Progesterone Mediates Its Anti-Mitogenic and Anti-Apoptotic Actions in Rat Granulosa Cells Through a Progesterone-Binding Protein with Gamma Aminobutyric Acid Receptor-Like Features

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ABSTRACT

Progesterone (P₄) inhibits small granulosa cell (GC) mitosis and large GC apoptosis. These actions are steroid specific and dose dependent and are inhibited by the progesterone receptor (PR) antagonist, RU-486. However, these cells do not express the nuclear PR but rather an ill-defined P₄-binding protein (P₄BP). This binding protein could function as a receptor and mediate P₄'s actions in GCs. Therefore, a series of studies was designed to characterize this P₄BP. First, an antibody directed against the ligand-binding site of the nuclear PR was used in a Western blot analysis. This analysis revealed the presence of a 60-kDa P₄BP within ovarian and GC lysates as well as within an ovarian membrane preparation. This protein was not observed in lysates of cells derived from the ovarian surface epithelium. In addition, this P₄BP was immunoprecipitated by an antibody to the alpha, chain of the gamma aminobutyric acid (GABAₐ) receptor, suggesting that the P₄BP could be the ovarian GABAₐ receptor. Since activation of the rat ovarian GABAₐ receptor increases intracellular cAMP levels, GCs were cultured with control medium supplemented with either 8-bromo-cAMP (8-br-cAMP), P₄, or muscimol (a GABA agonist). Increases in cAMP were detected by monitoring the cAMP-dependent phosphorylation of cAMP response element-binding protein (CREB). Phosphorylated CREB was not observed in control or P₄-treated cultures, but it was detected in the majority of both small and large GCs exposed to either 8-br-cAMP or muscimol. Since activation of the GABAₐ receptor with muscimol increases phosphorylated CREB but P₄ does not, this study indicates that P₄ does not activate the ovarian GABAₐ receptor. However, both bicuculline, a GABA antagonist, and the antibody to PR inhibited P₄'s ability to prevent both insulin-dependent mitosis and apoptosis. Collectively, these studies suggest that P₄ mediates its anti-mitotic and anti-apoptotic effects through this 60-kDa P₄BP, which has GABAₐ receptor-like properties and is localized within the surface membrane of GCs.

INTRODUCTION

The concept that progesterone (P₄) has an intraovarian site of action has been proposed for nearly 20 yr [1]. The data that support this concept come from both in vivo and in vitro experiments. In immature rats induced to ovulate with eCG and hCG, the 3α-hydroxysteroid dehydrogenase inhibitor, epoestane, reduces the number of ovulations [2]. Since epoestane could not have altered gonadotropin levels, these results suggest a direct intraovarian site of action for P₄. Similar studies have been also conducted in primates [3, 4]. In addition to having a role in ovulation, P₄ inhibits gonadotropin-induced follicular development in hypophysectomized hamsters [5], suggesting that P₄ directly affects granulosa cells (GCs) of developing follicles.

Numerous studies have demonstrated P₄'s direct effects on GC function in vitro. For example, P₄ enhances the ability of cultured rat GCs to respond to FSH with increased production of cAMP [6]. Further, the synthetic progestin, R5020, increases FSH- and LH-induced P₄ secretion in vitro [7]. In contrast, progestins inhibit FSH-stimulated estrogen production by cultured rat GCs [7–10]. Along with modulating steroidogenesis, P₄ suppresses insulin-dependent small GC mitosis [11, 12] and prevents large GCs from undergoing apoptosis [13, 14]. Both the anti-mitogenic and anti-apoptotic action of P₄ are dose dependent and steroid specific and are inhibited by the progesterone receptor (PR) antagonist, RU-486. In addition, P₄'s actions are not mimicked by dexamethasone [11], indicating that they are not mediated by the ovarian glucocorticoid receptor.

In spite of this biological evidence, a direct role for P₄ in regulating rat GC function still remains controversial. This is primarily due to the inability to detect PR mRNA or protein within GCs until just prior to ovulation [15–18]. Although PR mRNAs were not detected by Northern blot hybridization, in situ hybridization, or reverse transcription-polymerase chain reaction, the immature rat ovary expresses a protein that specifically binds P₄ [1, 19, 20]. This P₄-binding protein (P₄BP) is present within the cytosol/membrane fraction prepared from GCs of developing follicles. It is possible, then, that this P₄BP could function as a membrane receptor for P₄. If so, this putative membrane PR could possess the same P₄-binding site as the nuclear PR but not share any other sequence homology. To test this hypothesis, an antibody, directed against the P₄-binding site of the nuclear PR, was used to search for this putative membrane PR. The present studies identify a 60-kDa protein that is localized to the surface membrane of GCs and is involved in mediating P₄'s anti-mitogenic and anti-apoptotic action.

