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Lysophosphatidic Acid (LPA) Signaling in Vertebrate Reproduction

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Abstract

Lysophosphatidic acid (LPA) is a cell membrane phospholipid metabolite that can act as an extracellular signal. Its effects are mediated through at least five G protein-coupled receptors (GPCRs), LPA_{1-5}, and likely others as well. Studies in multiple species including LPA receptor-deficient mice and humans have identified or implicated important roles for receptor-mediated LPA signaling in multiple aspects of vertebrate reproduction. These include ovarian function, spermatogenesis, fertilization, early embryo development, embryo implantation, embryo spacing, decidualization, pregnancy maintenance, and parturition. LPA signaling may also have pathological consequences, influencing aspects of endometriosis and ovarian cancer. Here we review recent progress in LPA signaling research relevant to female and male reproduction.

Overview

Lysophosphatidic acid (LPA) is an extracellular lipid signaling molecule that produces a broad range of cellular influences including survival, differentiation, proliferation, morphological changes, migration, and others. Within vertebrates, LPA signaling has been implicated in numerous physiological and pathological processes affecting most, if not all, organ systems [1-4]. One key area LPA influences is reproduction, both male and female. This review briefly introduces LPA signaling and reviews its effects on reproduction. Additional details on LPA signaling can be found in recent reviews that also note a related lysophospholipid, sphingosine 1-phosphate (S1P) that has other influences on reproduction but will not be discussed here [5-12].

LPA production and metabolism

LPA is present in many biological fluids like serum (up to micromolar concentration), plasma, saliva, blister fluids, tears, chicken egg whites, follicular fluid, seminal plasma, and ascites fluids. LPA can be produced by myriad different cell types that include postmitotic neurons, adipocytes, mast cells, other lymphoid cells, endometrial cells, erythrocytes and cancer cells as well as activated platelets (reviewed in [2,13,14]). There are different species of LPA

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because of various acyl chain lengths and degrees of saturation, and position on the glycerophosphate backbone. A commonly used form in the laboratory is 18:1 or oleyl-LPA [5]. While precise and accurate mechanisms accounting for LPA metabolism within most cell types are still unclear, two general pathways of LPA production have been demonstrated. One pathway involves the generation of precursor lysophospholipids (LPLs) from various membrane phospholipids (PLs) by phospholipase A 1 and 2 ($PLA_{1/2}$), followed by the action of autotaxin (ATX) that is a lysophospholipase D. A second pathway involves formation of phosphatidic acids (PAs) from PL cleavage by phospholipase D (PLD) or from diacylglycerol by diacylglycerol kinase (DGK), and deacylation of PA by PLA-type enzymes. LPA can be metabolized to monoacylglycerol (MAG) by lipid phosphate phosphatases 1-3 (LPP1-3) and lysophospholipases. This process can be reversed by MAG kinase, while the actions of a variety of acyl transferases can also remove LPA from signaling or structural pools [12,14] (Figure 1). It has been reported that lysophospholipids are the main precursors for LPA production in serum, plasma, and adipocytes, whereas in the platelets and some cancer cells, LPA is mainly derived from phosphatidic acids (PAs) [14]. These differing routes of LPA biosynthesis likely reflect multiple levels of regulation – or disregulation in cancers – available to cells having different lineages or functions. These distinctions are reflected not only in the penultimate precursor molecule, but also in the employed enzymes that produce LPA, such as the lysophospholipase D called autotaxin or ATX that can also be produced locally and plays a key role in determining circulating LPA levels in adult animals [15,16] by acting on lysophosphatidyl choline (LPC) to produce LPA, vs. cytoplasmic phospholipase A2 (cPLA2) that acts on PAs. These respective enzymes are in turn regulated in different ways, e.g. through transcriptional or post-translational processes that are themselves influenced by both cell autonomous and non-cell autonomous signals. In addition to its biological significance, these different signaling pathways have clear therapeutic repercussions since compounds targeting ATX for pharmaceutical uses [3,17] would differ from those targeting other LPA biosynthetic pathways. Extracellular LPA is normally protein-bound to albumin, fatty acid binding proteins, other lipoproteins and gelsolin, all of which increase LPA's stability and aid in its transport [18-21], representing yet another level of control.

