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Minireview

Mapping PTGERs to the Ovulatory Follicle: Regional Responses to the Ovulatory PGE2 Signal[1]

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ABSTRACT

Prostaglandin E2 (PGE2) is a key intrafollicular mediator of ovulation in many, if not all, mammalian species. PGE2 acts at follicular cells via four distinct PGE2 receptors (PTGERs). Within the ovulatory follicle, each cell type (e.g., oocyte, cumulus granulosa cell, mural granulosa cell, theca cell, endothelial cell) expresses a different subset of the four PTGERs. Expression of a subset of PTGERs has consequences for the generation of intracellular signals and ultimately the unique functions of follicular cells that respond to PGE2. Just as the ovulatory LH surge regulates PGE2 synthesis, the LH surge also regulates expression of the four PTGERs. The pattern of expression of the four PTGERs among follicular cells before and after the LH surge forms a spatial and temporal map of PGE2 responses. Differential PTGER expression, coupled with activation of cell-specific intracellular signals, may explain how a single paracrine mediator can have pleotropic actions within the ovulatory follicle. Understanding the role of each PTGER in ovulation may point to previously unappreciated opportunities to both promote and prevent fertility.

follicle, ovary, ovulation, prostaglandin, receptor

PROSTAGLANDIN E2: AN ESSENTIAL PARACRINE MEDIATOR OF OVULATION

The ovulatory surge of LH is the initial endocrine stimulus for ovulation in mammals. LH acts at its receptors, which are located on a subset of follicular cells, including theca and the outermost mural granulosa cells [1, 2]. For the other cells of the follicle, the LH signal is transmitted indirectly via paracrine signals.

Prostasglandin E2 (PGE2) is a key paracrine mediator of ovulation. LH increases granulosa cell expression of essential prostaglandin synthesis enzymes, including the key enzyme PTGS2 (also known as COX2, [3]). The time interval between the LH surge and ovulation varies between rodents (12–16 h), cows (28–30 h), monkeys and women (37–42 h), and horses (39–48 h). However, in many (if not all) mammalian species, follicular levels of PGE2 reach peak levels in the hours just before ovulation (reviewed in [4]).

The interplay between PGE2 and other LH-stimulated paracrine mediators is unclear. For example, epidermal growth factor (EGF)-like growth factors such as amphiregulin have been reported to increase follicular PGE2, but PGE2-stimulated amphiregulin production has also been reported [5–7]. Similarly, progesterone has been reported to regulate PGE2 synthesis and also to be regulated by PGE2 [8–11].

Elevated follicular PGE2 is essential for successful ovulation. Mice lacking expression of PTGS2 or the PGE2 receptor PTGER2 fail to ovulate [12–14]. Administration of drugs that inhibit PTGS2 activity disrupts ovulatory events, such as cumulus expansion, follicle rupture, and oocyte release [15–35]. For many species, cotreatment with PGE2 restored ovulatory events [25, 35–37]. Based on these findings, it is widely accepted that PGE2 is the ovulatory prostaglandin and that PGE2 is an essential paracrine mediator of the LH surge in mammalian species. It is interesting to note that, at the time of ovulation, follicular concentrations of PGE2 are in the micromolar range, well in excess of the amount needed to bind to and activate >99% of PGE2 receptors. Of course, only cells with PGE2 receptors are able to respond directly to elevated PGE2 and initiate PGE2-stimulated ovulatory events.

PROSTAGLANDIN E2 RECEPTORS: THE PTGERS

Prostaglandin E2 receptors (PTGERs) transduce the PGE2 signal within individual cells of the follicle (Fig. 1). There are four PGE2 receptors: PTGER1, PTGER2, PTGER3, and PTGER4 [38]. Each of these G-protein coupled receptors activates different intracellular signaling pathways. Each cell type within the ovulatory follicle expresses a different subset of all PTGERs. In this way, each cell can respond to the ovulatory PGE2 stimulus with a unique series of intracellular signals, leading to cell-specific structural and functional changes. Finally, PTGER expression is regulated by the ovulatory LH surge, so PGE2 responses can be altered over the course of the interval between the LH surge and ovulation. For these reasons, mapping the spatial and temporal distribution of PTGERs to the cells of the ovulatory follicle is needed to fully understand the essential and complex role of PGE2 in the ovulatory cascade.

PTGERs are members of the guanine nucleotide-binding (G) protein-coupled family of receptors (GPCRs) [38, 39]. GPCRs are integral membrane proteins consisting of an

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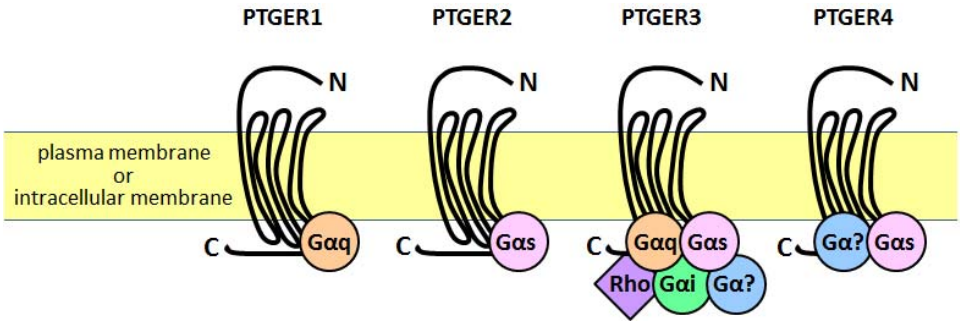

