Emerging roles of peroxisome proliferator-activated receptors in the pituitary gland in female reproduction

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Abstract Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear receptors that mediate numerous crucial physiological processes, including glucose and lipid metabolism, energy homeostasis, inflammation, cell proliferation, and differentiation. Therefore, targeting of PPARs for metabolic diseases, cardiovascular diseases, polycystic ovary syndrome, and cancer therapies is intensive. Over the last decade, studies on PPARs have shed light on their functional roles in the female reproductive system, including the hypothalamus–pituitary–gonad axis, placental development, and maternal-infant interactions. In this review we summarize PPAR genomic organization, molecular functions, and expression patterns, as well as insights from knockout mice and PPAR involvement in biological processes, with a focus on their novel role in the pituitary gland in female reproduction.

Introduction Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that are fundamentally involved in their involvement in fatty acid and lipoprotein metabolism, glucose homeostasis, cellular proliferation and differentiation, and the immune response. Accordingly, PPARs are
important targets in the treatment of metabolic disorders such as type 2 diabetes mellitus and are of interest in relation to chronic inflammatory diseases. Recent studies further identified novel functions of PPARs in blood pressure regulation, neuroinflammation, neuroprotection, and inflammatory pain reduction.

The abundant pleiotropic actions of PPARs suggest that PPAR agonists have enormous therapeutic potential. Ongoing research indicates that modulation of PPAR activity might be an effective therapy for additional conditions associated with the metabolic syndrome, including insulin resistance, inflammation, obesity, hyperlipidemia, hypertension, and heart disease. Moreover, PPARs play a role in the regulation of cancer cell growth, so specific PPAR agonists might have different effects on cancer incidence.

The impact of PPARs on female reproduction across vertebrates has recently been highlighted. Regulatory roles of PPARs in placental development during pregnancy, from gametogenesis to parturition, and in normal ovarian function have been identified. We found that PPARs are expressed in the pituitary gland and play a role in the hypothalamus–pituitary–gonad axis in female reproduction. Multifaceted roles of PPARs at the cellular and whole organism levels have been reviewed elsewhere.

The PPAR superfamily

The nuclear receptor family of transcription factors is quite large and can be categorized into three subgroups according to specific ligand binding: (1) classic hormone receptors (e.g., the glucocorticoid, estrogen, thyroid, retinoic acid, and vitamin D receptors); (2) sensor receptors (e.g., PPARs, liver X receptor, farnesol X receptor, and retinoid X receptor, RXR); and (3) orphan nuclear receptors (e.g., apolipoprotein A-I regulatory protein-1 and chicken ovalbumin upstream promoter transcription factor). The PPARs were originally named for their ability to induce hepatic peroxisome proliferation in mice in response to xenobiologic stimuli. The classic hormone receptors bind specific ligands with high affinity; by contrast, a broad range of lipophilic molecules bind to the sensor receptors, generally with low affinity compared to hormones that bind to the classic hormone receptors.

Across vertebrates, PPAR evolved into three paralogs: PPAR-alpha (PPARA), PPAR-delta (PPARD, also known as PPARβ) and PPAR-gamma (PPARG). Although the genomic organization of PPARs differs to some extent, each specific PPAR shares high protein sequence homology with PPARs in other species. Human PPARA, PPARD, and PPARG are encoded by three different genes. Alternative promoters give rise to two major PPAR/Pparg isoforms, γ1 and γ2, in mice, and rats. Studies at the protein level revealed that the three human PPARs share 64% and 85% homology in their ligand- and DNA-binding domains. As shown in Fig. 1C, the peroxisome proliferator response element (PPRE) is the DNA-binding sequence in PPARs. PPRE consists of a consensus DNA hexamer (A/GGGTCA) arranged as either a single half-site or, more often, as two tandem half-sites arranged as direct, inverted (palindromic), or everted repeats. In general, the majority of PPREs identified in genes responsive to PPAR ligands have a direct repeat separated by one spacer nucleotide structure (also known as DR1), even though other, more complex PPREs have been reported. PPARs only bind DNA as heterodimers with RXR alpha (RXRA, a sensor receptor) and cannot bind DNA as homodimers or monomers. DNA interaction is greatly enhanced by the binding of ligands to RXR and to PPAR. The PPAR–RXRA heterodimer is polar, with PPAR occupying the 5′ and RXRA the 3′ position when bound to the DR1/PPRE (Fig. 1C).