Receptor-mediated LPA signaling

The study of receptor-mediated LPA signaling mechanisms began with the cloning of LPA receptors and the establishment of receptor-mediated functions. So far five *bona fide* LPA receptors, $LPA_{1\text{-}5}$, have been identified [1,5,9,12,22]. Their human genes are designated *LPARx* with x=1-5 (human genome organization (HUGO)), while mouse names are *Lparx*, with x also =1-5 (Genome Informatix (MGI)) [5]. P2Y5 has been confirmed to be a new LPA receptor, LPA_6 [23,24]. Two additional possible LPA receptors, GPR87 and P2Y10, have also been reported [25,26], but await validation. LPA receptors are all cell-surface, seven transmembrane spanning G protein-coupled receptors (GPCRs). They can differentially couple with $G_{\alpha 12/13}$, $G_{\alpha q}$, $G_{\alpha i/o}$, or in one instance, $G_{\alpha s}$ to activate downstream signaling pathways leading to gene regulation and LPA-induced cellular functions (Table 1) [1,12,24,27]. In addition, LPA receptors may have preferences for certain chemical ligand structures. For example, LPA_3 has a relatively high affinity for 2-acyl-LPA containing unsaturated fatty acids (with the ester-linked fatty acid in the 2 position) whereas other receptors such as LPA_1 and LPA_2 do not discriminate 1-acyl- and 2-acyl-LPA [28-30]. LPA receptors also have overlapping and differential gene expression patterns [2]. These LPA receptor characteristics contribute to receptor activities and functions [10,31]. A critical, strategic approach in determining the biological roles for LPA signaling has been via the creation and study of mice null for one or more LPA receptors [5-12]. In this way, roles for receptor-mediated signaling in reproduction were identified, whereby LPA_3 was found to affect embryo spacing and embryo implantation [32,33], and three receptors, LPA_1, LPA_2, and LPA_3 combined to affect spermatogenesis [34] (Table 2).

LPA signaling in ovary

LPA signaling has been extensively studied in ovarian cells and the ovary. In one study, mRNA expression of three LPA receptors, LPA_1, LPA_2, and LPA_3 was detected in the granulosa-lutein cells from women undergoing *in vitro* fertilization (IVF) [35]. LPA_4 is highly expressed in human and mouse ovary [36,37]. LPA itself is present at significant levels in follicular fluid of the human preovulatory follicle [38]. Serum ATX/lysoPLD activity from patients receiving ovarian stimulation was higher than in women with natural cycles [35], and LPA was induced in incubated human follicular fluid by ATX/lysoPLD [38], suggesting that ovarian stimulation in women may increase LPA levels. High amounts of LPA are also known to be present in chicken eggs [39,40].

LPA signaling plays multiple roles in ovarian function (reviewed in [2]). LPA induces Ca^{2+}-activated Cl^- current in naked *Xenopus laevis* oocytes via $G_{\alpha i}$. Also, LPA receptor(s), $G_{\alpha i}$, and ERK (extracellular signal-regulated kinase)/p38 signaling pathways were implicated in mouse oocyte maturation *in vitro*. LPA induced expression of angiogenic cytokines, interleukin-6 (IL-6) and IL-8, in granulosa-lutein cells from women undergoing IVF. LPA_1, $G_{\alpha i}$, MAPK (mitogen-activated protein kinase)/p38, PI3K (phosphoinositol 3-kinase)/Akt, and NF-κB signaling pathways, and LPA_2, $G_{\alpha i}$, MAPK/p38, and NF-κB signaling pathways were shown to be involved in the LPA-induced IL-8 and IL-6 expression, respectively. Excessive induction of these angiogenic cytokines by LPA from multiple corpora luteae of stimulated ovaries may pathophysiologically contribute to ovarian hyperstimulation syndrome, a complication from some fertility medication with symptoms of abdominal bloating, nausea, to name a few [35]. LPA also induced Chinese hamster ovary (CHO) cell growth through $G_{\alpha i}$ [41], suggesting its physiological and pathological roles in CHO cells. LPA signaling also affects bovine ovarian theca cells and luteal cells, inducing ERK phosphorylation through the LPA_1-$G_{\alpha 12/13}$ signaling pathway; redistribution of protein kinase C delta (PKCδ) from the cytosol to the perinuclear area; morphological changes and steroid synthesis [42-44].

Available data from LPA receptor-null mice indicate that LPA signaling may not be critical for ovulation in mice. LPA_1, LPA_2, and LPA_4 are expressed in mouse ovary [37,45], while expression data for LPA_3 in mouse ovary are inconsistent [32,45]. However, deletion of any of these receptors as well as $LPA_{1/2/3}$ does not cause any obvious defect in ovulation [32,37, 46]. LPA signaling in human ovulation has yet to be determined.

