FULL PAPER

Physiology

In vivo evidence for possible up-regulating roles of lysophosphatidic acid around fertilization in rats

Tae TAKEDA[1,3], Miki SHIRASAKA[1], Makoto SUGIYAMA[2], Ryota TERASHIMA[1], Mitsumori KAWAMINAMI[1] and Shiro KURUSU[1]*

[1]Laboratory of Veterinary Physiology, Kitasato University School of Veterinary Medicine, Towada, Aomori 034-8628, Japan
[2]Laboratory of Veterinary Anatomy, Kitasato University School of Veterinary Medicine, Towada, Aomori 034-8628, Japan
[3]Present address: Laboratory of Veterinary Surgery, The University of Tokyo Graduate School of Agricultural and Life Sciences, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

ABSTRACT. Lysophosphatidic acid (LPA) produced by autotaxin (ATX) is recognized as a multi-functional mediator in mammalian reproduction. This study focused on possible effect(s) of LPA on ovulated cumulus-oocyte complexes (COCs) around fertilization in rats *in vivo*. Immunohistochemistry revealed the cell-type-dependent localization of candidates of synthetic enzymes, ATX and two phospholipases A_2 isofroms, and LPA receptors LPA_{1-4} in ovulated COCs and in oviductal epithelium. The eggs ovulated with a form of COCs became denuded of cumulus cells and underwent fragmentation in the absence of fertilization. *In vivo* experiments of local administration in non-copulated rats demonstrated that eggs denudation was increased by LPA and decreased by anti-ATX antibody and that fragmentation was inhibited by LPA and stimulated by an ATX chemical inhibitor. Furthermore, LPA administration in adult copulated rats increased the rate of cleaved embryos significantly. Obtained results suggest the presence of LPA synthesis and action system in ovulated COCs within the oviductal ampulla and positive actions of LPA possibly at multiple sites around fertilization in rats.

KEY WORDS: autotaxin, cumulus-oocyte complex, fertilization, lysophosphatidic acid, rat

Lysophosphatidic acid (LPA) is a primary lysophospholipid whose significant roles in physiology and pathology have recently been being elucidated [1, 3]. LPA can be generated through multiple pathways, the primary one of which involves the combinational activities of autotaxin (ATX)-dependent lysophospholipase D (lysoPLD) and phospholipase A (PLA) [2]. LPA exerts its actions through binding to six kinds of G protein-coupled receptors, LPA_{1-6} [1–3].

Reproduction in mammalian species is composed of sequential and integrated processes almost all of which occur in female reproductive tract. Early events of their pregnancy include 1) intra-ovarian events, such as follicular development, oocyte maturation, cumulus expansion and release of cumulus-oocyte complex (COC) (ovulation), and 2) intra-oviductal events, such as ovum binding with sperm (fertilization) and initial embryo development. These processes are further followed by early development, implantation, and others occurring within the uterus. As reviewed frequently [23, 24, 27, 32, 34, 35], basic animal studies and clinical human studies have implicated ATX/LPA system in various processes in female reproduction and embryo development. It has been established that LPA_3 signaling is critical for spatially- and temporally-regulated implantation in mice [8, 9, 36]. Our previous study with a rat model failed to detect any amount of ATX protein in growing and ovulatory follicles, suggesting little involvement of ATX/LPA system in the growth and ovulation of follicles in this rodent species *in vivo* [22]. However, ATX/LPA system in post-ovulation processes such as the dynamics of released COCs and fertilizing and dividing potential within the oviduct remains to be clarified. Very recent studies with a bovine model demonstrated mRNA expression of synthetic enzymes (ATX, Group IVA PLA_2, and $PLA_1\alpha$) and signaling receptors (all LPA receptors except for LPA_5) in the oviduct [29, 37]. Given these findings, we here focus on 1) expression of enzyme and receptor proteins in ovulated COCs and the oviduct and 2) possible *in vivo* functional role(s) of ATX/LPA system in COCs around fertilization in a rat model.
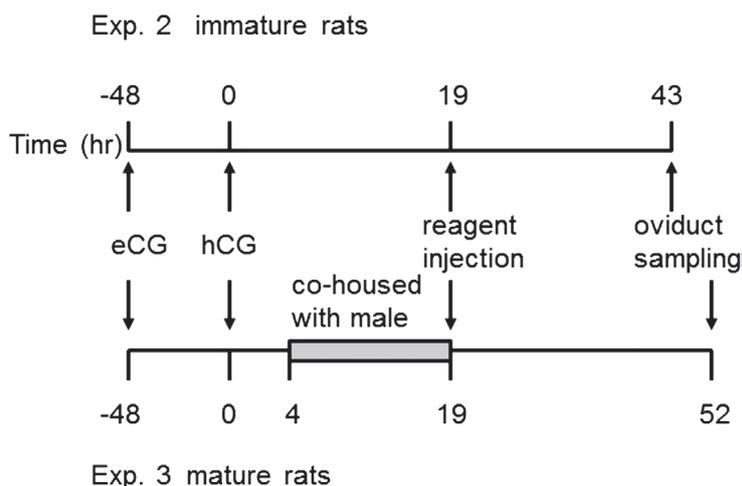
Fig. 1. Experimental schedule to test reagents' effect on COC. Immature and mature female rats were successively treated with eCG and hCG to induce time-controlled ovulation. Adult rats were then co-housed with fertile male from hCG4h to hCG19h. At hCG19h, rats were treated locally (into ovarian bursa) with LPA, anti-ATX antibody, or others. Twenty-four or 33 hr later, eggs in the oviductal ampulla were examined for occurrences of denudation and cytoplasmic fragmentation of eggs and the cleavage of embryos.

