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The effects of prostaglandin $F_{2\alpha}$ treatment on peripheral-type benzodiazepine receptors in the ovary and uterus during pseudopregnancy of rats

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ABSTRACT

A previous study by us indicated that peripheral-type benzodiazepine receptor (PBR) density may be increased in the ovaries and uterus of pregnant rats (Weizman R, Dagan E, Snyder SH, Gavish M. Impact of pregnancy and lactation on $GABA_A$ receptor and central-type and peripheral-type benzodiazepine receptors. Brain Res 1997;752:7–14). In the present study, the effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on PBR density in the ovary and uterus of pseudopregnant rats were assayed. Pseudopregnancy was induced on day 29 post-partum (PP) by s.c. injection of 50 IU pregnant mare serum gonadotropin (PMSG) and 3 days later by s.c. injection of 20 IU human chorionic gonadotropin (hCG). PBR ligand binding density was assayed with the specific PBR ligand [^{3}H]PK 11195. A two-fold increase in ovarian PBR density was observed 2 days after hCG administration compared with vehicle control rats and this effect was maintained for 3 weeks. In the uterus, a three-fold increase in PBR density was observed and this increase was maintained for 1 week after hCG administration. Pseudopregnancy did not appear to affect renal PBR density or affinity. Treatment with $PGF_{2\alpha}$, which causes luteolysis, resulted in an approximately 50% reduction of PBR density in the ovaries of pseudopregnant rats at day 53 PP compared to pseudopregnant control rats. Treatment with indomethacin, which prevents the formation of $PGF_{2\alpha}$, caused the PBR density in the uterus of pseudopregnant rats at day 53 PP to be twice as high as in pseudopregnant control rats. All the above treatments did not affect the affinity of [^{3}H]PK 11195 to ovarian and uterine PBR. These data suggest that PBR density in corpora lutea and uterus during pseudopregnancy is regulated by $PGF_{2\alpha}$.

1. Introduction

Peripheral-type benzodiazepine receptors (PBR) have been identified in virtually all tissues throughout the body [2] and are located primarily on mitochondrial membranes [3]. Three protein components constitute the PBR: the 18-kDa isoquinoline binding protein, the 32-kDa voltage dependent anion channel, and the 30-kDa adenine nucleotide transporter [4]. PBR protein component ratios appear to be tissue- and treatment-specific [5]. Interestingly, it was found that PBR

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are particularly abundant in steroidogenic tissues such as the adrenal gland, ovary, uterus, and testes (for review, see [6]). As a consequence, PBR's involvement in mitochondrial steroid production has been studied extensively [7]. In addition, PBR have been implicated in many other functions, including mitochondrial respiration [8], cell growth and differentiation [9], and apoptosis [10].

Several studies implied that the expression of PBR can be regulated by various hormones. For example, hypophysectomy of female rats vastly reduces PBR binding density in the adrenal cortex [11]. Replacement of the tropic hormone ACTH in this paradigm restores the binding density levels of PBR [11]. Similarly, PBR binding density in the ovary and uterus is reduced in hypophysectomized rats, while administration of pregnant mare serum gonadotropin (PMSG) or estradiol restores PBR binding density to normal levels [12]. It was also found that PBR density in the ovary and uterus is modulated during the rat oestrus-cycle. It increases just before pro-oestrus and decreases during oestrus and metoesetrus together with the increases and decreases of serum estradiol levels, respectively [13]. These studies also suggested that the pattern of the increase and decrease in PBR density in the ovary and uterus is associated with developmental and morphological changes within these organs. For example, it has been suggested that decreases in PBR density on the day of oestrus occur in association with the formation of preovulatory follicles and the subsequent corpora lutea in the ovary [13].

The degeneration of corpora lutea appears to be under hormonal control, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which is secreted by the uterus [14], is reported to play a major role in this process [15]. The luteolytic effects of $PGF_{2\alpha}$ occur when the released ova are not fertilized or following parturition. If pregnancy occurs, existence of the corpora lutea is extended and the corpora lutea then partake in the maintenance the pregnancy, including maintenance of the decidua in the uterus [16].