LPA signaling may play a significant role in ovarian pathology, especially relevant to ovarian cancer, through both LPA levels and LPA receptor upregulation (reviewed in [2,20]). LPA levels are elevated in the plasma and ascites of ovarian cancer patients. LPA_2 and LPA_3, but not LPA_1, were upregulated in ovarian cancer tissues. LPA promoted ovarian cancer cell proliferation and migration, and was suggested as a potential biomarker for ovarian cancers [5,47], although this relationship remains controversial [48]. A complex interplay of many other molecular factors modulate LPA signaling in ovarian cancer, examples of which include glycodelin, TRIP6 (thyroid receptor interacting protein 6), telomerase, granulin-epithelin precursor, Fas (which internalizes from the cell membrane to the cytosol), IL-6, IL-8, cyclooxygenase-2 (COX-2), growth-regulated oncogene alpha (GROα), and urokinase plasminogen activator (uPA) [49]. Several LPA-induced downstream signaling pathways have been identified in ovarian cancer cells. For example, uPA upregulation by LPA was mainly mediated through the $G_{\alpha i}$-Ras-PKCα-CARMA3-NF-κB signaling pathway [50,51]. LPA-induced IL-6 expression was via a $G_{\alpha i}$/PI3K-Akt/NF-κB pathway. Both $G_{\alpha i}$-Ras-MEKK1 (MAPK kinase kinase 1) and $G_{\alpha 12/13}$-RhoA-ROCK (Rho-associated kinase) signaling pathways contributed to LPA-stimulated ovarian cancer cell migration.

In addition, LPA signaling induces breast cancer cell proliferation and chemotaxis. LPA_2 is upregulated in mammary gland carcinoma tissue, and it has been suggested that LPA signaling may also play a role in breast cancer progression (reviewed in [2]). The involvement of LPA signaling in female reproductive organ function and dysfunction indicate common mechanistic threads that should provide insights into normal function, disease, and future therapeutic strategies. LPA signaling in both ovarian and breast cancers may allow the development of novel treatments, particularly in cases where these cancers are co-morbid.

LPA signaling in the oviduct

It was suggested that the receptor-mediated $G_{\alpha i}$-Ca^{2+} signaling pathway was involved in LPA-induced mouse ovum transport [52]. However, in mice, deletion of LPA_1, LPA_2, or LPA_3, the three $G_{\alpha i}$-coupled LPA receptors expressed in the oviduct, did not grossly appear to affect embryo transport to the uterus when the blastocysts were examined in embryonic day 3.5 (E3.5) mouse uterus (E0 is defined as the mating night) [32,46]. This suggests that 1) receptor-mediated LPA signaling is involved in embryo transport in the oviduct and the effect was not detectable at E3.5 but at an earlier time point not been determined by the authors; 2) LPA signaling is not critical for embryo transport in the oviduct under physiological conditions, and/or 3) if LPA signaling is indeed important for ovum transport, LPA_4 and/or other yet-to-be identified $G_{\alpha i}$-coupled LPA receptor(s) in the mouse oviduct can compensate for null mutations of normally expressed LPA_1, LPA_2, or LPA_3, a possibility that remains to be examined.

LPA signaling in uterus and pregnancy maintenance

LPA can be locally produced and released in the bovine endometrium during estrous and early pregnancy [53]. It is also detected in the ovine uterus at early pregnancy stages [54]. LPA containing varied fatty acid acyl chains was also detected in the porcine uterine lumen on day 12 of both the estrous cycle and pregnancy [55]. The production of LPA in mouse uterus has not been specifically determined; however, LPA-producing enzymes, PLD_1 and PLD_2, are expressed in young adult mouse uterus (unknown stage of estrous cycle) [56].

Gene expression patterns of LPA_3 in porcine uterus suggest that this isoform plays a role during early pregnancy, as the predominant LPA receptor subtype of four LPA receptors examined ($LPA_{1\text{-}4}$) and regulated by pregnancy and estrous cycle [55]. LPA_3 gene expression peaked on day 12 of pregnancy, when an embryo undergoes a dramatic elongation process prior to implantation [57], localized in the luminal and glandular epithelium, and induced by estrogen in the endometrium [55]. By comparison, studies in the mouse uterus indicate that LPA_3 is mainly localized in the luminal epithelium, upregulated by progesterone and unlike the porcine uterus, is downregulated by estrogens [32,58]. In addition, the presence of embryos induces porcine uterine LPA_3 expression [59], contrasting with that in mice, wherein LPA_3 had similar gene expression patterns in uteri from early pregnant and pseudopregnant (mating with a vasectomized male) females [32,58].