FIG. 1. PGE2 receptors (PTGERs) are members of the seven-transmembrane domain spanning family of receptors. PTGERs can be located in the plasma membrane or intracellular membranes, such as nuclear envelope, endoplasmic reticulum, and Golgi [43–46]. The ligand-binding domain includes the amino (N) terminus and is located in extracellular space or cytoplasm, respectively. The carboxy (C) terminus of each PTGER couples with a unique subset of G-proteins, which can include Gαq, Gαs, Gαi, an unknown pertussis-toxin sensitive G protein (Gα?), or the small GTPase Rho. Each PTGER3 isoform couples with a subset of the G proteins shown.

extracellular amino-terminal domain, seven-transmembrane α helices, and a cytosolic carboxyl-terminal domain [40, 41]. The GPCR forms a tertiary structure like a barrel, with the seven-transmembrane domain surrounding a pocket within the plasma membrane. This pocket and the extracellular domains interact with ligands based on multiple factors such as shape, size, and electrostatic properties [42]. In contrast, the intracellular domains and carboxyl-terminal portion participate in binding downstream proteins, including G proteins, that mediate intracellular signal transduction. PTGERs are most commonly found in the plasma membrane, but identification of functional PTGERs in the nuclear envelope and intracellular membranes has broadened the potential for signaling via PGE2 [43–47].

Four distinct PGE2 receptors have been identified: PTGER1, PTGER2, PTGER3, and PTGER4 (formerly known as EP1–EP4). PTGERs are products of different genes. For some PTGERs, splice variants give rise to functionally distinct isoforms. Each PTGER interacts with different Gα subunits of the heterotrimeric G proteins, leading to activation of different signaling pathways [48]. PTGERs have different affinities for PGE2 and other native eicosanoids [49, 50]. All PTGERs bind PGE2 with affinities in the low nanomolar range, with PTGER3 and PTGER4 binding PGE2 at 10-fold lower concentrations when compared to PTGER1 and PTGER2 [38]. Agonists and antagonists selective for individual PTGERs have been characterized [38–40], so selective activation or inhibition of individual PTGERs is an option for both experimental and therapeutic purposes (Supplemental Table S1; all Supplemental Data are available online at www.biolreprod.org). These differences between PTGERs consequently affect the intracellular response to a given concentration of PGE2.

PTGER1

Human PTGER is a 402-amino acid polypeptide with an estimated molecular mass of 42 kDa [51]. The rat PTGER1 shares 83% amino acid identity with the human PTGER1 [52]. PTGER1 transcript variants, including one which yields an inactive PTGER1 receptor variant [52], have been reported. Currently, PTGER1 cDNA and genomic sequence information is available for many mammalian species [51–54]. PTGER1 responds to PGE2 binding with activation of phospholipase C (PLC), elevated intracellular Ca^{2+}, and activation of protein kinase C (PKC), presumably via interaction with Gαq and generation of inositol trisphosphate (IP3) [51, 55–58]. PTGER1 is most often detected in the plasma membrane [56], but functional PTGER1 has also been localized to the nuclear envelope [43]. Plasma membrane and intracellular PTGER1 share similar PGE2-binding kinetics and activation of similar signal transduction intermediaries [43]. 17-Phenyl-trinor-PGE2 is commonly used as a selective agonist for PTGER1 [59]. Several PTGER1-selective agonists and antagonists have been reported [60–67]. Most notably, GW848687X is highly selective for PTGER1 over other PTGERs. GW848687X blocked PGE2-induced migration in monkey ovarian microvascular endothelial cells [68] and is a candidate for the treatment of acute and chronic inflammatory pain [60].

PTGER2

Human PTGER2 is a protein of 358 amino acids with an estimated mass of 53 kDa [69]. PTGER2 cDNA and genomic sequence information is available for many mammalian species [54, 70–74]. Regardless of species, PGE2 stimulation of PTGER2 leads to activation of the Gαs protein, which in turn stimulates adenylyl cyclase activity and increases cAMP production [38, 69, 73]. Indeed, PTGER2 stimulation is thought to alter cell function exclusively through cAMP generation [75]. PTGER2 shares 30% amino acid identity with PTGER4, so these PGE2 receptors may be inaccurately identified in the older literature [69]. Butaprost is a commonly used PGE2 analog that is an agonist and is highly selective for PTGER2 [50]; additional PTGER2-selective agonists have recently been characterized [64]. AH6809 is commonly used as a PTGER2 antagonist, but this molecule also has modest antagonist activity against PTGER1 and PTGER3 [64, 76]. Selective PTGER2 antagonists are rare [77–79]. However, a recent study described the novel PTGER2 antagonist PF-0441894 with over 2000-fold selectivity for PTGER2 when compared to other PTGERs and can block the ability of both native PGE2 and the selective PTGER2 agonist butaprost to activate PTGER2 [80].