We reported previously that the classical hormones regulating ovarian physiology, FSH/LH and estradiol, partake in the regulation of PBR density in the ovary, oviduct and uterus [11,12]. During pregnancy, PBR density is increased in the uterus and ovary of rats [1]. However, the type and mode of hormonal involvement in the induction of PBR during pregnancy has not been studied so far. For the present study, we utilized the model of pseudopregnancy, which is known to include prolongation of the presence of functional corpora luteua in the ovary [17] as well as uterine growth [18], closely mimicking events occurring during pregnancy. Thus, pseudopregnancy presents a powerful model to study the biochemical, morphological and hormonal interactions taking place during specific processes in the ovary and uterus. In the present study we examined the hormonal regulation of PBR densities in the ovary and uterus during pseudopregnancy. In particular, we studied changes in PBR densities in the ovary and uterus during pseudopregnancy as well as the effects of $PGF_{2\alpha}$ on these PBR densities. Therefore, we induced pseudopregnancy in rats with PMSG and human chorionic gonadotropin (hCG) and we used $PGF_{2\alpha}$ to interfere in these developmental processes. To prolong corpus luteum function, we applied indomethacin at a concentration known to reduce $PGF_{2\alpha}$ levels [19].

2. Materials and methods

2.1. Materials

hCG and PMSG were obtained from Organon (Oss, The Netherlands). $PGF_{2\alpha}$ was purchased from Upjohn (Brussels, Belgium). Polyvinylpyrrolidone (PVP) and indomethacin were obtained from Sigma (St. Louis, MO). [^{3}H]PK 11195 was purchased from NEN (Boston, MA). Lumax was purchased from Lumac (Schaesburg, The Netherlands). Ricinus oil was purchased from Ben Shimon Florish (Misgav, Israel).

2.2. Treatment of rats

Immature female Sprague-Dawley rats, i.e. before commencement of the estrous cycle, with an average weight of 75 g were treated according to the approval of the local Institutional Review Committee following government guidelines. The rats were housed in air-conditioned quarters with lights on between 07:00 and 19:00 h. Standard rat chow and water were available ad libitum. Our previous studies indicated that at this age the ovary contains an almost homogenous population of follicles (40–50% antral follicles) and there are no corpora lutea [20]. PMSG and hCG were dissolved in saline. To induce pseudopregnancy, according to methods applied previously [21], 50 IU of PMSG were injected s.c. on day 29 PP; followed by 20 IU hCG injected s.c. on day 32 PP (day 0 of pseudopregnancy). Rats were decapitated on days 34, 39, 46, and 53 PP. One group of pseudopregnant rats was injected s.c. daily for 3 days on days 39–41 PP with 0.2 mg $PGF_{2\alpha}$ dissolved in PVP, as described previously [22]. Another group of pseudopregenant rats was injected s.c. daily for 8 or 15 days with 0.5 mg indomethacin dissolved in ricinus oil from day 39 PP until decapitation on day 46 or 53 PP, according to methods described previously [23]. Control groups were treated with vehicle only (saline, PVP, or ricinus oil, as appropriate).

2.3. Radioimmunoassay (RIA) of progesterone levels

Serum progesterone levels were measured on day 53 PP by radioimmunoassay, using ImmunoChem double-antibody [^{125}I] progesterone RIA kit (ICN Biochemicals, Costa Mesa, CA), according to the instructions provided by the manufacturer.

2.4. PBR ligand binding

PBR ligand binding was assayed with the tritiated, PBR-specific ligand, [^{3}H]PK 11195, according to methods described previously [24]. Briefly, ovaries, uterus, and kidneys were homogenized separately in 50 vol. of 50 mM Tris–HCl buffer, pH 7.4, using a Brinkmann polytron (setting 10) for 15 s. The homogenate was centrifuged at 49,000 × g for 15 min at 4 °C. The pellet was suspended in 50 mM ice-cold Tris–HCl buffer, pH 7.4, to achieve 0.2–0.5 mg protein/ml, and used for binding assay. Protein content of the membrane homogenate was measured as described by Lowry et al. [25].

Specific binding of [^{3}H]PK 11195 at six concentrations (0.2–6 nM final concentration) was assayed in 50 mM Tris–HCl buffer, pH 7.4, in a final volume of 500 μl. The reaction mixture

consisted of 400 μl membrane homogenate (0.08–0.2 mg protein) and 25 μl [^{3}H]PK 11195 in the absence (total binding) or presence (nonspecific binding) of 75 μl unlabeled Ro5 4864 (10 μM final concentration). After incubation for 60 min at 4 °C, samples were vacuum-filtered over Whatman GF/B filters and washed three times with 4 ml of ice-cold Tris–HCl buffer. Filters were placed in vials containing 5 ml of a mixture of xylene–Lumax (3:1) and counted for radioactivity after 12 h. The maximal number of binding sites and equilibrium dissociation constant were determined by Scatchard analysis of saturation curves of [^{3}H]PK 11195 binding (0.2–6 nM final concentration).