**PPAR molecular functions and expression patterns**

According to their secondary structure conservation and Gene Ontology annotations, human PPAR paralogs are involved in intersecting molecular functions that include ligand binding, ligand-activated sequence-specific DNA-binding RNA polymerase II transcription factor activity, lipid binding, protein binding, and sequence-specific DNA-binding transcription factor activity (Fig. 2). According to the Expressed Sequence Tag database (dbEST; NCBI, Bethesda, MD, USA) and many studies, human PPARA is highly expressed (>50 per million ESTs) in adrenal gland, intestine, kidney, liver, muscle (skeleton), and nerve. PPARD is predominantly expressed in brain, nerves, ovary, placenta, prostate, spleen, thymus, thyroid, trachea, umbilical cord, and uterus. PPARG is highly expressed in adipose tissue, bone, cervix, intestine, nerves, and placenta. We and others recently found that PPARs are expressed in early embryos, developing fetuses, and various compartments of the reproductive system (hypothalamus, pituitary gland, ovary, uterus, and testis) in many species, including birds, fishes, and mammals such as rodents, cattle, pigs, and humans. Double immunostaining revealed that every human pituitary secreting cell lineage [growth hormone (GH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), and adrenocorticotropic hormone (ACTH)] expresses PPAR. Furthermore, pituitary gland PPAR expression has been observed across several species, including human, mouse, rat, sheep and chickens. The pituitary gland secretes nine hormones that regulate homeostasis and PPAR protein is highly expressed in every normal pituitary secreting cell lineage. Thus, PPARs might be involved in specific molecular functions in this organ.

**PPARs are involved in overlapping and distinct biological processes**

Each PPAR is involved in both specific and inter-related biological processes. For example, both PPARA and PPARD participate in the intracellular receptor-mediated signaling pathway. PPARA and PPARG negatively regulate cholesterol storage and macrophage-derived foam cell differentiation. PPARA also plays roles in negative regulation of receptor biosynthesis, sequestering of triglycerides, and RNA polymerase II promoter transcription. Both PPARD and PPARG are involved in apoptosis. PPARA has been involved in the regulation of cancer cell growth, so specific PPAR agonists might have different effects on cancer incidence. Therefore, modulation of PPAR activity might be an effective therapy for additional conditions associated with the metabolic syndrome, including insulin resistance, inflammation, obesity, hyperlipidemia, hypertension, and heart disease. Moreover, PPARs play a role in the regulation of cancer cell growth, so specific PPAR agonists might have different effects on cancer incidence.
implicated in the activation of cysteine-type endopeptidase activity in apoptosis,57 cell maturation,58 cellular responses to insulin stimulus, glucose homeostasis,40 lipoprotein transport, low-density lipoprotein (LDL) particle receptor biosynthesis, and monocyte differentiation.58

Ligands for PPARs

The main natural ligands for PPARs are the intermediate and end products of fatty acid and sterol metabolic pathways, including fatty acid derivatives and eicosanoids.38 These small lipophilic molecules can freely partition into cells. PPARA is activated by a variety of long-chain polyunsaturated fatty acids, including linoleic acid, branched, conjugated, and oxidized fatty acids such as phytanic acid, conjugated linolenic acid, and eicosanoids such as 8S-hydroxy-5Z,9E,11Z,14Z-eicosatetraenoic acid and leukotriene B4.59–62 Prostaglandin D2 (PGD2) and PGJ2 are naturally occurring ligands for PPARs.63 The endogenous PGD2 metabolite 15-deoxy-Δ12,14-prostaglandin J2 (15-d-PGJ2) is the most potent natural ligand of PPARG.64,65 Intriguingly, two pathophysiological oxidized metabolites of linoleic acid, 9-hydroxy and 13-hydroxy octadecadienoic acid (9- and 13-HODE), which are present in oxidized LDL, have been suggested as PPARG ligands.55,58 Linoleic acid, arachidonic acid (AA), eicosapentaenoic acid, and prostaglandin A1 bind to PPARD in the micromolar range.38,66