PTGER3

PTGER3 is unique among PTGERs in that multiple isoforms are formed by alternative RNA splicing, resulting in proteins of 40–45 kDa [81–91]. PTGER3 isoforms have essentially identical amino acid sequences in the ligand-binding region and, therefore, possess very similar ligand-binding properties. PTGER3 affinity for PGE2 is at least 40-fold higher than PGE2 affinity for PTGER1 and PTGER2 [50]. PTGER3 isoforms possess distinct sequences in their carboxyl-terminal (cytoplasmic) tails, a key region of the receptor for interaction with G proteins and other signal transduction

molecules [87, 92]. PTGER3 isoforms can increase cAMP, reduce cAMP, or elevate intracellular Ca^{2+} and IP3 response to PGE2, depending on individual isoforms and types of cells [38, 85, 87, 89]. In addition, certain PTGER3 isoforms can interact with the small G protein Rho [93, 94]. PTGER3 has been reported to regulate Gαi-induced adenylyl cyclase inhibition as well as intracellular Ca^{2+} mobilization via a pertussis toxin-sensitive G protein [38, 95]. Some PTGER3 isoforms interact with more than one type of G protein to regulate multiple signal transduction pathways. For example, the mouse PTGER3 isoform EP3γ can couple to both Gαs and Gαi proteins, with PGE2 binding able to both activate and inhibit adenylyl cyclase activity [85]. The ligand-binding domain is identical for all PTGER3 isoforms, so all PTGER3 isoforms are thought to bind a given agonist or antagonist with equal affinity. Sulprostone has been used extensively as a PTGER3 agonist, although sulprostone has moderate affinity for PTGER1 as well [50, 96, 97]. Several potent agonists for PTGER3 have recently been reported, but these molecules all possess some cross-reactivity with other EP receptors [64]. Among these newer PTGER3 agonists, MB28767 is perhaps most selective for PTGER3 [50, 97]. Several compounds with antagonist activities specific for PTGER3 have been reported [64, 67, 98–105]. Of note is L-798106, which prevented PGE2- and sulprostone-stimulated monkey ovarian endothelial cell migration [68].

PTGER4

The receptor currently known as PTGER4 was originally referred to as the EP2 receptor [69, 106]. Human PTGER4 is predicted to be 488 amino acids with molecular mass of 53 kDa [73]. In addition to the human, PTGER4 has been identified in many species including dog, mouse, rabbit, rat, and cow [70, 73, 106–110]. PTGER4 contains a longer carboxyl-terminal tail than that of PTGER2; this longer tail may participate in short-term, agonist-stimulated desensitization and interaction with multiple G proteins [111, 112]. PTGER4 stimulates cAMP production by coupling to the Gαs protein as its major signal transduction pathway [113]. PTGER4 also activates IP3-dependent intracellular signaling [48, 114–116]. Furthermore, PTGER4 can couple to a pertussis toxin-sensitive G protein that can reduce cAMP-dependent signaling in some cells [113]. These additional signal transduction pathways distinguish PTGER4 from PTGER2, which appears to utilize Gαs exclusively. PTGER4 binds PGE2 with at least 10-fold higher affinity than does PTGER2 [50, 112]. Pharmacologically, PTGER4 may be discriminated from other PTGERs by its insensitivity to both the PTGER1/PTGER3 agonist sulprostone and the PTGER2 agonist butaprost and its selectivity for PGE1 alcohol as an agonist [70, 76]. Several agonists that are selective for PTGER4 have also been reported [64, 117], including APS-999 that induced ovarian follicle growth and maturation in rats [118] as well as an orally active PTGER4 agonist (KAG-308; [119]). PTGER4-selective antagonists have also been reported [64, 117, 119–123]. Of specific interest is GW627368x, which is 100-fold selective for PTGER4 over other PTGERs but does have modest affinity for thromboxane receptors [118, 120, 121, 124].