A dramatic effect of LPA signaling in the uterus was identified in LPA_3-deficient mice, where loss of LPA_3 influenced embryo implantation and spacing [32,33]. Embryo implantation involves a competent embryo, a receptive uterus, and their reciprocal interaction [60]. Embryo spacing refers to the regular and approximately equidistant implantation of embryos along the uterus of polytocous (multiple offspring in a single birth) species. Deletion of LPA_3 in mice led to uneven embryo spacing and delayed embryo implantation [32]. This was associated with delayed embryonic development, prolonged pregnancy duration, and ~50% embryonic lethality. Decidualization was not adversely affected in LPA_3-deficient uterus. Embryo crowding and delayed implantation seem to be two segregated events, in that restoration of on-time implantation by exogenous prostaglandins PGE_2 and PGI_2 failed to correct embryo

crowding in LPA_3-deficient females while transferring a single embryo, which does not pose embryo crowding, to pseudopregnant LPA_3-deficient uterus does not restore delayed embryo implantation [32,33].

Prostaglandins (PGs) were identified to be at least partially responsible for the phenotypes of LPA_3–deficient females. Expression of COX-2, important in PG synthesis, as well as levels of the PG forms PGE_2 and PGI_2 were suppressed in pre-implantation E3.5 LPA_3-deficient uteri. Exogenous PGE_2 and PGI_2 rescued delayed implantation in the LPA_3-deficient females, reinforcing the importance of PGs in embryo implantation [61-63]. However, PGE_2 and PGI_2 failed to correct embryo spacing, suggesting that other PGs that control uterine contraction or possibly non-PG mechanisms may be responsible for embryo spacing [33].

LPA signaling, COX-2, and prostaglandins have also been examined in porcine and bovine uterus. LPA can induce COX-2 expression in porcine uterine endometrium, suggesting that LPA produced in the uterine endometrium may play a role in porcine uterine endometrial function [55]. In bovine uterus, LPA is locally produced and released from the endometrium. LPA can induce progesterone and PGE_2 secretion and increase the $PGE_2/PGF_{2\alpha}$ ratio. These effects can be inhibited by a dual LPA_1 and LPA_3 antagonist (Ki16425) [53,64]. Further study indicates that LPA induces PGE_2 production in endometrial stromal cells but not in epithelial cells. The effect is through upregulation of PGE_2 synthesis enzymes, COX-2 and PGE_2 synthase. LPA_1 is suggested to be the main receptor in mediating this process [65]. It is suggested then that LPA may play autocrine and/or paracrine roles in the maintenance of early bovine pregnancy [53,64,65].

In addition to data from non-human species, a recent report indicates that LPA_3 may also have function in human reproduction. This study demonstrates that LPA_3 levels decrease in the middle and later secretory endometrium (implantation occurs in the secretory phase) of patients with endometriosis [66], a medical condition in which the endometrial tissue grows outside of the uterus and that is associated with increased rate of subfertility, affecting 5 to 10% of women of reproductive age in the United States [67]. LPA_3 and other putative uterine receptivity biomarkers (glycodelin A, osteopontin, and HOXA10) examined in this study are all regulated by progesterone. Reduced expression of these genes may explain the progesterone resistance associated with endometriosis [66]. On the other hand, increased levels of COX-2-derived PGE_2 are detected in the endometrium, especially in ectopic endometriotic tissue from women with endometriosis [67]. In addition to the evidence that LPA signaling stimulates COX-2 expression in endometrial and endothelial cells [32,65,68], it is intriguing to speculate that LPA signaling may play a role in endometriosis as well as endometriosis-associated subfertility.

In human decidual cells, LPA can increase embryo outgrowth and induce actin stress fiber formation [69], a result consistent with the earliest known effects of receptor-mediated LPA signaling [70,71]. RhoA signaling mediates these LPA effects in decidual cells that may contribute to embryo development and differentiation after attachment [69]. LPA can induce IL-8 enhanced migration, permeability, capillary tube formation, and proliferation of human endometrial microvascular endothelial cells. Of the three LPA receptors examined, LPA_1 but not LPA_2 or LPA_3 are highly expressed in human endometrial stromal cells. LPA_1 mediates LPA-induced IL-8 expression via a NFκB-dependent pathway. A role of LPA in angiogenesis of endometrium and placenta through induction of IL-8 in endometrial stromal cells during pregnancy has thus been suggested [72].