PPARs have a large ligand-binding pocket (1300 Å), so they are capable of binding to several structurally diverse compounds, including industrial chemicals such as herbicides and plasticizers, as well as pharmaceutical compounds including hypolipidemic fibrates and antidiabetic

Figure 1 Peroxisome proliferator-activated receptors (PPARs) are highly conserved in vertebrates. (A) Functional domains of a typical PPAR member. N, amino-terminus; C, carboxy-terminus. Domains A/B, activation function 1 (AF1; transactivation); domain C, DNA-binding domain (DBD); domain D, flexible hinge region; domains E/F, ligand-binding domain (LBD) and activation function 2 (AF2) for transactivation, dimerization, and coactivator recruitment. Similar domain allocations are shown at the bottom for the human and mouse forms. Feature annotations are based on the UniProt database (http://www.uniprot.org/). Alternative splicing exists in both human PPARG and mouse Pparg genes: the first 30 amino acids are missing in isoform 1, but not isoform 2. (B) Phylogenetic tree of PPARs in human (h), rat (r), mouse (m), and chicken (c) demonstrates that a specific PPAR (PPARA, PPARD, or PPARG) is highly conserved across vertebrates compared to its corresponding paralogs; Xenopus PPARA served as the out-group. (C) Typical PPAR–retinoid X receptor alpha (RXRA) heterodimer complex.

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thiazolidinediones (TZDs; e.g., rosiglitazone, pioglitazone, and troglitazone). Transactivation assays have revealed that a variety of exogenous compounds selectively activate PPAR paralogs; for example, WY-14643 binds to PPARA and PPARG,31,68 clofibrate and eicosatetraenoic acid bind to PPARA,69 and troglitazone, rosiglitazone, and pioglitazone bind to PPARG.69,70 The synthetic ligand fibrate (or bezafibrate) is an agonist of all PPARs.72 In addition, PPARs may modulate gene transcription by directly interfering with other transcription factor pathways in a manner independent of DNA binding.69,73

Insights from Ppars knockout mice

Knockout of Ppar genes in mouse or rat models has provided evidence to support the pleiotropic effects of the Ppar gene family. For example, Ppara deficiency causes inflammatory responses74; abnormal fatty acid metabolism75–77; resistance to peroxisome proliferation76; a lack of hepatic cell proliferation in response to the agonist WY-1464375; a lack of Ppara-induced genes involved in fatty acid metabolism76; an increase in sensitivity to cellular oxidative stress78; a decrease in basal levels of mitochondrial fatty-acid-catabolizing enzymes and mediation of inducible mitochondrial and peroxisomal fatty acid beta-oxidation79; progressive dyslipidemia in sexually dimorphic obesity and steatosis80; and increases in maternal abortion, neonatal mortality, and T cell differentiation.81

Lack of Ppard in mice downregulates expression of apolipoprotein C-II (ApoC2) and Apoc3, both of which are components of very-low-density lipoprotein in the small intestine involved in triglyceride catabolism.82 During early tumorigenesis, Ppard-deficient mice are prone to colon polyps in adenomatosis polyposis coli mutants or chemically induced systems compared to wild-type counterparts.83 In vivo, Ppard mediates cyclin-dependent kinase inhibitor 1C (also known as p57) to inhibit cell proliferation and tumor angiogenesis.84 Opsonins such as complement component-1qb are suppressed in Ppard-e/e mice, which results in impairment of apoptotic cell clearance and reductions in anti-inflammatory cytokine production.85 Deletion of Ppard results in frequent (>90%) midgestation lethality due to defects of the placenta; surviving Ppard-e/e mice exhibit a striking reduction in adiposity relative to wild-type levels. Therefore, Ppard has an important role in normal adipose development and lipid homeostasis.86 Deiciency of Pparg results in two independent lethal phases, suggesting that Pparg is required for placental, cardiac, and adipose tissue development. Notably, dependence of the developing heart on a functional placenta was observed.87 Viable Pparg-null mice generated via specific and total epiblastic gene deletion demonstrated that this placental defect is the unique cause of Pparg-e/e embryonic lethality; however, vasculature defects were observed in placentas on embryonic day 9.5.88 Conditional disruption of Pparg in murine peritoneal macrophages by ligand treatment results in lower expression a series of Pparg-responsive genes, indicating that Pparg plays a critical role in the regulation of cholesterol homeostasis by controlling the expression of a network of genes that mediate cholesterol eflux from cells and its transport in plasma.89 Leptin-
deficient (Lep<sup>-/-</sup>) mice usually have severe obesity and a fatty liver, which can be remedied by co-knockout of Pparg. Heterozygous loss of Ppar causes an increase in beta-catenin levels and a greater incidence of colon cancer when animals are treated with azoxymethane. Using Cre/loxP technology, the expression of Ppar was disrupted in the ovary, rendering one-third of females sterile and the remaining females subfertile. Females that were subfertile took longer to conceive and had smaller litters. Conditional knockout of Ppar in pituitary gonadotrophs caused an increase in blood LH levels in female mice, a decrease in FSH in male mice, and a fertility defect characterized by reduced litter size. These data indicate that PPARs regulate normal placental embryonic development and PPAR regulates expression levels of gonadotropins (FSH and LH) in vivo.