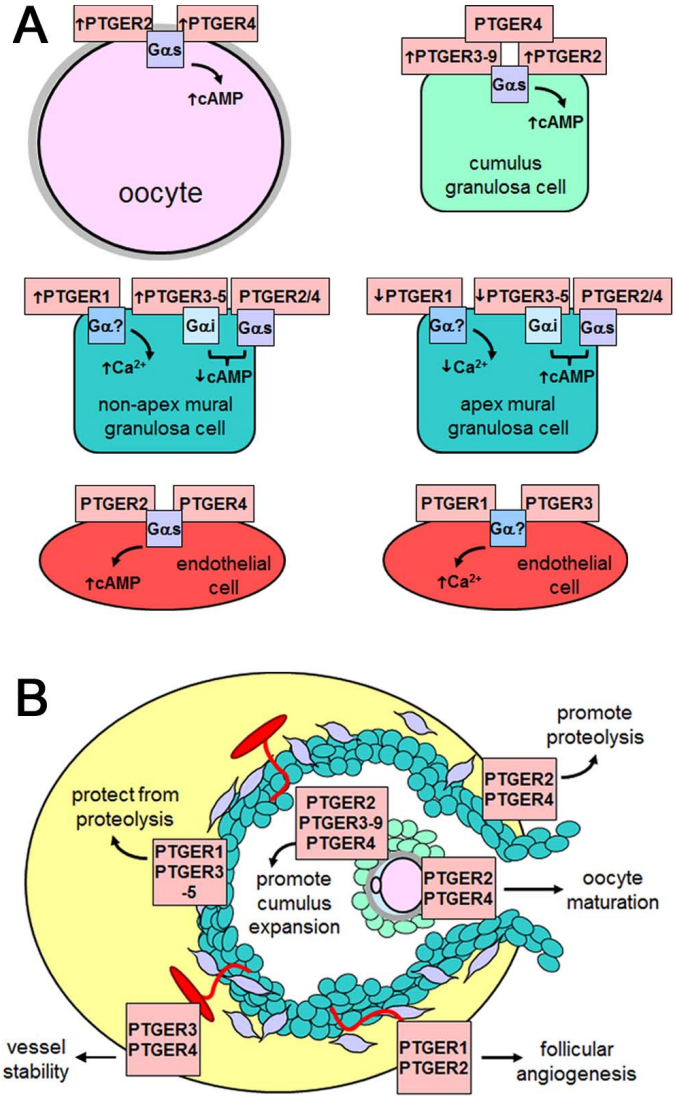

FIG. 2. Spatial map of PTGERs in the primate ovulatory follicle. The oocyte (pink) is surrounded by the zona pellucida (grey) and cumulus granulosa cells (light green). Mural granulosa cells (dark green) intermingle with invading endothelial cells forming capillaries (red lines). Theca cells (purple) and larger vessels (red ovals) are found in the connective tissue surrounding mural granulosa cells. Remainder of the ovary is yellow. **A**) Predominant PTGERs in each cell type are shown with G proteins and key intracellular signals. **B**) PTGER expression in the ovulatory follicle. Predominant PTGERs are listed in the boxes adjacent to the cell type, with arrows indicating the key PGE2-stimulated ovulatory events for each cell type. Oocytes express functional PTGER2 and PTGER4, which influence oocyte maturation. Cumulus granulosa cells express PTGER2, PTGER3 isoform 9, and PTGER4, which increase cAMP and likely promote cumulus expansion. Mural cells opposite the follicle apex have higher levels of PTGER1 and PTGER3 isoform 5, which promote expression of protease inhibitors. Mural granulosa cells at the follicle apex have relatively higher levels of PTGER2 and PTGER4, which increase protease expression. Stromal endothelial cells of established vessels express PTGER3 and PTGER4, which may promote vessel stability. Endothelial cells of invading capillaries express all PTGERs, but in vitro studies indicate that PTGER1 and PTGER2 are key receptors for new vessel formation in the granulosa cell layer. PTGERs in follicular theca cells (purple) have not been characterized and are, therefore, not indicated.

PTGER EXPRESSION AND FUNCTION IN FOLLICULAR CELLS

The ovarian follicle consists of a single oocyte surrounded by somatic cells, including granulosa cells and theca cells (reviewed in [125, 126]) (Fig. 2). The granulosa cells can be classified into distinct subpopulations based upon their location within the follicle and their functions in ovulatory events. Cumulus granulosa cells support the oocyte and expand to facilitate oocyte release at ovulation. Mural granulosa cells line the wall of the ovarian follicle, produce many paracrine

TABLE 1. PTGER functions in ovulation.

Receptor	Technique	Species	Ovarian Phenotype	References
PTGER1	Knockout	Mouse	Normal fertility	[129, 177]
	17-Phenyl-trinor-PGE2 (agonist)	Monkey	Increased follicular angiogenesis	[68]
	17-Phenyl-trinor-PGE2 (agonist)	Monkey	Increased follicle rupture, increased cumulus expansion	[25]
PTGER2	Knockout	Mouse	Reduced fertility, reduced cumulus expansion, decreased ovulation, failure of fertilization	[14, 127, 154]
	Butaprost (agonist)	Mouse	Increased cumulus expansion	[138]
	AH6809 (antagonist)	Mouse	Decreased cumulus expansion, decreased oocyte nuclear maturation	[138]
	Butaprost (agonist)	Mouse	Decreased oocyte nuclear maturation	[135]
	Butaprost (agonist)	Monkey	Increased follicular angiogenesis	[68]
	Butaprost (agonist)	Monkey	Increased follicle rupture, increased cumulus expansion	[25]
	Butaprost (agonist)	Monkey	Decreased oocyte nuclear maturation, decreased fertilization	[135]
PTGER3	Knockout	Mouse	Normal fertility	[129, 177]
	Sulprostone (agonist)	Monkey	Decreased follicular angiogenesis	[68]
PTGER4	Knockout	Mouse	Neonatal lethal	[131, 132]
	PGE1 alcohol (agonist)	Mouse	Decreased oocyte nuclear maturation	[135]
	AH23848 (antagonist)	Mouse	Decreased cumulus expansion, decreased oocyte nuclear maturation	[138]
	PGE1 alcohol (agonist)	Monkey	Increased follicle rupture	[25]
	PGE1 alcohol (agonist)	Monkey	Decreased oocyte nuclear maturation, reduced fertilization	[135]

