function of the corpus luteum (CL) is to synthesize and secrete progesterone (P4), which regulates the duration of the estrous cycle and maintains of pregnancy in many species. Both synthesis and action of this hormone is regulated by many luteotropic and luteolytic factors. Progesterone also affects its own synthesis by regulation of the activity and genes expression of crucial enzymes which control steroidogenesis. The physiological effect of P4 on luteal cells is mediated through the nuclear receptor which occurs in two specific A and B receptor isoforms and also by non-genomic pathways. The nature of non-genomic action of P4 has not been fully understood. It is possible that P4 can temporarily impair binding of oxytocin to its receptor or it can bind one of the three potential membrane receptors. It is assumed that one of these proteins, progesterone receptor membrane component 1 may be involved in regulation of CL function and it can participate in protecting bovine CL against luteolysis. This review summarize the data involving the molecular regulation of P4 synthesis, its intracellular and membrane receptor and the genomic and non-genomic action in the bovine CL.

K e y   w o r d s : *progesterone, corpus luteum, steroid receptor, steroidogenesis*

INTRODUCTION

The corpus luteum (CL) is a transient endocrine gland formed following ovulation from the secretory cells of the follicle. The main function of CL is production of progesterone (P4), which regulates various reproductive functions. Progesterone plays a key role in regulation of the length of estrous cycle and in the implantation of the blastocyst (1). Preovulatory surge of LH is crucial for the

luteinization of follicular cells and CL maintenance, however, CL is less dependent on LH stimulation during the early luteal phase. Since early CL requires luteotropic support for its growth and development, the other factors supports the role of LH to maintain CL development and its functioning. Indeed, it was found that products of luteal origin *i.e.* prostaglandins (PG) I2 and E2, oxytocin, noradrenaline and growth factors efficiently stimulate progesterone synthesis in bovine early CL (1, 2). Thus, it is accepted that though, hormonal and neural signals are critical for normal course of estrous cycle in domestic animals, CL has a broad area of autonomy. Corpus luteum autoregulate synthesis of P4 (3), which in turn, supports its own synthesis, affecting transcription of genes encoding steroidogenic enzymes (4, 5). Moreover, high P4 concentrations in luteal cells protect them against apoptosis, while disruption or impairment of steroidogenesis or reduced ability of P4 production and induced luteal cells death (6). This paper is focused on the molecular regulation of P4 synthesis and action within bovine CL.

MOLECULAR REGULATION OF PROGESTERONE SYNTHESIS IN CL

Cholesterol, which can be derived from the diet or be synthesized *de novo* (7, 8) and transported to the ovaries by lipoproteins (HDL and LDL) is a common precursor for steroids synthesis. Progesterone among others steroid hormones is the most important physiological regulator involved in the CL life span and implantation of the blastocyst. Ovarian steroidogenesis is regulated by several factors playing modulatory role during the estrous cycle. Centrally and locally produced factors modulate expression of genes encoding synthesis of steroidogenic enzymes and that way influence on the secretory function of CL.

The first step of the steroidogenesis occurs in mitochondria. Transport of the cholesterol into the mitochondrion is the rate-limiting step in P4 synthesis. The main protein which is responsible for the transport of cholesterol from the outer to the inner mitochondrial membrane is Steroidogenic Acute Regulatory Protein (StAR). It is synthesized as a 37 kDa protein precursor and processed to the 30 kDa mature protein after crossing mitochondrial membrane (9). Interaction of StAR with the outer mitochondrial membrane results in a conformational changes of protein and creates StAR's cholesterol binding pocket (10). Besides StAR, peripheral benzodiazepine receptor and endozepine, the natural ligand for this receptor also appears to be involved in the regulation of the rate of cholesterol transport (11). Inner mitochondrial membrane is linked with cytochrome P450scc, which is the first component of the enzyme complex that cleaves the side of chain from cholesterol to form pregnenolone. Thereafter, pregnenolone is converted to P4 by 3β-hydroxysteroid dehydrogenase/isomerase (3β-HSD), associated with the smooth endoplasmic reticulum (11).