MATERIALS AND METHODS

Animals

Immature female Wistar rats (22 days of age) were obtained from Charles River Laboratory (Wilmington, MA) and housed under controlled conditions of temperature, humidity, and photoperiod (12L:12D; lights-on at 0700 h). On the day of the experiment, immature animals were 23–25 days of age. In some experiments, immature animals (23 days of age) were injected i.p. with 20 IU of eCG. All rats were cervically dislocated between 0930 and 1000 h. The ovaries were removed, and either ovarian lysates were prepared for Western blot analysis or GCs were isolated.

A spontaneously immortalized GC line (SIGCs) and a rat ovarian surface epithelial cell line (ROSE-179) were also used in these studies. Both cell lines were provided by Dr. Robert Burghardt (Texas A & M University, College Station, TX). These cells were cultured as previously de-
scribed [21, 22]. Cell lysates were prepared from these cells and subsequently analyzed by Western blot.

**Western Blot Analysis**

Ovarian lysates were prepared from either fresh or frozen ovaries. GCs were isolated from the ovaries as described previously [23], and SIGCs and ROSE-179 cells were harvested from culture dishes. The cells were then centrifuged at 200 × g for 10 min. Homogenization buffer (50 mM Tris, pH 7.4, 1 mM EDTA, 2 mM PMSE; 2 mM sodium vanadate) was added to the ovarian tissue or cell pellet, and the tissue/cells were homogenized in a glass homogenizer. The lysates were centrifuged at 13 000 × g for 4°C for 5 min, and the supernatant was removed and stored at −20°C.

Protein concentration for each lysate was determined by the Bradford method, and equal amounts of protein were loaded onto a 10% polyacrylamide gel and electrophoresed at 100 V. Proteins were transferred to nitrocellulose and then incubated with Tris-buffered saline with 0.1% Tween 20 and 5% dry milk for 1 h. The nitrocellulose was incubated with either an antibody to the PR (1:500, C-262; StressGen, Victoria, BC, Canada) or an antibody to the alpha_1_ chain of the gamma aminobutyric acid_A (GABA_A) receptor (1:500; Boehringer-Mannheim, Indianapolis, IN). The nitrocellulose blot was washed twice with Tris-buffered saline and incubated with a 1:25 000 dilution of a peroxidase-labeled goat anti-mouse IgG (Kirkegaard and Perry, Gaithersburg, MD). The P₄BP and GABA_A receptors were detected by chemiluminescence using the detection system for P₄BP.

For quantitative analysis, the gels were scanned and analyzed using IP Lab Gel software (Signal Analytical, Vienna, VA). Density measurements were taken from both the Western blot and the negative control lane. The values from the negative control were subtracted from the values of the Western blot. This mixture was then centrifuged at 13 000 × g for 5 min and washed three times with single-strength immunoprecipitation buffer. The pellet was resuspended in 20 μl of loading buffer, boiled for 5 min, centrifuged, and loaded onto a 10% polyacrylamide gel. The proteins were then transferred to nitrocellulose and probed with the PR antibody using the Western blot procedure described above.

**GC Isolation and Culture**

GCs were isolated according to the procedure of Lederer et al. [23]. Ovaries were placed in Medium 199 containing 0.2% BSA and 9.1 mM EGTA (pH 7.4). The follicles were punctured with fine needles and then incubated for 5 min at 37°C in 5% CO₂ in air. The ovaries were transferred to Medium 199 containing 0.2% BSA, 2.1 mM EGTA, and 0.5 mM sucrose (pH 7.4) and incubated for 10 min at 37°C in 5% CO₂ in air. The ovaries were transferred to fresh Medium 199 containing 0.2% BSA and presssed to release the GCs. This GC preparation was used in the various experiments described except for those studies involving mitosis or apoptosis. For these studies, small and large GC populations were isolated by Percoll gradient centrifugation as previously described [23]. Small GCs were collected from fractions 3 and 4, while large GCs were isolated from fractions 6 and 7. The GCs were washed and resuspended in RPMI-1640. Small GCs were plated in 35-mm dishes for mitosis studies, and large GCs were plated in Lab-tek 8-chamber slides (Nunc, Naperville, IL) for apoptosis experiments.