2.5. Statistical analysis

Results are expressed as means ± S.E.M. The results were analyzed for statistical significance using one-way analysis of variance (ANOVA) and Student's *t*-test as a post hoc, as appropriate [26]. For each experimental group, $n \geq 5$. Statistically significant differences were considered to be indicated by $p < 0.05$.

3. Results

3.1. PK 11195 binding in ovary and uterus

We determined [^{3}H]PK 11195 binding characteristics in the ovary and uterus of the rats of our study. Representative saturation curves and Scatchard plots of [^{3}H]PK 11195 binding (0.2–6.0 nM) in the ovary and uterus of 39-day-old saline-treated female control rats are presented in Fig. 1. The B_{max} values in the ovary and uterus were 3784 and 2640 fmol/mg protein, respectively. The respective K_d values were, 1.18 and 1.05 nM. Non-specific binding was 15 and 14% of total binding in the ovary and uterus, respectively. The binding of [^{3}H]PK 11195 in both tissues was saturable and the Scatchard plots for both tissues are linear indicating a single population of binding sites.

3.2. Effect of pseudopregnancy on rat ovarian, uterine, and renal PBR binding characteristics

In rats, rendered pseudopregnant as described in Section 2, on days 34 PP (2 days after hCG administration), 39, 46 and 53 PP, respectively, PBR density in the ovaries was increased by 96% ($p < 0.05$), 166% ($p < 0.01$), 75% ($p < 0.05$) and 82% ($p < 0.05$), respectively, compared to control untreated rats (Fig. 2).

On days 34 and 39 PP, PBR density in the uterus was increased by 266% ($p < 0.001$) and 110% ($p < 0.05$), respectively, in pseudopregnant rats compared to control untreated rats (Fig. 3). On day 46 PP, pseudopregnant uterine PBR density was not significantly different from that of control rats (Fig. 3). On day 53 PP, uterine PBR density was decreased by 66% ($p < 0.05$), compared to control untreated rats (Fig. 3).

No differences in PBR binding affinity related to treatment and tissue was found in the ovary, uterus, and kidney in any of these experiments (data not shown). In the kidney, PBR density and affinity was determined on days 46 and 53 PP. No significant differences were found between the kidneys of control and pseudopregnant rats (day 46 PP: control 4035 ± 171 fmol/mg and pseudopregnant 5297 ± 607. Day 53 PP control 5661 ± 287 fmol/mg and pseudopregnant 5391 ± 500).

3.3. Effect of pseudopregnancy on rat serum progesterone levels

Pseudopregnant rats showed elevated serum progesterone levels on day 53 PP compared to control, 41 ± 7 ng/ml versus 23 ± 2.3 ng/ml, respectively ($p < 0.05$). Treatment with $PGF_{2\alpha}$ of pseudopregnant rats lowered serum progesterone levels to 30 ± 4 ng/ml.