MATERIALS AND METHODS

Antibodies and reagents

Ten kinds of antibodies and antisera were previously described [16, 22, 26] and summarized in Supplementary Table 1. LPA (1-oleoyl-sn-3-glycerophosphate) and S32826 (a chemical inhibitor of ATX) were purchased from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.). Equine chorionic gonadotropin (eCG) and human CG (hCG) were purchased from Shionogi (Osaka, Japan) and Daiichi-Sankyo (Tokyo, Japan), respectively. The Vectastain Elite ABC staining kit for immunohistochemistry was purchased from Vector Laboratories (Burlingame, CA, U.S.A.). Other standard reagents were of analytical grade.

Animals and tissue sampling

All procedures with animal treatment were carried out following the Guidelines of the Animal Care and Use Committee of Kitasato University School of Veterinary Medicine. Rats of Wistar-Imamichi strain were housed in an air- and light-conditioned room and free access to food and water. The estrous cyclicity and occurrence of copulation of adult female rats were examined by vaginal smear cytology. Mainly, immature female rats were used as a model of induced ovulation [17, 18] to study post-ovulatory events of COCs under the non-copulated/fertilized condition. Twenty-five days-old rats were treated with eCG (ip, 0.2 IU/g of body weight) followed 48 hr later by hCG (ip, 10 IU/rat) to induce ovulation. Adult female rats of 3–5 months-old were used to study ovulation and fertilization processes in the presence or absence of mating stimulus.

Oviducts and ovaries were sampled from animals euthanized with cervical dislocation under anesthesia in the time-course after ovulatory stimulus. The time of treatment and tissue sampling was depicted in this paper as that relative to hCG injection (Fig. 1; for example, 24 hr after hCG was depicted as hCG24h). For immunohistochemical examination of intact tissues, they were harvested from immature rats at hCG8h and hCG24h, because, in a rat model, ovulation-related biochemical and genetic events in ovulatory follicles culminate around hCG4h ~hCG8h, and because COCs are released from ruptured follicles around hCG12h and very rapidly moves to and stay in oviductal ampulla by hCG24h. Ovaries and oviducts under the natural condition were sampled from non-mated adult cycling rats at 10:00 hr of the estrous day. For evaluation of the dynamics of ovulated COCs under the non-mated condition, oviducts were harvested from only gonadotorpins-treated immature rats at hCG19h, hCG24h, hCG36h, and hCG43h.

Experimental procedure and evaluation of COCs and eggs

Exp. 1: In the oviductal ampulla, just ovulated ova remained surrounded by cumulus oophri for some time and became denuded of them with time (Supplementary Fig. 1). In the absence of copulation and fertilization, ova underwent fragmentation, a hallmark of cellular degeneration and decreased quality, with time. In the time-course of post-hCG treatment, the COCs present in the ampulla were evaluated under a light microscope. The numbers of ova were counted with considering the occurrences of denudation of cumulus cells and fragmentation.