**PPARs and proper placenta development**

In rat, mouse, cattle, and human species, PPARs are highly expressed in various placental locations. Their predominant expression in trophoblasts suggests specific roles of PPARs in trophoblast differentiation and functions. Studies on cellular models revealed that human PPAR, PPARD, and/or PPARG inhibit cell growth, which might be regulated by suppression of 11-beta-dehydrogenase activity, stimulate the synthesis and secretion of chorionic gonadotropin and trophoblast differentiation, increase free fatty acids uptake, and inhibit cell invasion.

Clinically, enhanced expression of PPAR and the proliferative index protein Ki-67 in villous and extravillous trophoblasts has been documented in missed abortion. Secreted by white adipocytes, LEP plays a major role in the regulation of body weight through the LEP receptor. In the placenta, LEP also has several endocrine functions and is involved in the regulation of immune and inflammatory responses, hematopoesis, angiogenesis, and wound healing. Decreased LEP levels are associated with miscarriage and LEP homeostasis is connected to PPARG via a negative feedback loop. LEP-mediated secretion of proinflammatory cytokines such as interleukin 1 beta, interleukin 6, tumor necrosis factor, and prostaglandin E2 is inhibited by PPARG activation. Therefore, it has been suggested that PPARs are key messengers responsible for the translation of nutritional or metabolic stimuli into changes in gene expression pathways with high spatial and temporal specificity for placenta development.

**PPARs and the HPG axis in female reproduction**

In vertebrates, reproduction is primarily controlled by the HPG axis and this endocrine pathway is highly conserved in jawed vertebrates (gnathostoma). The hypothalamic neuroendocrine system, especially gonadotropin-releasing hormone (GnRH), regulates the synthesis and release of gonadotropins from the pituitary, and these in turn stimulate gonadal development, in particular via the induction of sex steroid synthesis. Sex steroid feedback to the hypothalamus and the pituitary regulates gonadotropin synthesis and release.

Once released into the bloodstream, gonadotropins exert their biological activities via G-protein-coupled receptors (GPCRs). The presence of FSH and LH from the pituitary gland and of their corresponding FSHR and LHR receptors in gonads is well documented in male and female vertebrates. The primary function of the HPG axis is to facilitate the production of germ cells and to coordinate reproductive events in relation to body condition and environment. In addition to its function in adult animals, the HPG axis regulates the differentiation of sex-specific phenotype during early development. In female mammals an dbirds, FSH stimulates the maturation of ovarian follicles and the LH surge causes release of an egg from mature follicles. FSH, LH, and another hormone secreted by the anterior pituitary, TSH, are heterodimeric glycoprotein hormones consisting of a non-covalently linked common glycoprotein hormone alpha-subunit (also known as CGA) and a specific beta-subunit (FSHB, LHB, or TSHB) that confer biological activity. In Chinese hamster ovary cells, LHB synthesis has been identified as the critical step in LH formation. Accordingly, LHB transcription and translation may play vital roles in LH synthesis and surges and the consequent ovulation across vertebrates.