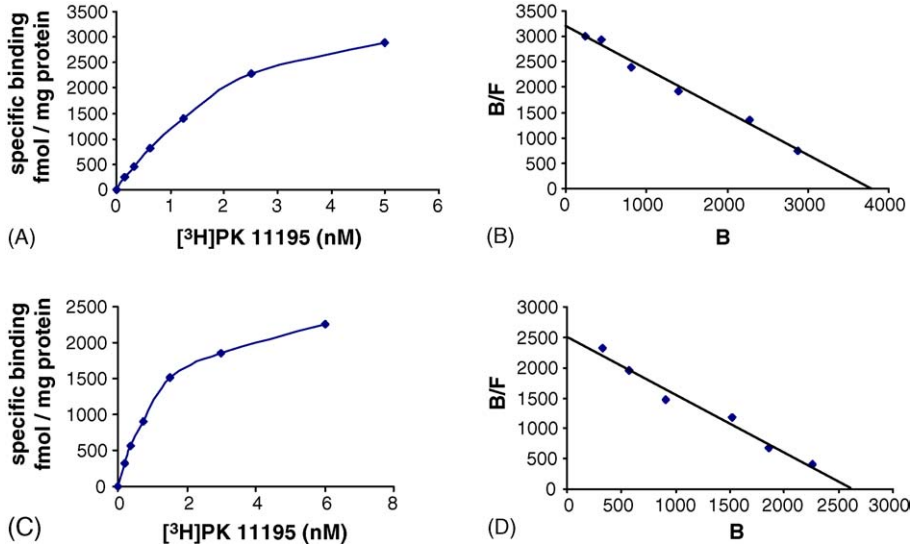

Fig. 1 – To the left, representative examples of saturation curves of [^{3}H]PK 11195 binding to membranes of the ovary (A) and uterus (C). To the right, Scatchard plots of the saturation curves of [^{3}H]PK 11195-specific binding to membranes of the ovary (B) and uterus (D); B: bound; B/F: bound/free.

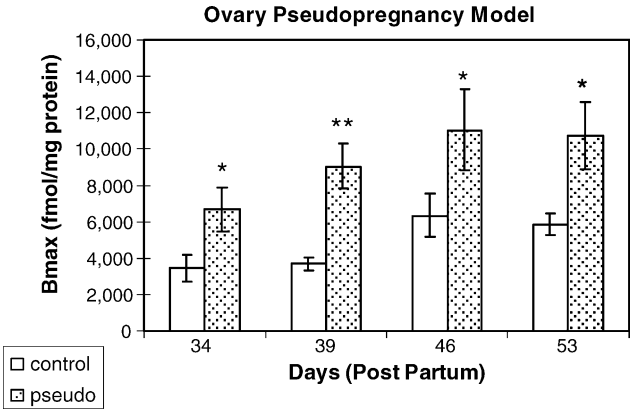

Fig. 2 – Increased PBR density in rat ovary of pseudopregnant rats. Rats were injected with PMSG (day 29 PP) and hCG (day 32 PP) or vehicle (control) and ovaries were removed for PBR density measurement on the days PP as indicated on the figure. When data were subjected to one-way ANOVA, the following statistical values were observed $F = 18.678$ $p < 0.001$. The * and ** indicate $p < 0.05$, respectively, $p < 0.01$, vs. control at the same day by Student's *t*-test.

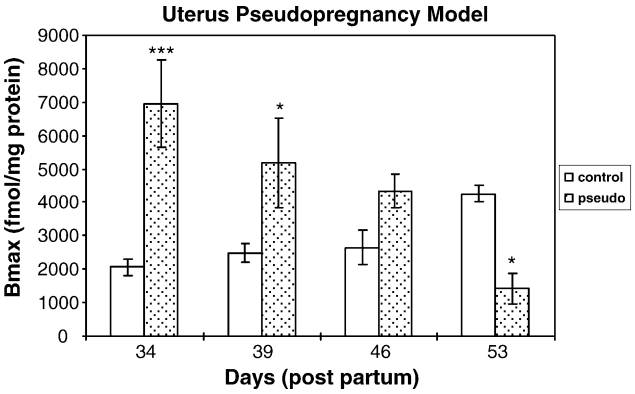

Fig. 3 – Increased PBR density in rat uterus of pseudopregnant rats. Rats were injected with PMSG (day 29 PP) and hCG (day 32 PP) or vehicle (control) and uterine horns were removed for PBR density measurement on the days PP as indicated on the figure. When data were subjected to one-way ANOVA, the following statistical values were observed $F = 18.024$ $p < 0.001$. The * and * indicate $p < 0.05$, respectively, $p < 0.001$, vs. control at the same day by Student's *t*-test.**

3.4. *Effect of $PGF_{2\alpha}$ and indomethacin on ovarian and uterine PBR density in pseudopregnant rats*

Regarding PBR density in the ovary, uterus and kidney, no significant differences were detected between the rats injected with the vehicles PVP or ricinus oil alone. Therefore, the PVP and ricinus oil injected groups were pooled.

The effects of treatment with $PGF_{2\alpha}$ or indomethacin of pseudopregnant rats on ovarian and uterine PBR density on day 53 PP are presented in Table 1. Ovarian PBR density in the $PGF_{2\alpha}$-treated pseudopregnant rats (at day 53 PP, i.e. 12 days after the last administration of $PGF_{2\alpha}$) was decreased by 60% ($p < 0.01$) compared to the control group of pseudopregnant rats (Table 1). However, ovarian PBR density of pseudopregnant rats on day 53 PP remained high following treatment with indomethacin (Table 1).