mediators of ovulation, and remain behind at ovulation to form the corpus luteum. Mural granulosa cells at the follicle apex likely play a key role in follicle rupture, whereas mural granulosa cells located away from the apex may be more involved in steroidogenesis and contribute to the formation of the corpus luteum. Theca cells are steroidogenic cells located outside the granulosa cell basement membrane. Vascular endothelial cells branch from stromal vessels to form the extensive vascular network of the corpus luteum. PTGER localization has been described within many of these follicular cell types. Each PTGER shows a different pattern of expression that varies by cell type and exposure to the ovulatory gonadotropin surge. For some cell types of the ovulatory follicle, specific PGE2-regulated functions have been associated with individual PTGERs.

Global knockout of individual PTGERs has provided useful but limited information on the role of each PTGER in ovulatory processes. Mice lacking PTGER2 demonstrate reduced or failed ovulation, reduction in oocyte fertilization, and reduced litter size from multiple studies [14, 127, 128]. No reproductive abnormalities were reported for mice lacking expression of PTGER1 or PTGER3 [129, 130]. Mice lacking PTGER4 die soon after birth, so adult reproductive function has not been examined [131, 132]. The observation of a reproductive phenotype in only one PTGER knockout focused attention on PTGER2 as the key PGE2 receptor involved in mediating the ovulatory effects of PGE2. However, the role of other PTGERs in ovulation could not be effectively examined using the global knockout strategy. More recent studies using PTGER-selective agonists and antagonists have provided additional and important information to support roles for each PTGER in ovulatory events. Ovulatory functions linked to individual PTGERs are summarized in Table 1 and discussed below.

Oocytes

Multiple PTGERs are expressed in oocytes of several mammalian species, including mouse, cow, and monkey [133–135]. Messenger RNA for each PTGER was detected in monkey oocytes, while mRNA for PTGER2, PTGER3, and PTGER4 was detected in mouse oocytes [135]. Importantly, only PTGER2 and PTGER4 proteins have been detected in oocyte plasma membranes, and both mouse and monkey oocytes responded to PTGER2 and PTGER4 agonists with increased cAMP, demonstrating that these PTGERs are functional in oocytes [135].

Little is known regarding the actions of PGE2 via oocyte PTGERs. PGE2 treatment of monkey and mouse oocytes in vitro delayed nuclear maturation from the germinal vesicle to metaphase II stage [135]. Monkey (but not mouse) oocytes treated with PGE2 also showed reduced rates of fertilization in vitro [135]. Interestingly, treatment of bovine oocytes with the PTGER2 antagonist AH6809 reduced nuclear maturation [136]. These disparate findings are difficult to reconcile, especially in the context of studies that show elevated cytoplasmic cAMP maintains meiotic arrest in the oocyte [137]. Many studies report that treatment of cumulus-enclosed oocytes with PGE2 improves oocyte maturation [136, 138–140], but the effects of PGE2 on the oocyte cannot be distinguished from the effect of PGE2 on cumulus cells, which also express PGE2 receptors. Oocyte PTGERs may also play a role in the development of the ovary. In situ hybridization analysis showed the signal for PTGER4 mRNA within oocytes of mouse preantral follicles [133]. PTGER2 and PTGER4 have also been reported in germ cells in the developing human ovary, where PGE2 regulates expression of key germ cell genes [141].

Cumulus Granulosa Cells

Cumulus cell expression of PTGERs has been examined for many mammalian species, including mouse, rat, cow, pig, horse, monkey, and human [118, 133, 138, 140, 142–145]. While sensitive microarray techniques can detect expression of most or all PTGERs in cumulus cells, patterns of PTGER expression have emerged that cross species. PTGER1 is rarely detected in cumulus cells. Expression of PTGER2 and PTGER4 is commonly measured, and expression of these PTGERs is elevated after the ovulatory gonadotropin surge in many species [54, 133, 140, 143, 144]. Cumulus expression of PTGER3 has been reported for mouse, cow, and monkey [138, 142, 144], but increased PTGER3 mRNA has been reported after the ovulatory gonadotropin surge only in primates [144]. In monkey cumulus, the PTGER3 isoform 9 was the predominant PTGER3 isoform detected, with mRNA levels elevated 100-fold after the ovulatory gonadotropin surge [91]. The presence of receptor protein for PTGER2 [54, 138, 143], PTGER3 [138, 144], and PTGER4 [138, 144] has been confirmed for cumulus from several species. In monkey

cumulus, the ovulatory gonadotropin surge increased the levels of PTGER2 and PTGER3 proteins. PTGER2, PTGER3 isoform 9, and PTGER4 respond to PGE2 stimulation with increased cAMP (discussed above), consistent with a role for elevated cAMP mediation of essential cumulus functions such as expansion (discussed below). However, increased cAMP production by cumulus cells via receptor-selective agonist has only been confirmed for PTGER2 stimulation of monkey cumulus [146].