Exp. 2: To evaluate effects of local ATX/LPA system on ovulated COCs in immature rats, the intra-ovarian bursal treatment with LPA or related reagents was done at hCG19h (Fig. 1). Animals under anesthesia were subjected to bilateral abdominal incisions and the ovarian bursa was exposed. A 50 μl of vehicle (physiological saline), LPA (10 μM), S32826 (1.0 mM), normal rabbit serum

(1:50 dilution with saline) or anti-ATX serum (Sigma-Aldrich Japan, 1:50 dilution) was injected into one ovarian bursa using a syringe and repeated in another side of ovarian bursa. The doses of LPA and other reagents were decided according to a number of previous studies *in vitro* [4–6, 10, 12, 13, 15, 38] and our study *in vivo* [22]. No visible leakage of the injected solution and swelling of the bursa were ascertained in this procedure. After the injection, ovaries were positioned back to the abdominal cavity and muscles and skins were sutured separately. At hCG43h (24 hr after the reagent administration), animals were sacrificed and tissues were harvested.

Exp. 3: Finally, the effect of LPA administration on post-fertilization process was tested in adult mated rats. Rats were treated with eCG (ip, 20 IU/head) on metestrus and 48 hr later with hCG (ip, 50 IU/head) (Fig. 1). From hCG4h to hCG19h, hormonally treated female rats were co-housed with a fertile male for them to copulate and achieve fertilization. At hCG19h, female rats were examined for vaginal smear cytology and checked for the presence of sperm (a sign of successful copulation and ejaculation). In rats, COCs are released around hCG12h and very instantly move to and stay in the ampulla, and the ejaculated sperm in natural condition reach the ampulla and gain capacitation within a few hours. Therefore, most eggs must have been fertilized by hCG19h in this experimental protocol. Then, the rats were locally treated as described above. A bolus of LPA (10 μM) or saline was administered at the dose of 100 μl per one ovarian bursa. At hCG52h, oviducts were sampled, and eggs seen in the ampulla were examined for occurrence of cleavage. One cell-stage of eggs were judged as the sum of unfertilized ones and fertilized, but not uncleaved ones. Two and occasionally 4 cell-stages of eggs were judged as cleaved ones. The cleavage rate was calculated as the ratio of cleaved eggs to total ones in number.

Immunohistochemistry

Immunohistochemistry of seven different antigens was performed as reported previously [22, 26]. Concerning PLA isoforms that associate with ATX to produce LPA, group IVA and VIA PLA$_2$s were chosen, as their metabolic association was demonstrated in a mouse model of neuropathic pain [11, 21]. Concerning LPA receptors, LPA$_{1-4}$ were examined as their specific antibodies were available. Tissues fixed in Bouin's solution overnight were dehydrated and embedded in paraffin. Tissues were serially sectioned (3–5 μm in thickness), deparaffinized, and examined immunochemically. Endogenous peroxidase was quenched by pretreatment with 0.3% H$_2$O$_2$ in methanol for 30 min. Characterization of the antibodies and their dilution at use were described in Supplementary Table 1. Anti-ATX antibody used for immunohistochemistry was from Cayman Chemical. Serial sections for each sample were incubated with these primary antibodies at 4°C overnight. Negative controls were performed with normal mouse IgG. The antigen/antibody complex was visualized as a brown color of peroxidation of 3, 3-diaminobenzidine tetrahydrochrolide. Slides were counter-stained with hematoxylin. The signal intensity was semi-quantitatively evaluated (blind test by two independent investigators) with a scale graded from negative (−), faintly positive (±), moderately positive (+), to intensely positive (++). They are shown in Supplementary Tables 2 and 3.

Statistical analysis

The values among different groups were analyzed by Tukey's WSD test [25]. A P value less than 0.05 was considered significant.