In the uterus, $PGF_{2\alpha}$ treatment did not alter PBR density of pseudopregnant rats, compared to the already decreased uterine PBR density found in pseudopregnant control rats at day 53 (Table 1). In addition, uterine PBR density remained elevated in the indomethacin-treated pseudopregnant rats, i.e. twice as high as in pseudopregnant control rats ($p < 0.05$) at day 53 PP (Table 1).

On day 46 PP, in pseudopregenant rats treated with $PGF_{2\alpha}$, or indomethacin, no significant changes in ovarian or uterine PBR density were observed, compared to pseudopregnant control groups (data not shown).

4. Discussion

In the present study, rats were rendered pseudopregnant by injections of PMSG and hCG. We observed increases in PBR density in the ovary of pseudopregnant rats during the 3 weeks following injections of PMSG and hCG. This increase in PBR density in the ovary follows a time course comparable to that seen for pregnant rats [1]. Also in the uterus of pregnant rats an increase in PBR density is observed [1]. In our pseudopregnant rats, PBR density was increased for approximately 10 days in the uterus. Since no changes in PBR binding affinity were detected in these organs, the changes in binding density appear to reflect a change in PBR number. The lack of changes

Table 1 – Effect of $PGF_{2\alpha}$ or indomethacin administration on uterine and ovarian PBR density of pseudopregnant rats on day 53 PP

Organ	Pseudopregnancy (fmol/mg protein)	$PGF_{2\alpha}$ (fmol/mg protein)	Indomethacin (fmol/mg protein)
Ovary	10721 ± 1819 ($n = 12$)	4720 ± 410 ($n = 6$)[a]	9304 ± 713 ($n = 6$)
Uterus	2343 ± 358 ($n = 13$)	2629 ± 280 ($n = 5$)	5238 ± 841 ($n = 8$)[b]

[^{3}H]PK11195 binding densities in the ovary and uterus of non-pseudopregenant rats at age 53 PP, as presented in Figs. 2 and 3, are: 5859 ± 616 and 5261 ± 255, respectively.

Data were subjected to one-way ANOVA and the following statistical values were observed: $F = 19.47$, $p < 0.001$ for ovary, and $F = 8.52$, $p < 0.001$ for uterus, and Student's *t*-test was used as a post hoc giving the following *p*-value: (a) $p < 0.001$ vs. pseudopregnant vehicle-treated rats; (b) $p < 0.05$ vs. pseudopregnant vehicle-treated rats.

in renal PBR density during pseudopregnancy suggests that the changes detected in the uterus and ovary are specific.

The present study shows that treatment with $PGF_{2\alpha}$ resulted in reduced PBR ligand binding in the ovary on day 53 PP, compared to vehicle control pseudopregnant rats, which typically display elevated PBR ligand binding. $PGF_{2\alpha}$ is reported to be involved in the regulation of normal corpus luteum regression [27]. Possibly, the enhanced levels of PBR in the ovary actually reside in the corpora lutea and their regression due to $PGF_{2\alpha}$ may consequently lead to a reduction in PBR levels and serum progesterone levels. Our previous studies suggested similar effects on follicular growth, which was found to be associated with increased PBR density [13], while follicular regression entailed a significant reduction in PBR density [12].

In the pseudopregnant rat model, luteolysis usually starts on day 6 (considering the day of hCG administration as day 0), however, another cohort of corpora lutea is formed on day 9 and pseudopregnancy is continued [28]. It is well known that the corpus luteum produces progesterone [29]. In particular, enzymes critical for progesterone biosynthesis in luteal cells such as 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (3β-HSD) and cholesterol side-chain cleavage cytochrome P450 (P450scc) are at their highest level during pseudopregnancy [30]. In the present study, the high levels of serum progesterone detected on day 21 of pseudopregnancy (day 53 PP) suggest the presence of steroidogenic active corpora lutea. Thus, the presence of high ovarian PBR density until day 53 PP (Fig. 2) may be correlated with the presence of steroidogenically active corpora lutea. The luteal precursor for progesterone is pregnenolone, which is formed from cholesterol in the mitochondria [31]. PBR and StAR have been implicated in the transfer of cholesterol from intracellular stores into the mitochondria, which is the rate limiting step for pregnenolone formation [32].