**Preparation of Culture Medium and Reagents**

RPMI-1640 without phenol red (Gibco Laboratories, Grand Island, NY) was used in all culture experiments. This medium was supplemented with penicillin (0.14 g/L), streptomycin (0.27 g/L), Heps (4.76 g/L), BSA fraction V (2 g/L), sodium selenite (5 ng/ml), sodium bicarbonate (2.2 g/L), and transferrin (5 μg/ml). The pH was adjusted to 7.3, and the medium was filtered through a 0.2-μm filter. Insulin (5 μg/ml; Sigma Chemical Co., St. Louis, MO), P₄ (200 ng/ml; Sigma), PR antibody (10 μg/ml; C-262; StressGen), and bicuculline (10 μM; Sigma) were added to cultures depending on the experimental design. P₄ was made as stock solution in ethanol at 1 mg/ml and then diluted in RPMI-1640 to the desired concentration.

**Assessment of GC Mitosis and Apoptosis**

**Mitosis.** Small GCs were plated at a density of 1.5–2 × 10⁵ cells/ml of RPMI-1640 and incubated at 37°C in 5% CO₂ in air with RPMI-1640 supplemented with either 1) insulin, 2) insulin and P₄, or 3) insulin, P₄, and PR antibody. In some experiments, the effect of insulin and P₄ was assessed in the presence of IgG. This served as a control for the nonspecific effects of the PR antibody. After 2 h, the media were replaced in order to remove any nonattached GCs. The number of remaining GCs was counted within 8 different 160-μm² grids within the 35-mm dish [11, 12]. The grids were located at the ends of horizontal and vertical axes and at the center of each dish. After 24 h of culture, the number of GCs present in these same areas was determined. Cell proliferation was expressed as the percentage increase in cell number over 2-h control values. A similar study was conducted in which the ability of bicuculline to block P₄'s anti-mitotic action was assessed.

**Apoptosis.** Large GCs were plated in 8-chamber Lab-tek slides at plating density of 7 × 10⁴ cell/400 μl. GCs were
cultured in RPMI-1640 (control) supplemented with P4 or P4 plus PR antibody. To assess the nonspecific effects of the PR antibody, additional studies were conducted in which the effect of P4 was determined in the presence of IgG. Cells were incubated for 24 h at 37°C in 5% CO2 in air and then stained with hydroethidine (14 μg/ml of PBS; Polysciences, Warrington, PA) [13]. The percentage of apoptotic single cells was counted under epifluorescence as previously described [13].

Detection of cAMP Response Element-Binding Protein (CREB) and Phosphorylated CREB

GCs were plated in 8-chamber Lab-tek slides at a density of 2 × 10⁵ cells/400 μl of RPMI-1640, which was supplemented with 10% FBS. After a 3-h incubation at 37°C in 5% CO2 in air, the cells were washed three times with serum-free RPMI-1640 and treated with P4 (200 ng/ml; Sigma). The cells were then incubated for an additional 30 min. GCs were washed three times with PBS and fixed in 4% paraformaldehyde for 30 min at 4°C. The cells were stained for CREB and phosphorylated CREB using the PhosphoPlus CREB Antibody kit from New England BioLabs (Beverly, MA) and the HistoMark streptavidin-horseradish peroxidase system from Kirkegaard and Perry. The cells were stained using a brown/orange stain. For each trial, a sample was stained in the absence of a primary antibody. This served as a negative control.

Statistical Analysis

All experiments were repeated two to three times. Those experiments that involved the assessment of mitosis or apoptosis were run in duplicate and replicated at least three times. These data were evaluated by an ANOVA followed by a Student-Newman-Keuls test; p values ≤ 0.05 were considered to be significantly different.

RESULTS

Using an antibody directed against the P4-binding site of the nuclear PR, Western blot analysis revealed the presence of a 60-kDa protein in the ovaries and GCs of immature rats (Fig. 1A). This protein was also detected within SIGCs (Fig. 1A) but not in ROSE-179 cells (data not shown). Treatment with eCG only slightly altered the expression of this protein, increasing the levels by approximately 140% at 24 h and decreasing the levels to 80% of initial values at 48 h after treatment (Fig. 1B). The 60-kDa protein was also detected within the membrane fraction prepared from ovarian lysates (Fig. 2A).