Luteinizing hormone (LH) is accepted as the most important regulator of lutel steroidogenesis, though this process is also regulated by several others luteotropic factors. Membrane receptors for LH are located mainly in small luteal cells. Binding of LH to its receptor, leads to cAMP-dependent activation of the protein kinase A (PKA) and increases P4 production. The amount of LH receptors varies in the course of the estrous cycle. It is low in early and late days of the estrous cycle and high in mid CL (12). In bovine and human CL LH increases simultaneously the expression of genes encoding StAR, cytochrome P450scc and 3β-HSD synthesis (4, 13).

Involvement of noradrenergic system in ovarian steroidogenesis

Bovine ovaries are supplied with adrenergic nerves, which essentially support steroidogenesis in the granulosa and luteal cells (14). Denervation of ovaries markedly reduced the secretion of ovarian steroids (14). Luteal concentration of noradrenaline (NA) and its precursor - dopamine (DA) varies throughout the luteal phase (15), and CL can synthesize NA from DA (16, 17). The highest amounts of DA and NA were found in the early CL, but they decreased in mid CL and again increased in the late CL. Beta-receptor concentrations, however, in bovine CL was highly correlated with plasma concentrations of P4 during the course of the estrous cycle (18). Stimulation of the ovarian β-receptor in cows by infusion of NA, which mimics of a short-term stress, increased P4 and ovarian OT secretion within a few minutes (19, 20, 21). Furthermore, NA stimulated activity of 3β-HSD and cytochrome P450scc (15) and peptidyl glycine-amidating mono-oxygenase (PGA) (22). PGA is a crucial enzyme involved in post-translational processing of OT synthesis. At the same time, P4 reduced the activity of mono-amino-oxidase and catechol-O-methyl transferase, the enzymes primarily responsible for an intracellular degradation of catecholamines (23). Thus this way, P4 prolongs the half-life of NA and the duration of stimulatory influence on P4 synthesis. However, NA affects neither StAR, cytochrome P450scc and 3β-HSD gene expression nor the level of functional proteins encoding by these genes (4). Therefore we assume that short-term stimulation of CL by NA can rather stimulate P4 secretion only, but not its synthesis. Schematic involvement of some hormones in luteal cell function is presented on *Fig. 1*.

Noradrenaline may also interact in bovine CL with nitric oxide (NO), which is involved in a modulation of NA output and synthesis in vascular tissue (24). Furthermore, NO caused dose-dependent decrease of P4 in human granulosa cells (25). This effect was elicited through the inhibition of cytochrome P450 action (26) and through activation of luteolytic mediators such as prostaglandin (PG) $F_{2\alpha}$ in bovine luteal cells (27). These results were supported by observed decrease of StAR protein, cytochrome P450scc and 3β-HSD genes expression and by the low level of their products in response to NO in bovine luteal cells (4). Changes observed on the gene level were followed by the reduction of P4 secretion from luteal cells and by
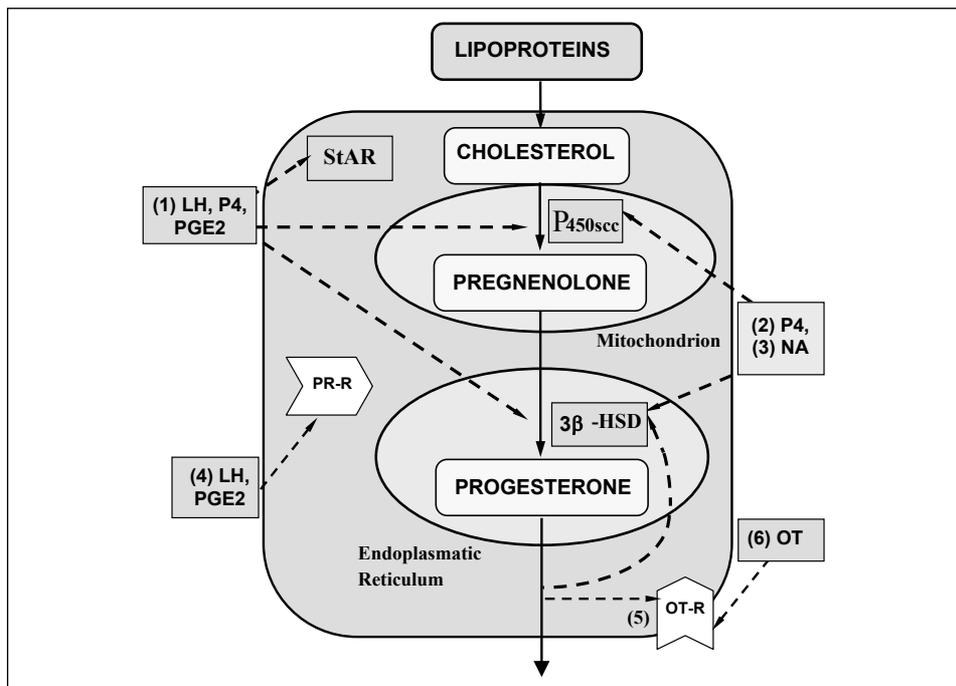
Fig. 1. Molecular regulation of progesterone synthesis in the bovine luteal cell. LH, P4, PGE2 increase the gene expression of StAR, cytochrome P450scc and 3β-HSD, which are crucial enzymes of steroidogenesis (1). Progesterone influences on its own synthesis by increasing of P450scc and 3β-HSD activity (2). Even though noradrenaline (NA) stimulates progesterone secretion and increases P450scc and 3β-HSD activity (3) it does not affect gene expression of these enzymes. LH and PGE_2 (on days 6-10 of the estrous cycle) increases of gene expression of PR-R (4). P4 increases protein level for OT-R only on days 6-10 (5), while OT stimulates of its own gene expression on days 11-16 (6).