RESULTS

Immunolocalization of putative proteins for generation and signaling of LPA in COCs and oviducts (Figs. 2 and 3)

First, we examined, by immunohistochemistry, the expression and localization of seven kinds of proteins in the COCs in preovulatory follicles and oviducts of immature rats sampled at hCG8h. The specimen with non-specific antibody application served as negative control and exhibited no reaction (data not shown). Immunoreactivities of PLA$_2$s that are both relatively ubiquitous enzymes were moderate in oocytes and cumulus and mural granulosa cells of preovulatory follicles (Fig. 2A1 and B1). Immunoreactive ATX was absent in preovulatory follicles (Fig. 2C1). These staining behaviors are consistent with those of our original description [17, 22]. Among LPA receptors examined, immunoreactivities for LPA$_2$ (Fig. 2E1) and LPA$_3$ (Fig. 2F1) were moderate in granulosa cells. LPA$_4$ reactivity was marked in oocytes of preovulatory follicles (Fig. 2G1) and of any stages of growing follicles (data not shown). In the oviducts, epithelia were evidently positive for GIVA (Fig. 2A2 and 2A3) and GVIA (Fig. 2B2) PLA$_2$s, ATX (Fig. 2C2 and 2C3), LPA$_1$ (Fig. 2D2 and 2D3), LPA$_3$ (Fig. 2F2 and 2F3), and LPA$_4$ (Fig. 2G2 and 2G3) and faintly positive for LPA$_2$ (Fig. 2E2 and 2E3). In the smooth muscle layers of the oviducts, immunoreactive LPA$_3$ (Fig. 2F2 and 2F3) was evident, and immunoreactivities of LPA$_2$ (Fig. 2E2 and 2E3) and LPA$_1$ (Fig. 2D2 and 2D3) were also significant. Compared to those in epithelia and smooth muscle layers, the immunostaining for most antigens in the stroma was modest or negligible. The relative intensity of the immunoreactivities of each antigen was summarized in Supplementary Table 2. It can also be noted that, in general, the immunoreactivities of ATX and LPA receptors seemed to be more intense in the isthmus than in the ampulla.