To determine whether this protein was capable of binding P4, an ovarian lysate was divided into equal aliquots. In one aliquot, the Western blot procedure was modified such that the dry-milk blocking solution was supplemented with P4 (0.32 μM). The other aliquot was processed with the standard dry-milk blocking solution, which was supplemented with 12% ethanol. A comparison of the Western blots revealed that the P4 supplement reduced the amount of P4BP detected by Western blot by ~80% (Fig. 2B). Further, supplementing the blocking solution with 0.32 μM dexamethasone, estradiol-17β, testosterone, 5α-pregnane-3α-ol-20-one, or 5α-pregnane-3β-ol-20-one did not impair the ability of the PR antibody to detect the 60-kDa protein (Fig. 3). These data indicate that the 60-kDa protein binds P4 in a steroid-specific manner.

The ability of this antibody to attenuate P4's anti-mitotic and anti-apoptotic action was also assessed. The mitotic studies confirm previous work demonstrating that insulin induces GC proliferation and P4 blocks insulin's action.

![FIG. 1. Expression of a P4BP as detected by Western blot analysis. Lysates from immature ovary (Ovary), granulosa cells (GC) isolated from immature oocytes, or spontaneously immortalized granulosa cells (SIGC) were prepared. A) A P4BP of ~60 kDa was detected in each lysate using an antibody directed against the P4-binding domain of the nuclear PR. B) Levels of this P4BP were slightly increased (~140%) after 24 h of eCG stimulation.](image)

![FIG. 2. The membrane localization (A) and P4-binding ability (B) of the 60-kDa P4BP. A) Western blot of a lysate prepared from an ovarian membrane fraction. Note that the 60-kDa P4BP is detected in this membrane preparation. B) To confirm that this 60-kDa protein binds P4, lysates from the same sample were electrophoresed, transferred to nitrocellulose, and then incubated with a buffered 5% dry-milk solution (Cont.) supplemented with progesterone (Prog.). The control was supplemented with 12% ethanol. A comparison of the Western blots revealed that the P4 supplement reduced the amount of detectable P4BP by ~80%.

![FIG. 3. The P4-binding specificity of the 60-kDa P4BP. A single lysate was run using a preparative comb. The proteins were then transferred to a single sheet of nitrocellulose. The nitrocellulose sheet was cut into strips, and each strip was incubated with a dry-milk blocking solution that was supplemented with either 12% ethanol (EtOH), progesterone (Prog), 5α-pregnane-3β-ol-20-one (5α3β), 5α-pregnane-3α-ol-20-one (5α3α), dexamethasone (Dex), estradiol-17β (Est), or testosterone (Test). All steroids were used at a concentration of 0.32 μM. A negative (Neg) control was also run in which the primary antibody was omitted. Note that only the P4 treatment reduced the amount of P4BP detected.](image)
was plated in 8-chamber Lab-tek slides in RPMI-1640 (control) supple-
over the 2-h control value. To assess apoptosis, the large
small
gation. The populations were approximately 80% pure. An increase in
populations of either small or large GCs using Percoll gradient centrifu-
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dition, the PR antibody did not affect the ability of basic
observed in the presence of IgG (data not shown). In ad-
(Fig. 4B). P 4 's anti-mitotic and anti-apoptotic actions were
neutered P 4 's ability to block insulin-induced mitosis
[45x404]added, one of these being ~60 kDa (marked by an arrow). This band can
best be seen in the densitometry trace. B) When the ovarian lysate was
immunoprecipitated (IP) with the antibody to the GABA A receptor and
then probed with the antibody to the PR, a 60-kDa band was detected.
This indicates that the 60-kDa P 4 BP shares some immunological charac-
teristics with the GABA A receptor. The identity of the 69-kDa protein is
not known.

FIG. 4. The effect of P 4 and PR antibody on insulin-induced mitosis (A)
and apoptosis (B). For these studies, GCs were separated into enriched
populations of either small or large GCs using Percoll gradient centrifug-
[44x580]lution. For these studies, GCs were separated into enriched
populations of either small or large GCs using Percoll gradient centrifu-
[45x457]
lyzed with P 4 and/or the PR antibody. After 24 h, the percentage of
apoptotic single cells was determined. In some mitosis and apoptosis ex-
periments, both control and treatment groups were supplemented with
the appropriate amounts of IgG. The presence of IgG did not alter the
results of these experiments.