Over the past 10 years, a number of in vivo and in vitro studies have strongly suggested that PPARs play significant roles from gametogenesis to parturition, including gestation and the connection between mother and fetus. Fuel sensors, such as glucose, insulin, and LEP, directly regulate fertility at each level of the HPG axis, and it is proposed that PPARG acts as a fuel sensor in reproductive compartments to inform cells of the energy status. In this case, PPARG may be a link between energy metabolism and reproduction, as both are frequently associated with insulin resistance in polycystic ovary syndrome (PCOS) and gestational diabetes.

**Hypothalamus**

The rat hypothalamus expresses PPARG, which is activated by its natural ligand 15-d-PGJ<sub>2</sub> in cerebrospinal fluid to play at least a partial role in antipyretic action. However, PPARG was not detected in a hypothalamus extract from sheep. The role of PPARs in reproduction remains elusive. So far, studies on hypothalamic PPARs are limited.

**Pituitary gland**

Across vertebrates, the pituitary gland regulates homeostasis via the secretion of numerous hormones. In the anterior pituitary, GH, TSH, corticotropin, lactotrophin, FSH, and LH are synthesized and secreted under the control of the hypothalamus. Hypothalamic hormones are secreted to the anterior lobe via a special capillary system, the hypothalamic—hypophyseal portal system. Another GPCR, GnRH receptor (GnRHR), is synthesized by gonadotrophs and expressed on the surface of pituitary gonadotrope cells as well as some other tissues. Following binding to GnRH, GnRHR associates with G proteins that activate a phosphatidyl inositol—calcium second messenger system. Activation of this receptor ultimately causes the synthesis and release of FSH and LH. Therefore, GnRH and GnRHR play a pivotal role in the control of reproduction in vertebrates.
Compared to mammals, the chicken serves as an excellent model to observe ovulation in a noninvasive manner. We found significantly elevated expression of hematopoietic prostaglandin D synthase (HPGDS) transcripts in the pituitary glands of hens with high egg production, laying, and ovulation rates. The HPGDS gene is highly conserved through mammals and its encoded protein belongs to the AA metabolism pathway. This enzyme catalyzes conversion of the short-half-life prostaglandin H$_2$ (PGH$_2$) to PGD$_2$ and plays a role in the production of prostanooids in the immune system and mast cells. PGD$_2$ can be further metabolized to the F and J series of prostaglandins, including PGJ$_2$.15

Studies by our group and others indicate that this conservation can be further extended to vertebrate species. It was previously reported that PGD$_2$ induces LH release from the rat pituitary gland without modulating hypothalamic GnRH. Therefore, a high HPGDS level might enhance ovulation via a mechanism other than the GnRH-dependent pathway. In a cellular model using primary culture of chicken pituitary cells, HPGDS downstream metabolites PGD$_2$ and PGJ$_2$, or a PPAR pan agonist, GW9578, significantly elevate LHB mRNA and protein levels in a dose- and time-dependent manner; treatment with AA alone significantly induced 15-d-PGJ$_2$ and LHB mRNA and protein expression. Furthermore, HPGDS overexpression in these cells in medium containing AA dramatically increases 15-d-PGJ$_2$ and LHB mRNA and protein levels. In the pituitary gland of ovulating hens, there are significant correlations between HPGDS and PPAR and between HPGDS and PPARA mRNA levels. PPARA- and PPAR-specific antagonists (GW6471 and T0070907, respectively) significantly suppress endogenous LHB mRNA and protein levels. Moreover, both antagonists attenuated AA-induced LHB mRNA and HPGDS-induced (in the presence of AA) LHB mRNA and protein levels in primary cultures of chicken pituitary cells. Higher LHB mRNA/LHB protein ratios in PGD$_2$-, PGJ$_2$-, AA-, HPGDS-, and GW9578-induced and in GW6471- and T0070907-suppressed pituitary cells suggest that LHB transcription occurs before translation. Therefore, HPGDS induces LHB transcription and subsequent translation via the PPAR signaling pathway in the chicken pituitary cellular model.23

In the AA metabolic pathway, AA is oxidized by constitutive prostaglandin endoperoxide synthase 1 (PTGS1, also known as cyclooxygenase-1/COX-1) and inducible PTGS2 (also known as COX-2) to yield PGG$_2$, which is in turn sequentially converted to the di-unsaturated prostaglandins PGH$_2$ and PGD$_2$ by hydroperoxidase and HPGDS, respectively. PTGS1 or PTGS2 is a rate-limiting enzyme in prostaglandin synthesis. The final product of this pathway, PGJ$_2$, is intracellularly converted to albumin-dependent $\Delta^{12}$-PGJ$_2$ and nonenzymatically deactivated 15-d-PGJ$_2$. The natural precursor of PGJ$_2$, derivatives appears to be PGD$_2$, as its administration leads to a large increase in $\Delta^{12}$-PGJ$_2$ in vivo. Alternatively, 15-d-PGJ$_2$ is capable of being produced extracellularly via non-enzymatic conversion of PGD$_2$ and may function as an autocrine and/or paracrine factor during inflammatory processes.