apoptosis of these cells (6). However, it was also suggested that NO shows antiluteolytic effect acting as activator of cyclooxygenase pathway followed by increase secretion of PGE2 (26). So, it was assumed that NO may be a luteolytic factor but may also play indirectly, antiluteolytic or luteotropic role in luteal cells, affecting the increase of PGE2 secretion. This effect is suppose to be a dose-dependent, but further studies are needed to elucidate the role of NO in bovine CL.

Progesterone receptor isoforms

Progesterone affects the target cells through the specific receptor (PR) isoforms in CL of human (29) and bovine (3) CL, mouse ovary (30, 31), and rat brain (32) which occurs in A (PR-A) and B (PR-B). Both isoforms are transcribed from the same gene but are controlled by two promoters. Human PR-A is shorter by about 164 nucleotides than the PR-B (33). It was established that PR-B acts mainly as an activator of progesterone-responsive genes, while PR-A acts as a modulator or
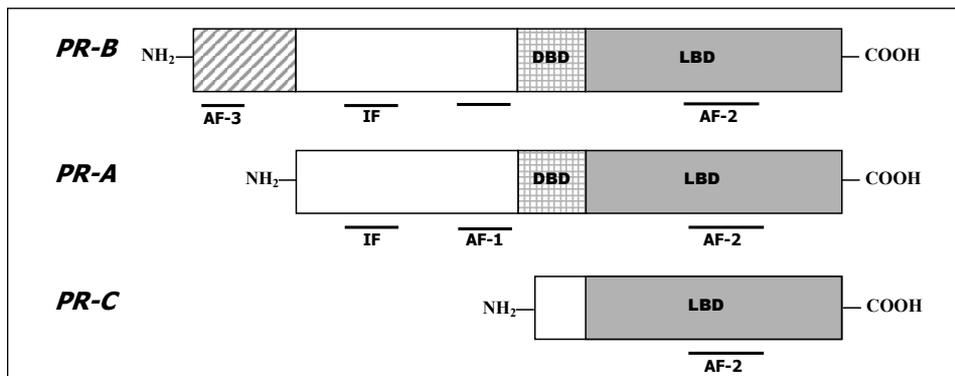
Fig. 2. Schematic representation of human progesterone receptor (PR)-B, PR-A and PR-C isoforms. DBD - DNA binding domain, LBD - ligand binding domain, AF1-AF3 activation domains, IF - inhibitory domain.

repressor of PR-B activity (33). PR-A isoform has also revealed a similar inhibitory effect on other nuclear receptors like glucocorticoid, androgen, and mineralocorticoid receptor-mediated gene transcription (34). This suggests that the ratio of both isoforms (A and B) during the estrous cycle can modulate P4 influence on the function of female reproductive tract. Inactive steroid receptor is located in the cell cytoplasm and connected with heat shock proteins (HSP). After passing the cell membrane, hormone is bound to the receptor, which is subsequently released from HSP and translocates to the nucleus. There, the receptor dimerizes and binds the DNA sequences in target gene promoter called Hormone Responsive Elements (HRE). Next, receptor recruits a number of coactivators or repressors, resulting in enhanced or decreased gene transcription (35).