PGE2 is well-established as a paracrine mediator of cumulus cell function. PGE2 treatment stimulates expansion of cumulus-oocyte complexes in vitro in mouse, pig, horse, and monkey [13, 140, 146–148]. PGE2 is known to increase cumulus cell synthesis of proteins such as hyaluronan synthase 2 (HAS2) and hyaluronan, which play important roles in the production of the novel extracellular matrix of the expanded cumulus [140, 146, 147]. Inhibition of PTGS2 activity and PGE2 synthesis in porcine cumulus-oocyte complexes blunted FSH-stimulated production of the EGF-like factors amphiregulin and epiregulin as well as TNFα-converting enzyme/A disintegrin and metallopeptidase domain 17 (TACE/ADAM17) [140]. PGE2 cotreatment restored FSH-stimulated synthesis of these important ovulatory mediators in porcine cumulus-oocyte complexes [140], indicated a key role for PGE2 in these ovulatory events. In addition, the expression of the cumulus expansion-related HAS2 and tumor necrosis factor α-induced protein 6 (Tnfaip6) genes were decreased by inhibition of prostaglandin production with suppression of cumulus expansion, which was restored by PGE2 as well as EGF [140].

PTGER2 is the only PTGER that shows a reproductive deficit with global deletion [14, 127, 128, 149], so examination of PGE2 receptors in mice has focused on PTGER2. PTGER2-deficient mice were found to have a failure of cumulus expansion after an ovulatory gonadotropin stimulus in vivo, and cumulus-oocyte complexes from mice lacking PTGER2 expression failed to undergo cumulus expansion in response to PGE2 in vitro [14]. In monkeys, cumulus-oocyte complexes experienced cumulus expansion when an agonist selective for either PTGER1, PTGER2, or PTGER4 was co-administered with a PGE2 synthesis inhibitor in vivo [25], but the specific cellular target of these PTGER-selective agonists is not known in these in vivo studies. In vitro, stimulation of either PTGER2 or PTGER4 resulted in full expansion of mouse cumulus-oocyte complexes [138], while PTGER2 was shown to mediate cumulus expansion in primate cumulus-oocyte complexes in vitro [146]. Proper expression of cumulus expansion-related proteins is disrupted in mice lacking PTGER2 expression [150, 151]. While studies continue to focus on PTGER2 as the key receptor to mediate PGE2-stimulated cumulus expansion, PTGER1, PTGER3, and PTGER4 may participate in this process as well.

Mural Granulosa Cells

Considerably less attention has been paid to individual PTGERs in mural granulosa cells. Expression of many or all PTGERs have been reported in granulosa cells from ovulatory follicles of mouse, rat, cows, sheep, horse, monkey, and human [118, 133, 143, 144, 152–157]. PTGER2 is expressed in mural granulosa cells of mouse follicles [133], but, perhaps surprisingly, little or no information is available for expression of other PGE2 receptors specifically in mural granulosa cells of rodent follicles. Mural granulosa cell expression of PTGER2 and PTGER4 was shown to increase in response to the ovulatory gonadotropin surge in horse and monkey [143, 144]. Additional studies identified five distinct PTGER3 isoforms with increased mRNA levels after the ovulatory gonadotropin surge in monkey mural granulosa cells [91]. The ovulatory gonadotropin surge also led to significant increases in granulosa cell levels of PTGER1, PTGER3, and PTGER4 proteins in monkey [144] and PTGER2 protein in horse [143]. In summary, all four PTGERs are expressed by mural granulosa cells in a dynamic fashion, with changes in mRNA and/or protein reported in response to the ovulatory gonadotropin surge.

Few studies have examined differential PTGER expression in subpopulations of mural granulosa cells. Recently, laser capture microscopy was used to successfully separate mural granulosa cell subpopulations based on their location within monkey ovarian follicles and examine PTGER expression in mural granulosa cell at the apex of the follicle and mural granulosa cells not at the follicle apex [91, 144]. While the ovulatory gonadotropin surge increased PTGER1 protein levels in all mural granulosa cells, PTGER1 protein was present at higher levels in granulosa cells not at the follicle apex when compared to granulosa cells at the follicle apex just before ovulation. PTGER4 protein levels were lower in apex when compared to nonapex mural granulosa cells before the ovulatory gonadotropin surge, but no differences in PTGER4 protein levels were noted between these subpopulations after the gonadotropin surge. No differences in PTGER2 protein levels were noted between mural granulosa cell subpopulations before or after the ovulatory gonadotropin surge [144]. While overall mural granulosa cell PTGER3 protein levels were significantly higher after the ovulatory gonadotropin surge, this increase was not specific to an individual granulosa cell subpopulation [144]. Interestingly, mRNA for PTGER3 isoform 5 increased in apex mural granulosa cells in response to the ovulatory gonadotropin surge, while PTGER3 isoform 5 mRNA levels were not altered in nonapex mural granulosa cells, suggesting preferential expression of this PTGER3 isoform in subpopulations of mural granulosa cells [91].