Surprisingly, we found that treatments with higher concentrations of PGD$_2$, PGJ$_2$, or GW9578 caused reverse effects on induction of LHB mRNA and protein levels in chicken primary pituitary cells. Prostaglandins belong to a family of biologically active molecules with a diverse range of actions, depending on the prostaglandin type and cell target. The prostaglandin 15-d-PGJ$_2$ is a potent anti-inflammatory agent that represses the expression of a number of inflammatory response genes, including inducible nitric oxide synthase, tumor necrosis factor alpha, matrix metalloproteinase 9, and PTGS2 in activated macrophages. Some of these repressive actions are mediated by high-affinity binding of 15-d-PGJ$_2$ to PPAR. Additional studies have demonstrated that 15-d-PGJ$_2$ inhibits transcriptional activation by polypeptide gene enhancer in B-cells 1 (NFkB1, also known as NFkB) and Jun activation domain-binding protein (JUN, also known as AP-1) in a PPAR-dependent manner. As a transcriptional factor, NFkB1 protein resides in the cytoplasm in association with its repressor NFKBIA. Upon cellular signaling, NFKBIA can be phosphorylated by the conserved helix–loop–helix ubiquitously kinase (CHUK, also known as IKB kinase), resulting in release of NFKB1 and its migration to the nucleus. The prostaglandin 15-d-PGJ$_2$ inhibits NFKB1, either by hindering CHUK or by directly interacting with NFKB1 protein. Furthermore, PTGS2 is transactivated by NFkB1 protein14 and NFkB1 transcription is inhibited by 15-d-PGJ$_2$. Taken together with our results, these findings suggest that higher PGD$_2$, PGJ$_2$, and downstream metabolite levels, as well as the PPAR agonist GW9578, negatively regulate PTGS2 expression via a PPAR-dependent pathway, resulting in suppression of LHB mRNA and protein levels and thus acting as a negative feedback loop. This might explain why conditional knockout of Ppard in pituitary gonadotrophs caused an increase in blood LH levels in female mice and the defect in fertility characterized by reduced litter size is due to disruption of the HPG axis.

It was recently revealed that PPAR is abundantly expressed and is therefore an important novel molecular target in pituitary adenoma cells, given that the PPAR ligand rosiglitazone inhibits tumor cell growth and ACTH, GH, PRL, and LH secretion both in vitro and in vivo. In the GH3 rat pituitary cell line, which synthesizes and secretes acromegalic GH, treatment with the synthetic PPAR ligands ciglitazone or pioglitazone arrests cell proliferation and decreases GH mRNA levels via binding to PPRE with RXRA in the GH promoter. It was also reported that although pioglitazone has a potency similar to that of rosiglitazone for inhibition of the proliferation of GH3 (PRL- and GH-releasing) cells, the PPAR antagonist GW9662 does not lead to significant reversal of the antiproliferative effect of rosiglitazone; in fact, GW9662 suppresses proliferation by itself. Therefore, the anti-proliferative action of rosiglitazone probably does not occur via the PPAR pathway. Instead, in the mouse gonadotropinoma cell line LITZ2, PPAR activation differentially regulated gonadotrope cell proliferation and gonadotropin transcription in a ligand-dependent manner, that is, the PPAR and PPAR ligands fenofibric acid and pioglitazone, respectively, directly suppressed the transcriptional activities of FSHB, LHB and GnRHR. Our earlier study129 and that of Takeda et al29 differed in terms of the types and concentrations of PPAR ligands used. Besides, because Takeda et al did not use antagonists, the doses of fenofibric acid or pioglitazone they applied may have suppressed LHB transcriptional activities via a PPAR-independent pathway, the negative feedback loop, or suppression of GnRHR. In contrast, we applied PPARA- and PPAR-specific antagonists and found that both antagonists significantly suppressed endogenous