[11, 12, 25]. The addition of the PR antibody completely neutralized P 4 's ability to block insulin-induced mitosis
(Fig. 4A). Similarly, the PR antibody attenuated P 4 's capacity to prevent large GCs from undergoing apoptosis
(Fig. 4B). P 4 's anti-mitotic and anti-apoptotic actions were
observed in the presence of IgG (data not shown). In addition, the PR antibody did not affect the ability of basic
fibroblast growth factor to prevent large GC apoptosis (data
not shown).

In sperm, the effects of P 4 can be influenced by GABA A receptor antagonist, bicuculline [26–28]. Bicuculline also
blocked P 4 's anti-mitotic and anti-apoptotic action in GCs
(Fig. 5, A and B). The alpha, chain of the GABA A receptor,
a 50-kDa protein [29, 30], was detected within the immature
rat ovary by Western blot. In addition to this 50-kDa protein, the GABA A receptor antibody detected minor pro-
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tein A, B, and C forms of the nuclear PR [34–36]. However,
this 60-kDa protein is not the A (~90 kDa) or B (~120
kDa) form of the PR [37]. It is also unlikely that this 60-
kDa form is the C form of the PR, although the two proteins
have similar molecular masses [34–36]. The C form of the
PR is generally absent in PR-negative cells and requires
high-salt conditions for nuclear extraction and subsequent
[44x383]FIG. 5. Effect of P 4 and bicuculline on insulin-induced small GC mitosis
(A) and large GC apoptosis (B).

GABA increases the intracellular levels of cAMP [31],
which ultimately induces the phosphorylation of CREB
[32]. To determine whether P 4 activates the ovarian GABA A receptor, the effect of various agents on the
phosphorylation of CREB was assessed immunocytochemically.
As shown in Figure 7E, both small and large cells expressed CREB. Phosphorylated CREB was not detected in
small or large GCs under control culture conditions (Fig.
7A). As expected, both 8-br-cAMP (Fig. 7B) and musci-
mol, a GABA agonist (Fig. 7D), induced the phosphory-
lation of CREB in small and large GCs. However, P 4 did
not induce the phosphorylation of CREB in either size of
GC (Fig. 7C). In addition, two P 4 metabolites that are
known to activate the GABA A receptor, 5a-pregnan-3a-ol-
20-one and 5a-pregnane-3β-21-diol-20-one [33], did not inhib-
the detection of the 60-kDa P 4 binding protein (Fig. 3).

DISCUSSION

On the basis of previous ligand-binding studies, various
investigators have predicted that GCs of immature and de-
veloping follicles would express a protein that specifically
binds P 4 and functions as a PR [1, 19, 20]. By using an
antibody directed against the P 4-binding domain of the nuclear
PR, the present study identifies such a protein. This protein has
a molecular mass of 60 kDa and is expressed at relatively
constant levels during eCG-induced follicular development.
Further, this protein is expressed in GCs but not in cells
derived from the rat ovarian surface epithelium, demonstrat-
ing ovarian cell-type specificity.

Since this 60-kDa protein was detected using an anti-
body directed against the P 4-binding domain of the nuclear
PR, it appears that it possesses the same P 4-binding site as
the A, B, and C forms of the nuclear PR [34–36]. However,
this 60-kDa protein is not the A (~90 kDa) or B (~120
kDa) form of the PR [37]. It is also unlikely that this 60-
kDa form is the C form of the PR, although the two proteins
have similar molecular masses [34–36]. The C form of the
PR is generally absent in PR-negative cells and requires
high-salt conditions for nuclear extraction and subsequent
detection was observed only with P₄ and not with several P₄ BP detected by Western blot. This suppression of P₄ BP is demonstrated by supplementation of the dry-milk blocking solution with P₄ prior to Western blot analysis. This experiment showed that supplemental P₄ reduces the amount of P₄ BP detected by Western blot. This suppression of P₄ BP detection was observed only with P₄ and not with several other steroids, thereby demonstrating steroid specificity. It is assumed that the reduced ability of the PR antibody to detect the 60-kDa protein is due to the binding of the supplemental P₄ binding to a P₄-binding domain within the 60-kDa protein. As a result, the PR antibody cannot bind to this site within the 60-kDa protein.

While the classic PRs are localized to the nucleus [36, 37], the 60-kDa binding protein is localized to the surface membrane. The membrane localization of this 60-kDa protein was confirmed by the two different experimental approaches. First, the 60-kDa binding protein was detected in an ovarian membrane preparation. Although containing the membranes associated with cytoplasmic organelles, this preparation is enriched with plasma membranes. Second, P₄'s biological actions were inhibited by PR antibody. Since the PR antibody cannot enter nonpermeabilized GCs, it is likely that it interferes with P₄'s actions by binding to the P₄-binding site of the 60-kDa protein that is apparently present on the extracellular surface of the plasma membrane.