It is impossible to determine mRNA for PR-A, since its all sequence is a part of mRNA for PR-B (*Fig. 2*). Therefore amount of mRNA for PR-A is showed as a ratio of mRNA expression for PR-B to the total amount of mRNA for PR described as PR-AB together (36). The level of PR-B mRNA in human CL was 100-1000-fold lower than PR-AB mRNA and it was lower in mid luteal phase than in early and late luteal phase (29). The proportion of PR isoforms mRNA concentrations depends on steroid concentrations. It is suggested (36) that a high concentration of P4 within luteal cell induces the expression of PR-A mRNA, which represses the transcription of PR-B mRNA, and as a result PR function and P4 effect is suppressed. On the other hand, a low P4 concentration might suppress the expression of PR-A mRNA followed by the increase of PR-B mRNA transcription. This will induce of PR function and the effect of P4 within target cell (*Fig. 3*). In contrast, the treatment of mouse granulosa cells with RU486, P4 antagonist (30) resulted the down-regulation of both PR isoforms and simultaneous increase of caspase-3 activation, decrease of proliferating cell level and the reduction the rate of ovulation. These results indicate that elimination of PR isoforms are some part
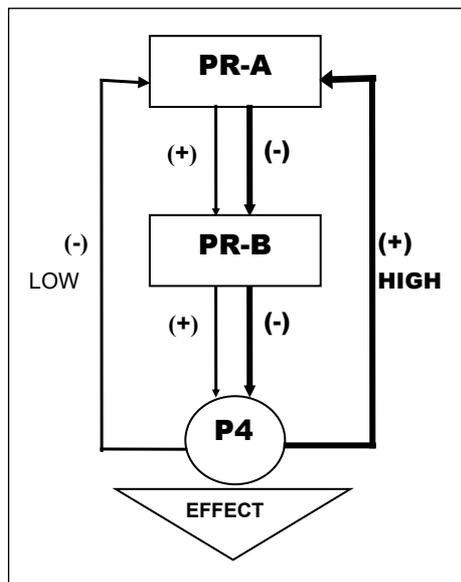
Fig. 3. Schematic representation the influence of high (thick line) and low (thin line) concentrations of P4 on proportion of PR isoforms. High amount of P4 induces the expression of mRNA for PR-A followed by the repression of the transcription of mRNA for PR-B and as a result decline of P4 effect. Low P4 concentration suppresses the expression of mRNA for PR-A and increases transcription of mRNA for PR-B, resulting the enhance of P4 effect.

of inhibitory mechanism of RU486 action upon ovary. It is also assumed that overexpression the one of PR isoform leads to disruption of P4 signalling and may play a role in development and progression of breast cancer (37).

There is also the third PR-C isoform identified in human myometrium (38) or placenta (39). This is N-terminally truncated isoform of PR-C with molecular mass of about 60 kDa and resides in the cytoplasm of the expressing cells. PR-C lacks the first zinc finger of the DNA binding domain but can still bind of the P4 (40). This isoform can be bound to the PR-B isoform thereby reducing the capacity of PR-B to bind transcriptional factors and reducing their transcriptional activity (38).

Autoregulation of progesterone synthesis

Progesterone has also been shown to regulate its own synthesis in CL of sheep (41) and cow (3) and in rat granulosa cells (42). Treatment of bovine luteal slices with P4 increased 3β-HSD activity, with an intensity comparable to LH, whereas P4 antagonist diminished stimulatory effects of P4 on 3β-HSD activity (3). Moreover, P4 also stimulated gene expression for StAR protein, cytochrome P450scc and 3β-HSD on days 6-10 and 11-16 of the estrous cycle (4) and this way increased its own synthesis in luteal cells (3). Increase P4 concentrations in luteal cells protected them from apoptosis, while disruption of steroidogenesis and reduced ability of luteal cells to produce P4 induced cell death (6).

Luteolysis in cows, depends on pulsatile secretion of PGF2α from the uterus (1) and is followed by rapid decrease of P4 synthesis in CL. Soon thereafter, CL undergoes structural regression and cells become apoptotic. There are a few

symptoms of cell apoptosis and the expression of *BAX* and *BCL-2* regulatory genes belong to them (43). High expression of *BAX* gene in CL is associated with the cell death, while high expression of *BCL-2* gene protects the cell from apoptosis (44). Moreover, decrease of P4 synthesis in cells treated with PGF2α causes a marked increase of mRNA for caspase-3, pivotal executor of apoptosis (6). Our data showed also that P4 stimulates the expression of anti-apoptotic bcl-2 mRNA on days 6-15 of the estrous cycle in the cow and decreases the ratio of bax/bcl-2 gene transcription. Whereas decrease of caspase-3 activity was observed in luteal cells treated with P4 (6).