LPA signaling has also been suggested to have pathological roles during pregnancy. Although LPA_2 and LPA_3 are not detectable in human endometrial stromal cells, high levels of LPA_2 and LPA_3 gene expression were detected in the placentas of patients with gestational hypertension and preeclampsia (pregnancy-induced hypertension in association with

significant amounts of protein in the urine) [73]. In addition, LPA can potentiate endothelin-1 induced vasoconstriction [74]. These data suggest that LPA signaling might contribute to gestational hypertension and preeclampsia. These hypertensive disorders can worsen with pregnancy progression, and LPA levels are known to increase during pregnancy [75]. It is unknown whether even higher levels of LPA are present in pregnant patients with complicated hypertensive disorders. LPA levels increase during blood clotting that can promote wound healing processes. However, aberrant accumulation of LPA in blood may lead to adverse effects such as endothelial barrier dysfunction [76] and platelet aggregation[77], which can contribute to thrombosis during pregnancy. LPA signaling also may be associated with preterm labor or pre-eclampsia [78] and influence the growth of uterine tumors such as leiomyomas or fibroids (tumors of uterine smooth muscle) [79]. LPA signaling may also play a role in endometrial cancer. A recent study indicates that LPA can promote endometrial cancer invasion. LPA_2 and matrix metalloproteinase-7 (MMP-7) are implicated in this process [80]. The functions of LPA signaling in these processes await further exploration.

LPA signaling has also been suggested in maintenance of human pregnancy. Serum ATX/lysoPLD activity, a key enzyme for LPA production, and LPA levels were shown to increase during pregnancy [75]. High lysophospholipase activity was present in human placental tissues, with the highest in the amnion [81]. Although the amnion has been heavily implicated in the initiation of labor, presumably through the release of arachidonic acid, the high lysophospholipase activity in the amnion suggests that lysophospholipid substrates, including LPA, might also be involved in the regulation of labor. These issues deserve further examination.

LPA signaling could potentially regulate uterine contractility as well as load-bearing during pregnancy and labor. An early study indicated that LPA had similar effects as $PGF_{2\alpha}$ on rat smooth muscle contraction and intrauterine pressure [82]. Although the effect of LPA signaling in parturition *per se* has not been established *in vivo*, deletion of FP, the GPCR for $PGF_{2\alpha}$, led to parturition failure [83]. The potential roles of LPA signaling in uterine smooth muscles can be further demonstrated by the following studies. LPA stimulated myosin light chain phosphorylation through RhoA signaling in myometrial tissue from pregnant women [84], specifically through $G_{\alpha 12/13}$-Rho kinase signaling [85]. In addition, the $G_{\alpha i/o}$ signaling pathway involving $[Ca^{2+}]_i$ regulation regulates LPA-induced cell proliferation of human myometrial smooth muscle cells [86]. A follow-up study identified the critical role of Ca^{2+}/calmodulin-dependent protein kinase in this effect as well as the detection of LPA_1, LPA_2, and LPA_3 in the human myometrial smooth muscle cells [87]. The involvement of LPA_3 in uterine smooth muscle contraction has been demonstrated in mice using LPA_3 specific agonist T13 and LPA_3-deficient uterus [33]. These data indicate that LPA signaling can regulate uterine contractility, which may render its roles not only in embryo spacing in mice but also in load-bearing during pregnancy and eventually labor in humans.

LPA might also have relevance to infection-related preterm labor. Significantly higher levels of lysophosphatidylcholine (LPC), the substrate for ATX to produce LPA, was detected in human uterine endometrial cells upon exposure to extracts from common anaerobes involved in intrauterine infection, accompanied with elevated arachidonic acid, a key precursor for PG synthesis in regulating labor. PLA_2 activity was involved in the reported lipid metabolism [88,89]. Since PLA_2 and LPC are important components in the LPA metabolism pathway [19], it is reasonable to expect that LPA signaling might also be involved in infection-related preterm labor.

In the bovine reproductive tract, LPA has been demonstrated to stimulate progesterone and PGE_2 secretion, which may indicate a supporting role for LPA in the corpus luteum. Hence, it was suggested that LPA may be involved in the maintenance of early bovine pregnancy

[53]. LPA signaling has also been implicated in the establishment and maintenance of porcine pregnancy [90].

LPA signaling in testis and prostate

Three lines of evidence suggested that LPA signaling may have potential roles in male reproduction (reviewed in [2]). First, LPA biosynthetic enzymes, including PLA_1, PLA_2, and autotaxin/lysoPLD are present in the testis. Second, *Lpar1-3* are highly expressed in the mouse testis [34,91] while *LPAR1-4* are detected in human testis [5,36]. Third, transgenic mice overexpressing LPP1, which degrades LPA, show impaired spermatogenesis [92]. *In situ* hybridization demonstrated *Lpar1-3* expression in male germ cells. Deletion of these receptors in mice led to a testosterone-independent reduction of mating activity and sperm count, with an increased prevalence of azoospermia in aging animals. Increased germ cell apoptosis was responsible for the consequent reduction of germ cell proliferation and diminished sperm count, implicating LPA signaling as a germ cell survival factor in spermatogenesis [34].