Along with a limited immunological similarity to the nuclear PRs, this 60-kDa protein also has immunological characteristics of a GABA receptor. The present studies show that this protein is immunoprecipitated by an antibody directed against the alpha₁ chain of the GABA receptor. In addition, the GABA receptor antagonist, bicuculline, attenuates P₄'s anti-mitotic and anti-apoptotic action. However, P₄ does not mediate its action through the ovarian GABA receptor, since the GABA receptor agonist muscimol, but not P₄, stimulates the phosphorylation of CREB. Finally, neither 5α-pregnan-3α-ol-20-one nor 5α-pregnane-3β,21-diol-20-one interferes with P₄ binding to the 60-kDa P₄ BP. Since both of these P₄ metabolites bind to and activate the GABA receptor [33], the inability to compete with P₄ for the binding site within the 60-kDa P₄ BP suggests that the 60-kDa P₄ BP is not the GABA receptor. Rather, it appears that the P₄ BP has some but not all the features of a GABA receptor. P₄ likely mediates its action via this P₄ BP whose action can be modulated by the GABA antagonist, bicuculline. The mechanisms through which bicuculline interacts with the P₄ BP remain to be determined.

The signal transduction pathway that is activated by P₄ binding to this putative membrane receptor remains to be determined. In some cell types, P₄ increases intracellular levels of cAMP [38, 39]. In GCs, P₄ does not act in this manner, since it does not enhance the phosphorylation of CREB. P₄ has also been shown to stimulate a transient increase in intracellular calcium [2, 33, 40]. Although this cannot be ruled out, prolonged treatment with P₄ does not increase intracellular calcium levels within GCs but rather maintains intracellular calcium levels within a physiological range [13]. Similarly, it is not likely that P₄ activates the protein kinase C (PKC), since 1) P₄ inhibits GC mitosis [11] and 2) an activator of PKC, the phorbol ester 12-O-tetradecanoylphorbol 13-acetate, promotes GC mitosis [25]. Several other signal transduction pathways have been associated with P₄'s action in non-ovarian tissue. These signal transduction pathways include the suppression of adenylate cyclase, activation of a G protein-linked phosphatidylinositol-specific phospholipase C, induction of diacylglycerol and inositol 1,4,5-triphosphate, stimulation of tyrosine kinase activity, and regulation of chloride channels [2, 33, 41, 42]. The ability of P₄ to activate these pathways in GCs is presently being investigated.

Since multiple signal transduction pathways have been associated with P₄'s action, it is possible that there exist a
family of membrane PRs with each receptor linked to a specific signal transduction pathway. Putative membrane PRs have been identified in other tissues. For example, Meyer et al. [43] isolated specific PR-BPs with apparent molecular masses of 28, 56, and 85 kDa from the membrane fraction of porcine liver. Photoaffinity labeling studies have shown that the brains of mice express PR-BPs of 29, 54, 60, 64 kDa [44]. Using a similar technique, Tischkau and Ramirez [45] identified a 40- to 50-kDa PR-BP within rat brains. Finally, Saebur et al. [46], using the same PR antibody that was used in the present studies, detected a 50- to 52-kDa PR-BP in human sperm. None of these PR-BPs have been cloned and sequenced. Thus, whether the 60-kDa antibody that was used in the present studies, detected a 50-kDa PR-BP expressed in a tissue- and/or cell type-specific manner. If so, this 60-kDa protein may represent a GC-specific form of a membrane PR. Considerably more research is needed to validate this concept.

In summary, these studies support the concept that GCs express a 60-kDa PR-BP that appears to function as a membrane receptor for P4 and mediates P4's anti-mitotic and anti-apoptotic actions. Since GCs of immature and developing follicles do not express nuclear PRs, the existence of this protein is very important in that it provides a putative mechanism to account for P4's direct effects on these GCs. Further characterization of this protein should lead to a better understanding of P4's intracellular role in regulating ovarian function.

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REFERENCES

17. Park Sarge OK, Mayo KE. Regulation of the progesterone receptor gene by gonadotropins and cyclic adenosine 3',5'-monophosphate in rat granulosa cells. Endocrinology 1994; 134:709–718.
35. Wei LL, Miner R. Evidence for the existence of a third progesterone...
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