Prostaglandin E_2 (PGE$_2$) synthesized in CL is also an important luteotrophic/antiluteolytic factor. It prevents apoptosis of luteal cells, supposedly by the support of luteal P4 production, which increases of bcl-2 gene expression. Similar mechanism is turned on by LH, resulted in increase P4 concentration in medium but do not affect bcl-2 gene expression (6). The highest production of PGE$_2$ was observed in the early CL (45), suggesting the possibility of neutralization of PGF2α action and increase of P4 synthesis. On the other hand, P4 stimulates PGE$_2$ secretion from bovine CL suggesting that there is a positive feedback loop between P4 and luteal PGE$_2$ during the early luteal phase of the estrous cycle in cow (46). It is worthy to notice that PGE$_2$ stimulates steroidogenesis like LH does. Binding of PGE$_2$ to specific receptors causes an increase of cyclic AMP followed by an activation of protein kinase A (PKA), which subsequently phosphorylates the regulatory proteins and affects the transcription of selected genes (47). Therefore, the high luteal concentration of PGE$_2$ stimulates StAR protein, 3β-HSD and cytochrome P450scc gene expression and increases of their protein products (4), that leads to an increase of P4 synthesis.

Changes of gene expression encoding hormone receptors in the luteal cells varies in course of the estrous cycle and depend on the intensity of luteal steroidogenesis. Our studies revealed that LH stimulates the abundance of mRNA for oxytocin receptor (OT-R) in luteal cells from days 6-10 of the estrous cycle, and the abundance of mRNA for P4-R in luteal cells from days 6-16 of the estrous cycle. Since P4 can stimulate its own synthesis, this would suggests that LH amplifies this process. However, P4 alone was not able to affect the expression of the gene for its own receptor on either 6-10 or 11-16 days of the estrous cycle, but enhanced the transcription of the OT-R gene. These relationship between transcription of PR-R and OT-R gene suggests that there is a positive feedback mechanism between these hormones, and that each of these hormones play as a local, intra-ovarian factor that improves the function of the CL (3, 5). We have also looked for some relationship between OT and β$_2$-receptor (β$_2$-R) stimulation in CL (5). It was found that transcription of OT-R gene during the estrous cycle was the highest at the beginning of the estrous cycle, declined in mid cycle and rised again toward the end of the estrous cycle. The β$_2$-R gene transcription was also highest at the beginning of the estrous cycle, however, continuously decreased and reminded the lowest values at the end of the estrous cycle.
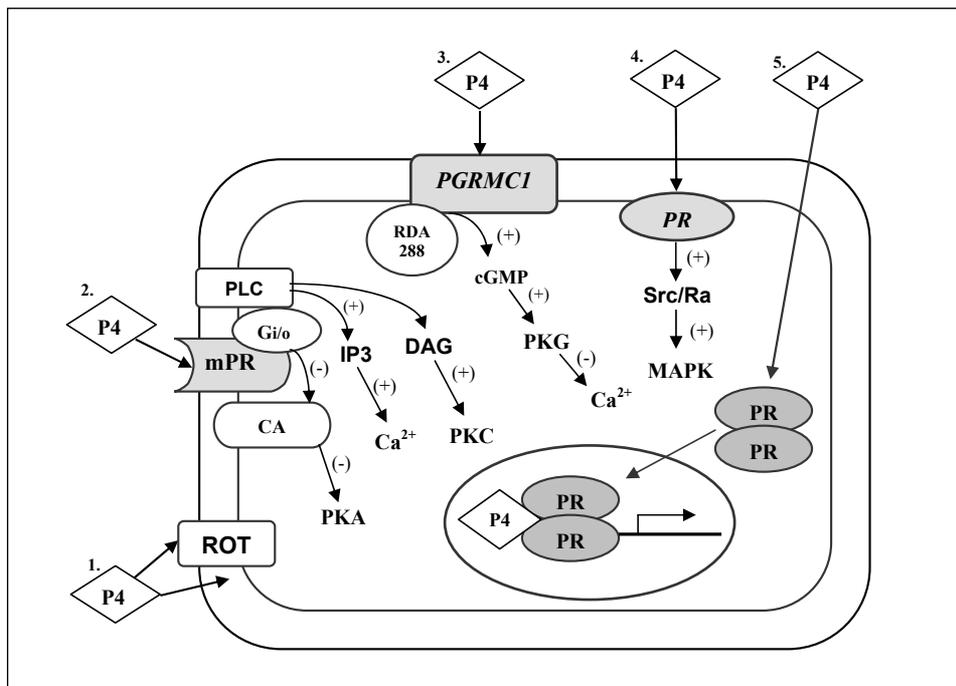
Fig. 4. Non-genomic (1-4) and genomic (5) pathways of progesterone (P4) action on luteal cells: 1. influence of P4 on oxytocin receptor (OTR) or permeability of cell membrane and this way affect the affinity of OT to its receptor, 2. P4 binding with membrane progestin receptor (mPR), 3. P4 stimulation of progesterone membrane component 1 (PGRMC1) or complex of PGRMC1/RDA 288 protein, 4. effect of P4 on intracellular progesterone receptor (PR) localized near cell membrane, or 5. P4 activation nuclear progesterone receptor (PR).