LPA signaling may have functions in the pathology of the prostate. *Lpar1-3* were detected in the prostate, and significantly higher expression levels were reported in human prostate malignancies compared with benign tissues [93,94]. LPA signaling may have multiple roles in prostate cancer by facilitating early prostate cancer development, inducing prostate cancer cell proliferation, survival, morphological changes, migration, and invasion. LPA_1 seems to be the key receptor in mediating LPA-induced prostate cancer cell proliferation and migration because the expression of this receptor is correlated with these LPA-induced cellular events in cultured prostate cancer cells [94]. NF-κB is constitutively activated in prostate cancer but not in benign prostate tissues. LPA can activate Akt-NF-κB pathway in cultured prostate cancer cells. It is suggested that LPA receptor-Akt-NF-κB signaling axis may mediate LPA-induced prostate cell survival [95]. LPA may also play roles in prostate cells via secondary factors, such as cytokines, CYR61, RhoA, etc (reviewed in [2]).

LPA signaling in fertilization

The spermatic acrosome reaction is a main step in fertilization, involving the binding and fusion of sperm and egg. LPA can activate spermatic PKCα, implicated in the acrosome reaction, and could promote actin polymerization, a process necessary for spermatozoa penetration into the egg cytoplasm (reviewed in [2]). LPA and Rho GTPases are involved in the latter process consistent with receptor-mediated phenomena [96,97]. However, the LPA receptor(s) mediating this process is (are) unknown. LPA signaling does not appear to alter sperm motility in either mice deficient for three LPA receptors, $LPA_{1/2/3}$ or in studies of bovine motility. No obvious deficiencies in fertilization were observed in LPA_3-deficient mice [32]. To determine the potential function of LPA signaling in fertilization, especially the acrosome reaction, systematic study needs to be carried out. Currently, expression levels of different LPA receptors in the sperm remain unknown, although several are detected in the testis [5,34,36].

LPA signaling in preimplantation embryo development

LPA signaling has been implicated in post-implantation embryo developmental processes such as vascular formation, vascular maturation and maintenance, heart development, and brain formation (references in [2]). In a study preceding LPA receptor identification, culturing of embryos from the pronuclear stage in the presence of LPA significantly increased the success rate of the development of 2-cell and 4-cell stage embryos to blastocysts via a $G_{\alpha i}$-protein receptor mechanism [98]. A more recent study reported *Lpar1* mRNA expression in differentiating mouse blastocysts, expression of *Lpar2* in late-stage blastocysts and no expression of *Lpar3* at any of the examined stages [99]. One potential mechanism could be that LPA elevates $[Ca^{2+}]_i$ levels to accelerate murine blastocyst differentiation. LPA induces

the transient accumulation of heparin-binding EGF-like growth factor (HB-EGF) on the embryo surface, and interfering with HB-EGF signaling through EGF receptors ErbB1 or ErbB4 could attenuate LPA-stimulated blastocyst differentiation [99]. However, there is no obvious defect in blastocyst development in LPA_3-deficient or LPA_1/LPA_2-double deficient mice [32]. Delayed post-implantation embryo development in LPA_3-deficient uteri reflects delayed embryo implantation that is maternal in origin [32]. Nevertheless, LPA signaling can influence post-implantation embryo development. Deletion of ATX, a key enzyme in LPA production, leads to embryonic lethality due to defects in blood vessel formation, brain development, etc [15,16], suggesting roles for LPA signaling in embryo development. Expression patterns of LPA receptors in embryo suggest potential functions of LPA signaling in organogenesis [31]. We have observed embryonic hematoma and embryonic lethality with incomplete but increased penetrance in LPA_1, $LPA_{1/2}$ double knockout, and $LPA_{1/2/3}$ triple knockout mice [34,46,100]. However, these phenotypes can't represent what are observed in ATX knockout mice. Considering the fact that more LPA receptors are being identified, any single or a few LPA receptors may not be able to represent the function of ATX.

LPA signaling may play a role in reproduction for other species as well. $XLPA_1$ and $XLPA_2$ receptor-mediated LPA signaling is important for early embryo development in *Xenopus* via the maintenance of overall rigidity and shape of the embryo [101,102]. LPA_3 was detected in porcine concepti of day 12 and 15, and it was suggested that LPA produced in porcine uterine endometrium influences conceptus development during implantation and establishment of porcine pregnancy [55]. A recent study shows multiple effects of LPA on ovine trophectoderm cells, such as activation of MAPK ERK1/2 phosphorylation, promotion of proliferation and cytoskeletal rearrangement, as well as release of $PGF_{2\alpha}$ and PGE_2, suggesting a potential role of LPA signaling in the ovine conceptus at the time of implantation [54].