and HPGDS/AA-induced LHB mRNA and protein levels. GW6471 is a PPARA-specific antagonist that completely inhibits GW409544-induced activation of PPAR; it also induces a PPARA conformation that interacts efficiently with corepressors. T0070907 is a potent and selective antagonist of the human PPARG receptor that inhibits binding to rosiglitazone; T0070907 covalently binds to Cys313 of PPARG and induces conformational changes that block the recruitment of transcriptional cofactors to the PPARG—RXR heterodimer. Our results strongly suggest that in the chicken primary pituitary cell model, both endogenous and HPGDS/AA-induced LHB mRNA and protein levels are regulated via the PPAR signaling pathway (Fig. 3).

To examine whether this is also true for mammals, we tested rat primary pituitary cells. Quantitative reverse transcription-polymerase chain reaction and immunoblotting analysis revealed that Lhb mRNA and protein levels were significantly upregulated after treatment with GW9578 or transfection of plasmids carrying human PPARA-turbo GFP (unpublished data). Further knockdown experiments will provide more in vitro evidence.

Ovary

Our studies revealed significantly higher HPGDS mRNA and protein levels in ovaries than in testes of adult chickens. We also found that all Ppars are expressed in the rat ovary during follicular development and periovulatory periods, confirming the presence of a HPGDS—PPAR pathway in the ovary in vertebrates. The presence of active endogenous PPAR in rat and sheep ovaries was confirmed by an increase in reporter activity in granulosa cells transiently transfected with reporter constructs whose expression was driven by PPREs, in the absence or presence of agonists for PPARG. In rat granulosa cells, Pparg also binds to DNA. All these results demonstrate that PPARG is functional in granulosa cells and its endogenous ligand is also present in these cells.

In sheep and rats, PPARG is detected early in folliculogenesis (at the primary/secondary follicle stage), increases until the large follicle stage, and decreases after the LH surge. Most notably, Pparg expression is primarily limited to granulosa cells in developing follicles and is downregulated by LH, suggesting a similar negative feedback loop between PPAR and LH in the ovary to that observed in pituitary cells. According to the above findings and evidence from conditional/ovary Ppar knockout mice, Pparg potentially affects somatic cell/oocyte communication not only by impacting granulosa cell development but also via direct effects on oocytes. In reality, the species and the status of granulosa cell differentiation (follicular phase, before or after the gonadotropin surge) can modulate the actions of synthetic PPAR ligands on steroidogenesis. For example,
treatment with rosiglitazone or pioglitazone stimulated progesterone secretion by a mixture of human granulosa, theca, and stroma cells obtained from premenopausal or perimenopausal patients without gonadotropin treatment at the time of oophorectomy. The effects of PPARs on steroidogenesis in ovaries have been reviewed elsewhere.

Targeting PPARs for female fertility disorders

In addition to the fatty acid metabolic pathway, PPARs have been associated with many human diseases including obesity, insulin resistance, type 2 diabetes, atherosclerosis, hypertension, inflammation, and cancer. It has been suggested that ligands for these receptors have therapeutic potential for selected human diseases. Synthetic ligands for one family member, PPARG, are currently widely used as treatment for chronic diseases such as type 2 diabetes and another condition in which insulin resistance is a factor (PCOS).

Conclusions

PPAR family members play key roles in regulating energy homeostasis, particularly fatty acid oxidation and carbohydrate metabolism. An increasing body of in vivo and in vitro research has recently revealed that PPARs also have functions in reproductive organs including the HPG axis and the placenta during pregnancy in several species. These studies strongly suggest that PPARs might play critical roles in reproduction and development, in addition to their central actions in energy homeostasis. The exact mechanism by which PPARs regulate LHβ transcription remains to be elucidated. However, in the near future, natural or synthetic ligands of PPARs might be used in a broad spectrum of reproduction treatments such as improvement of follicular development and ovulation, infertility associated with metabolism disorders, regulation of steroidogenesis, certain complications of pregnancy, and sex-hormone-sensitive cancers affecting reproductive tissues, such as breast, ovary, and pituitary adenomas.

References