Surprisingly, the highest protein level encoded by these two genes is accompanied by the lowest transcription of these genes. The results suggest that OT-R takes part in both luteotropic and luteolytic processes, while β_2-R may be involved mainly in the formation of a newly-formed CL.

Concluding, LH plays a key role in both CL formation in the place of ruptured follicle and its functioning throughout the estrous cycle. However, a lot of factors produced locally within CL are also essentially involved in P4 synthesis as an auto-paracrine factors.

MOLECULAR MECHANISM OF PROGESTERONE EFFECT IN CL

Non-genomic influence of progesterone on the target cell

Except the physiological effects of P4 mediated through interaction with specific nuclear progesterone receptor (PR-A and PR-B) on the target cell (48),

P4 can also elicit the cell response within seconds or minutes which is too short to activate the genomic way (*Fig. 4*). The non-genomic effect of P4 has been found in the number of tissues from the female reproductive tract (49-53) including cows (54-61). The nature of this mechanism has not been fully understood. It is possible that P4 may temporarily modify or impair binding of some ligands to their membrane receptor (49, 53, 54), or that P4, as lipophilic substance, can change permeability of cell membrane and this way affect the affinity of other ligands to their membrane receptors (62, 63). Presumably, P4 can be bound by specific membrane receptors (52, 55, 64-66). At least three different proteins, which can be potential membrane progesterone receptor: membrane progestin receptor (mPR), RDA 288 protein and progesterone membrane receptor component 1 (PGRMC1) (52, 66).

Membrane progesterone receptor

Membrane progestin receptor (mPR) has characteristic seven transmembrane domain and activates an inhibitory G protein. It suggests that these proteins may be G-protein coupled receptors (GPRC)s (67). For the first time GPRC was isolated from the sea trout ovary, but it was showed also in different tissues of human, pig, mouse (67, 68), sheep (69) and rat (70). There are known three izoforms of this receptors: mPRα, mPRβ, mPRγ (67). Expression of mPR is highly tissue-specific. Expression of mPRα subtypes in human was found mainly in reproductive system, mPRβ was found in nervous system and mPRγ in digestive system (67). Similar tissue distribution was reported for mPRβ and mPRγ mRNA in rat (70). Expression of mPR in sheep was observed in reproductive system, as well as in hypothalamus and pituitary. Localization of mPR isoforms in animals suggests that these receptors may participate in control of female reproductive function (69).

The mechanism of P4 action *via* mPR involves a decrease of tissue adenylate cyclase activity or increase of MAP kinase activity in target cells (52, 67). It is suggested that decrease of intracellular cAMP would suppress steroidogenesis, while MAPK activation could be a part of apoptotic mechanism in many rat cell types (52). Therefore, P4 acting *via* mPR can increase cell apoptosis in different organs.