Summary

Progress over the past decade has revealed the importance of LPA signaling in female and male reproduction, as well as fertilization and pre-implantation embryo development. All examined vertebrate species utilize LPA receptor-mediated mechanisms, and this form of lysophospholipid signaling influences most elements of reproduction directly or indirectly. Numerous issues remain to be addressed in the future (Box 1), a partial list that underscores both the multitude of pathway elements to consider, along with selected questions within each group. The therapeutic potential of LPA signaling represents an area of opportunity that awaits future investigations.

Box 1. Outstanding Questions

LPA ligand-related issues

What chemical forms of LPA exist within the reproductive system?

What are local LPA concentrations?

Do LPA concentrations vary with reproductive stage or age?

Are there LPA concentration gradients?

Which cells produce LPA?

What is most critical for LPA signaling with respect to ligand: synthesis or degradation?

Are there physiological or disease conditions that significantly alter LPA levels or gradients (e.g., obesity)?

LPA biosynthetic and degradative enzyme-related issues

Which enzymes are most important in the reproductive system?

How is their expression and activity controlled?

Do LPA precursors and/or products create positive and negative feedback loops affecting activity?

Are there unidentified enzymes that contribute to LPA enzymatic pathways?

LPA receptor-related issues

Have all LPA receptors involved in reproduction been identified?

What are rate-limiting mechanisms in controlling LPA receptor activity?

Which downstream signaling pathways are dominant for LPA-mediated reproductive effects?

What is the relationship between LPA signaling and other lysophospholipid pathways?

What is the relationship between LPA signaling and other signaling pathways involved in reproduction?

LPA-based therapeutic issues

Which molecular targets are tractable for intervention?

What physiological or disease indications could be therapeutically and safely accessed?

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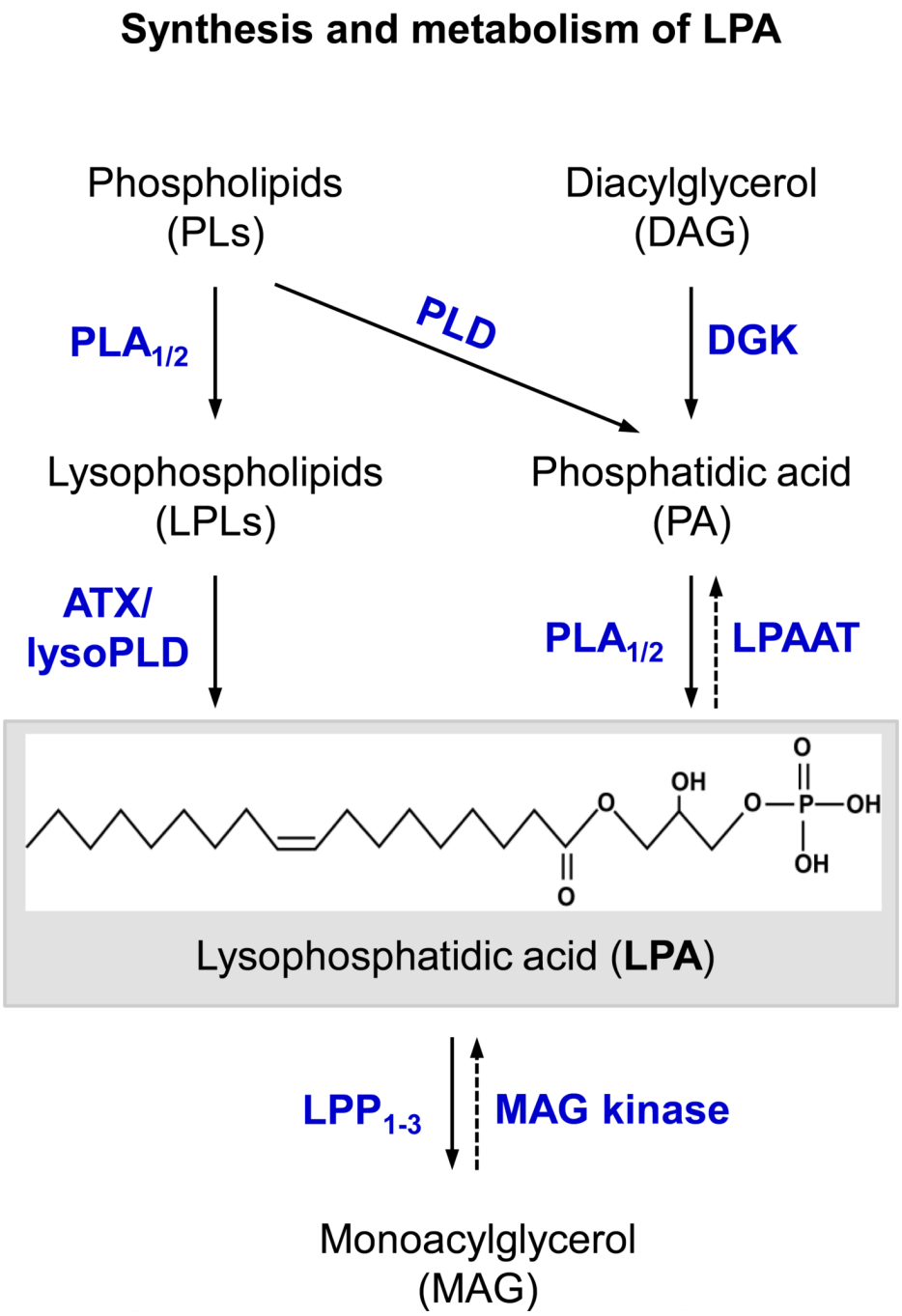