Certain PGE2-stimulated follicular events have been attributed to mural granulosa cells. In vivo studies using an ablate-and-replace model have demonstrated unequivocally that PGE2 is involved in follicle rupture [32, 35]. Despite this critical role, very few PGE2-regulated mRNAs or proteins have been identified. Certain proteases, protease inhibitors, and components of the extracellular matrix are regulated by PGE2, including matrix metalloproteinases 1 (MMP1), tissue-type plasminogen activator (PLAT, also known as tPA), PA inhibitor type 1 (SERPINE1, also known as PAI-1), glucosamine, and some forms of collagen [158–161]. In monkey mural granulosa cells, regulation of PLAT and SERPINE1 were attributed to specific PTGERs, with PTGER2 and PTGER3 mediating increased PLAT protein while PTGER1 and PTGER3 stimulation increased SERPINE1 protein in vitro [159].

Steroidogenesis is widely believed to be a key function of mural granulosa cells. PGE2 increased both aromatase (CYP19) expression and progesterone production by rat granulosa cells [162, 163]. PGE2 stimulated progesterone production by bovine cells from both small (<5mm) and large (>8mm) ovarian follicles while inhibiting estradiol secretion [164]. In human follicular fluid, elevated PGE2 concentrations correlated with elevated progesterone concentrations, and PGE2 increased STAR mRNA and protein as well as progesterone production by human granulosa-luteal cells in vitro [165, 166]. Overall, these findings suggest that PGE2 may enhance progesterone synthesis by the ovulatory follicle or young corpus luteum. In contrast, PGE2 did not alter estrogen or progesterone production by pieces of human ovulatory

follicle wall in vitro [167]. In vivo administration of a PGE2 synthesis inhibitor without or with replacement of PGE2 or an individual PTGER agonist did not alter indicators of mural cell progesterone synthesis, including mural granulosa cell levels of 3β-hydroxysteroid dehydrogenase (HSD3B), follicular fluid progesterone levels, or serum progesterone levels in species as diverse as cows, monkeys, and women [25, 27, 33, 34]. While widely studied in vivo and in vitro, the effects of PGE2 on steroidogenesis are equivocal and may vary by species.

Theca Cells

Theca cells have been suggested as targets for PGE2 action. Expression of PTGER2, PTGER3, and PTGER4 has been reported in bovine theca cells surrounding ovulatory follicles [168]. PTGER2 mRNA has also been detected in theca cells of horse and mouse ovulatory follicles [14, 133, 143]. No change in PTGER2 mRNA was observed in theca cells of mouse preovulatory follicle in response to the ovulatory gonadotropin surge [14, 133]. In contrast, PTGER expression is regulated by the ovulatory gonadotropin surge in domestic animal species. The ovulatory gonadotropin surge increased PTGER2 mRNA and protein in theca surrounding horse ovulatory follicles, where PTGER2 mRNA and protein increased from pre-hCG levels to peak late in the ovulatory interval [143]. In detailed studies of theca PTGER expression, it was reported that the ovulatory gonadotropin surge increased theca expression of PTGER2 while decreasing PTGER3 and PTGER4 expression levels [155, 156]. There are no reports of PTGER1 detection in theca for any species.

The role of PGE2 action at theca PTGERs has received little attention. PGE2 stimulates steroidogenesis in isolated theca cells from a variety of mammalian species, with increases in production of progesterone, estradiol, androstenedione, and testosterone reported [155, 156, 169, 170]. However, PGE2-stimulated steroid hormone production has not been attributed to individual PTGERs.

Vascular Endothelial Cells

The ovulatory LH surge promotes angiogenesis of the luteinizing follicle in mammals [171], and blockade of follicular angiogenesis prevents ovulation [172], supporting an essential role for new capillary formation in the ovulatory cascade. Recently, PGE2 was shown to mediate some actions of the ovulatory gonadotropin surge to increase capillary formation in the luteinizing follicle [68].

Endothelial cells from monkey and human ovulatory follicles expressed all PTGERs in vitro [68, 173]. Immunodetection of PTGERs in monkey ovarian tissues confirmed spatial differences in PTGER distribution. PTGER1 and PTGER2 were only detected on endothelial cells of capillary-like structures forming in the granulosa cell layer of the ovulatory follicle while PTGER3 and PTGER4 were present in stromal vessels and in follicular endothelial cells forming capillary-like structures [68]. Agonists selective for PTGER1 and PTGER2 promoted new capillary formation while stimulation of PTGER3 inhibited capillary formation sprouting in monkey ovarian endothelial cells in vitro and in monkey ovulatory follicles in vivo [68]; similar findings were recently reported using human ovarian endothelial cells in vitro [173]. In rat luteal endothelial cells, PTGER2 antagonist inhibited PGE2-induced tube formation and partly suppressed the VEGF-induced tube formation in vitro [174]. These findings indicate that PTGER1 and PTGER2 may be most important for new capillary formation in the ovulatory follicle.