Treatments with indomethacin are known to reduce endogenous $PGF_{2\alpha}$ levels within the ovary and thereby to extend corpus luteum function [19,33]. With the present study, we did not observe indomethacin effects on PBR density in the ovary of pseudopregnant rats on days 46 and 53 PP. Since the corpora lutea are still functionally active during this time period of pseudopregnancy, $PGF_{2\alpha}$ levels are not yet increased [30]. Thus, indomethacin would not decrease $PGF_{2\alpha}$ levels during this same period. $PGF_{2\alpha}$ is known to interfere in corpora lutea functionality and reducing progesterone secretion [30], which apparently significantly decreases PBR density in the ovary (Table 1).

The uterine PBR density does not follow exactly the same time course as in the ovary and is reduced to control levels within the first 2 weeks of pseudopregnancy (Fig. 3). It would be interesting to study whether the enhanced abundance of PBR in the uterus during pseudopregnancy may be associated with the steroidogenic capacity of this organ. Usually, endometrial-stroma tissue undergoes a process involving proliferation and differentiation into distinct large cells, named decidual cells. In the decidua of pregnant mice and humans progesterone appears to be formed from pregnenolone [34,35]. In rats, mRNA and protein expression of key steroidogenic enzymes such as P450scc and 3β-HSD was observed in the uterine wall upon decidualization, but this expression ceased at mid-pregnancy [36].

In particular, following 8 days of pregnancy in rats a significant reduction in the expression of the key steroidogenic enzymes (P450scc and 3β HSD) was noted in the uterine stromal/deciduas cells [37]. The time course of these key steroidogenic enzymes is similar to that of the PBR density reported in the present paper, conceivably suggesting that the changes in PBR density are correlated with the steroidogenic capability of the stromal/decidua cells.

In the uterus, $PGF_{2\alpha}$ inhibits vascular permeability and endometrial decidualization [38,39]. The lack of an effect of $PGF_{2\alpha}$ treatment on uterine PBR levels on day 53 PP in our study may be because PBR levels at this stage of pseudopregancy are already below the levels observed in the control animals (Fig. 3). On the other hand treatments with indomethacin are known to reduce $PGF_{2\alpha}$ levels within the uterus in pseudopregnant rats and extend decidua life [23,40]. Thus, indomethacin may have prevented the effect of endogenous $PGF_{2\alpha}$, and there maintained the level of PBR binding on day 53 PP as present at earlier stages of pseudopregnancy, such as on days 39 and 46 (Fig. 3). The lack of an effect of indomethacin on day 46 is in accord with this conclusion, since at this day the level of PBR binding is still relatively high (Fig. 3). This suggests that endogenous $PGF_{2\alpha}$ indeed partakes in the reduction of PBR levels in the uterus, which we prevented by treatment with indomethacin. We hypothesize that this reduction in the level of PBR binding is possibly related to the life cycle of large decidual cells [41].

$PGF_{2\alpha}$ has been reported to induce apoptosis of the corpora lutea, i.e. luteolysis [42]. $PGF_{2\alpha}$ also has been associated with apoptosis in the uterus, i.e. regression of the deciduas following luteolysis [43]. We do not know whether the effects of $PGF_{2\alpha}$ in reducing PBR levels is due to a reduction in the number of the cells carrying PBR or due to the reduction in number of receptor per cell or that both processes occur simultaneously. Due to its inhibition of $PGF_{2\alpha}$ formation, indomethacin prevents the reduction of cell number. As indicated in Table 1, this effect of indomethacin is accompanied by a high PBR density in the uterus. Thus, indomethacin also could prevent the regression of cells carrying PBR or induce increases in PBR number per cell.

In conclusion, pseudopregnancy in rats increases PBR density in the ovary and uterus compared to non-pseudopregnant rats. We believe that these increases in PBR density are associated with the steroidogenic activity of these organs. This increased PBR density during pseudopregnancy appears to concur with the reported presence of active corpora lutea in the ovary and the development of the decidua in the uterus. Our treatments with $PGF_{2\alpha}$, and its inhibitor, indomethacin, suggest that $PGF_{2\alpha}$-induced reductions in PBR binding density in the ovary are due to the regression of the corpora lutea. Thus, we believe that the increases in PBR binding we see during pseudopregnancy in rats, are due to the presence of PBR carrying corpora lutea and LDC, in the ovary and the uterus, respectively.

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