Figure 1.

Synthesis and metabolism of LPA. LPA can be generated via two general pathways. (i) One involves the generation of LPLs from PLs by $PLA_{1/2}$, and transformation of LPLs to LPA by ATX/lysoPLD. (ii) The other involves the formation of PAs from PLs by PLD or from DAG by DGK, and transformation of PA to LPA by $PLA_{1/2}$. (iii) LPA can be metabolized to MAG by LPP_{1-3} and lysophospholipases, a process that can be reversed by MAG kinase. LPA can also be removed by a variety of LPAATs. ATX/lysoPLD, Autotaxin/lysophospholipase D; LPA, lysophosphatidic acid (shown for the 18:1 chemical species of LPA); LPAAT, LPA acyltransferases; LPPs, lipid phosphate phosphatases; LPLs, lysophospholipids; MAG,

monoacylglycerol; PA, phosphatidic acid; PLD, phospholipase D; $PLA_{1/2}$, phospholipase A_1 and A_2; PLs, phospholipids.

Table 1

LPA receptors and their coupling to G proteins.

Receptor (synonyms)	G Protein(s)	References
LPA_1 ($EDG\text{-}2/VZG\text{-}1/LP_{A1}$)	$G_{\alpha 12/13}$, $G_{\alpha i/o}$, $G_{\alpha q}$	[70]
LPA_2 ($EDG\text{-}4/LP_{A2}$)	$G_{\alpha 12/13}$, $G_{\alpha i/o}$, $G_{\alpha q}$	[103]
LPA_3 ($EDG\text{-}7/LP_{A3}$)	$G_{\alpha i/o}$, $G_{\alpha q}$	[28,93]
LPA_4 ($p2y_9$/GPR23)	$G_{\alpha 12/13}$, $G_{\alpha i/o}$, $G_{\alpha q}$, $G_{\alpha s}$	[36]
LPA_5 (GPR92)	$G_{\alpha 12/13}$, $G_{\alpha q}$	[104,105]
LPA_6 (P2Y5)	$G_{\alpha 12/13}$	[23,24]

Table 2

LPA receptor-mediated signaling in reproduction.

Site	Effect(s)	Identified receptor(s)	Reference(s)
Testis	Germ cell survival	LPA_1 LPA_2 LPA_3	[34]
Oocyte	Maturation	LPA_1? LPA_2?	[106]
Xenopus oocyte	Early embryo shape maintenance	$XLPA_{1-2}$	[101]
Ovary	IL-6, IL-8, and growth factor induction	LPA_1 LPA_2	[45]
	Cancer cell growth, survival, migration, and invasion	LPA_2 LPA_3	[107-110]
Blastocyst	Differentiation	LPA_1? LPA_2?	[99]
Uterus	Uterine receptivity	LPA_3	[34]
	Embryo spacing	LPA_3	[32,33]
	Uterine contraction	LPA_1? LPA_2? LPA_3	[33,82,87]
	Angiogenesis of endometrium	LPA_1?	[72]
Placenta	Angiogenesis of placenta	LPA_1?	[72]
	Pregnancy hypertension	LPA_2? LPA_3?	[73]

? Indicating proposed but not fully confirmed receptor(s).