A second potential membrane progesterone receptor is RDA 288 protein called also plasminogen activator inhibitor RNA binding protein (PAIRBP1). Its expression was showed in rat ovarian follicle and luteal cells (52) as well as in human granulosa and luteal cells (71). It is proposed that PAIRBP1 can take part in the anti-apoptic influence of P4 in granulosa cells (52, 71). PAIRBP1 may bind to a transmembrane protein PGRMC1 and form a P4 receptor-membrane complex (72, 73). This complex is localized in the extracellular surface of the cell membrane and takes part in anti-apoptic and anti-mitotic action of P4 (72, 73). Moreover, it was determined that activation of this complex by P4, increased the level of cAMP and activities protein kinase G (PKG). This leads to the reduction

of intracellular Ca^{2+} concentration in the cell and this way the receptor-membrane complex can participates in anti-apoptotic effect of P4, as observed earlier in granulosa cells (52, 71).

Another membrane receptor is PGRMC1 protein, which was isolated for the first time from pig liver (74). Expression of PGRMC1 was also detected in preovulatory mouse follicles (75), porcine granulosa cells (76), rat granulosa and luteal cells in rat (52, 70) and human (77). Moreover, this receptor is also localized in the endoplasmic reticulum and in Golgi apparatus (52, 78, 79). It was found that the expression of PGRMC1 protein is regulated by P4 in the brain regions involved in the female reproductive behavior (80). Overexpression of PGRMC1 in Chinese hamster ovary cells increased P4 binding to the cell membrane (52). In ovarian cells of rats overexpression of ovarian PGRMC1 enhanced P4 responsiveness, while PGRMC1 antibody blocked the antiapoptotic action of P4 (52). These data do not allow to specify the role of PGRMC1 precisely. It is assumed that PGRMC1 may be involved in the regulation of luteal function affecting the transcription of genes responsible for anti-apoptotic processes and the synthesis of proteins which participate in the cells cross-talk (70, 71). The possible mechanism of PGRMC1 action may involve an activation of various kinases after P4 binding with the SH2 and SH3 receptor domains. Moreover, it is suggested that activation of protein kinase G (PKG) and phosphorylation of numerous proteins can be the first sign of intracellular events associated with the ligand activation of PGRMC1 (52).

Recently, we have found that P4 can affect the function of bovine endometrial and luteal cells without modulation of transcription, because the actinomycin D (blocker of transcription process) did not change the effect evoked by P4 (54, 58). We also found that P4, its precursor-pregnenolone (P5) and metabolite-17β-hydroxyprogesterone (17βOHP4) suppressed the influence of OT on the secretion of PGF2α, but not PGE2, from bovine endometrial cells and reduced $[Ca^{2+}]$ release from this cells *via* non-genomic pathway (54, 57, 59). Moreover, we observed that P4 reduced $[Ca^{2+}]$ release from bovine luteal (56) and myometrial cells (58) after short time (30-240 min) of preincubation of the cells in the present of this steroid. This inhibitory effect of P4 appeared after short-term culture, indication non-genomic mechanism of P4 action. It is also possible that the non-genomic effect of P4 observed in bovine endometrial and luteal cells was evoked partly *via* membrane progesterone receptor. The results of our studies demonstrated the expression of PGRMC1 mRNA in bovine CL (61) and endometrial cells (Kowalik and Kotwica - unpublished data). PGRMC1 mRNA is expressed during CL development (61). This data suggest that PGRMC1 protein may be involved in non-genomic mechanism of P4 action on luteal and endometrial cells function in cow (56, 58, 60). But it may also participate in the regulation of luteal function affecting transcription of genes responsible for anti-apoptotic processes and synthesis of proteins which play a role in regulating cell

survival through a cell-contact-mediated mechanism (70, 71). It is also possible that PGRMC1may have influence on regulation of steroids synthesis (81).

The data from the presented studies indicate that P4 and other steroids affect the target cells by both genomic and non-genomic mechanism. It is suggested that non-genomic pathway of steroids influence on the cell can inhibit secretion of luteolytic $PGF_{2\alpha}$ and this way support action of PGE_2 and further CL function. Therefore, it is possible that non-genomic action of P4 on endometrial secretion of $PGF_{2\alpha}$ and PGE_2 is the one more mechanism involved in the maintenance of an early pregnancy (59).

Conflicts of interest statement: None declared.

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Author's address: Dr Robert Rekawiecki, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, ul. Tuwima 10, 10-740 Olsztyn; Phone: 89-539 3117, Fax: 89-539 3146; e-mail: Robert@pan.olsztyn.pl