SUMMARY: THE PTGER SPATIAL AND TEMPORAL MAP

Each type of cell within the ovulatory follicle possesses a unique complement of PTGERs. Functional studies, when available, support the concept that a subset of all PTGERs mediates PGE2 action within a specific type of cell. In addition, PTGER levels often change in response to the ovulatory gonadotropin surge, providing another variable in the ovulatory follicle's response to rising PGE2 levels.

We have summarized the expression of key PTGERs within distinct cell types of the monkey ovulatory follicle, which shares many features of PTGER expression with other mammalian species. As PGE2 levels peak and ovulation approaches, the oocyte, cumulus cells, apex mural granulosa cells, nonapex mural granulosa cells, and vascular endothelial cells each express a different subset of all PTGERs. The PTGERs anticipated to dominate PGE2 responses in each cell type are shown in Figure 2.

In the oocyte, PTGER2 and PTGER4 dominate, and both receptors couple to Gαs to increase cAMP. Stimulation of these receptors delays nuclear maturation and fertilization of the oocyte in vitro and may play a similar role in vivo.

Cumulus cells express primarily PTGER2 and PTGER4, with evidence of PTGER3 isoform 9 expression specifically in primates. These PTGERs couple to Gαs to increase cAMP in cumulus cells. PGE2 action via these receptors may promote cumulus expansion and may also support oocyte health, maturation, and ultimately fertilization.

Mural granulosa cells express all PTGERs. All four PTGERs may be expressed in the majority, if not all, mural granulosa cells. However, PGE2 action via PTGER2 and PTGER4 likely dominates granulosa cell responses at the follicle apex while PGE2 action via PTGER1 and PTGER3 isoform 5 likely dominates in mural granulosa cells elsewhere along the follicle wall. The importance of this spatial distribution is highlighted in studies examining components of the plasminogen activator family of proteases. PTGER2 and PTGER4 couple to Gαs to increase cAMP levels [54]; this signaling pathway increased PLAT levels in monkey granulosa cells [159] and is consistent with the presence of multiple rupture sites in monkey ovulatory follicles injected with agonists selective for PTGER2 and PTGER4 [25]. In contrast, PGE2 stimulation of PTGER1 and PTGER3 isoform 5 overall reduces cAMP while increasing intracellular Ca^{2+} and presumably additional downstream intracellular signals [54, 91]. These pathways were linked to enhanced expression of SERPINE1 [159], consistent with limited proteolysis in regions of the follicle other than the follicle apex. In this way, the distribution of PGE2 receptors may regulate proteolysis in a regional fashion to ensure that a single rupture site occurs at ovulation.

The vascular endothelial cells of stromal vessels express PTGER3 and PTGER4 [68] and may participate in maintenance of stable vessels. Endothelial cells invading the granulosa cell layer as ovulation nears continue to express PTGER3 and PTGER4 but also express PTGER1 and PTGER2. PTGER1 and PTGER2 promoted ovarian endothelial cell sprout formation in vitro and angiogenesis in the ovulatory follicle in vivo [68, 173], supporting the concept that PGE2 action via PTGER1 and PTGER2 are critical for ovulatory angiogenesis.

Information regarding PTGER expression in primate theca cells is not available. However, data obtained from other species suggest that PTGER2 and likely additional PTGERs respond to PGE2 stimulation to regulate steroid hormone production.

CONCLUSIONS AND PERSPECTIVES

PGE2 action via PTGERs within the ovarian follicle is clearly a necessary step in the ovulatory cascade. However, very limited information is available regarding specific, critical actions of PGE2. Few gonadotropin-regulated gene products have been shown to be dependent on elevated intrafollicular PGE2 levels. Microarray approaches have identified few PGE2-regulated gene products [175]. Use of technologies to eliminate expression of a single PTGER in individual follicular cell types may be helpful to clarify the physiological changes mediated via each PTGER during the ovulatory cascade.

Assessment of PGE2 action at each follicular cell type has provided some insight into possible ovulatory functions of PGE2. This review considers effects of PGE2 on oocytes, cumulus cells, mural granulosa cells, theca cells, and vascular endothelial cells. However, the ovulatory follicle includes other cell types including stromal fibroblasts, lymphatic endothelial cells, and invading cells of the immune system. Virtually nothing is known about PTGER localization and PGE2 action at these cells in the context of the ovulatory follicle. However, important roles for PGE2 in the regulation of inflammatory and immune responses in immune cells are well established, and work in this area includes identification of individual PTGERs responsible for mediating PGE2 actions [48, 176].

The spatial and temporal map of PTGER distribution within the ovulatory follicle may be useful to target therapeutics to enhance or reduce ovulation. Infertility in mice with global deletion of PTGER2 focused researchers on the critical role of PTGER2 in ovulatory events. Recently, Peluffo and colleagues demonstrated the potential for a selective PTGER2 antagonist as a novel method to block ovulation for contraceptive use [146]. Conversely, agonist action at PTGER2 may promote fertility. In monkeys, PTGER1 has been specifically implicated in both follicle rupture and follicular angiogenesis [25, 159], suggesting that this PTGER is also a potential target for promoting fertility or for development of novel contraceptives.

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