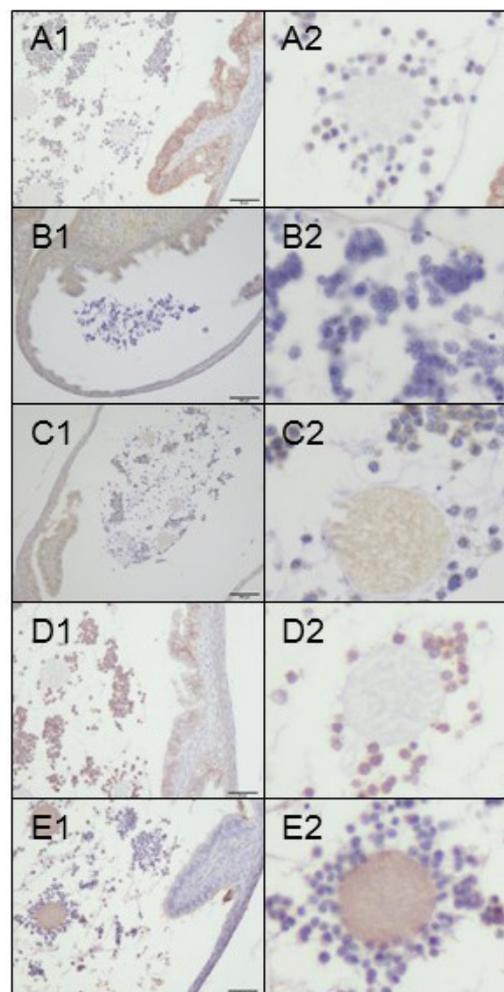
In ovulated COCs in the ampulla of immature rats at hCG24h, ATX immunoreactivity (Fig. 3A1 and 3A2) remained almost negative. Among LPA receptors, LPA$_4$ (Fig. 3E2) and LPA$_2$ (Fig. 3C2) immunoreactivities were present in eggs, and cumulus cells were positive for LPA$_2$ (Fig. 3C2), LPA$_3$ (Fig. 3D2), and LPA$_4$ (Fig. 3E2). LPA$_2$ immunoreactivity in COCs appeared after ovulation (Supplementary Table 3).
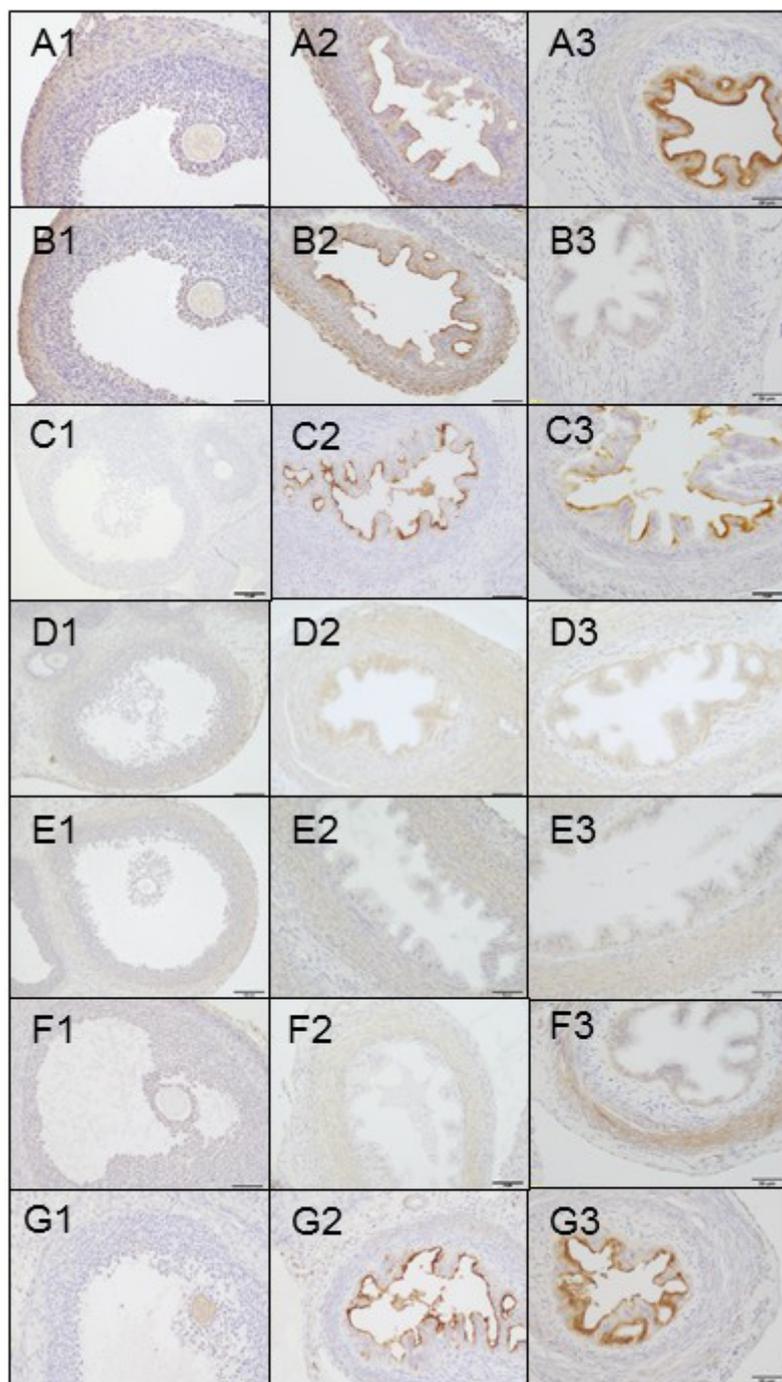
Fig. 2. Immunolocalization of candidate proteins for generation and signaling of LPA in preovulatory follicles and oviducts. Immunoreactivities of GIVA PLA$_2$ (A), GVIA PLA$_2$ (B), ATX (C), LPA$_1$ (D), LPA$_2$ (E), LPA$_3$ (F) and LPA$_4$ (G) were examined for ovaries (A1-G1) and oviducts (A2-G2) of immature rats at hCG8h and for oviducts of adult estrous rats (A3-G3). Cell type- and tissue-dependent localization of each protein is noted. NRS-stained samples with no positive reaction are not shown. Scale bars, 100 μm (ovaries, left column) and 50 μm (oviducts, center and right columns).

Fig. 3. Immunolocalization of ATX and LPA$_{1-4}$ in oviducts and ovulated COCs of immature rats at hCG24h. Epithelial cells in the ampulla of oviducts in immature rats are positive for all of ATX (A) and LPA$_1$ (B), LPA$_2$ (C), LPA$_3$ (D) and LPA$_4$ (E). In COCs (A2, B2, C2, D2 and E2 magnified from A1, B1, C1, D1, and E1, respectively), cumulus cells appear negative for ATX (A2) and LPA$_1$ (B2) but are positive for LPA$_2$ (C2), LPA$_3$ (D2) and LPA$_4$ (E2). An egg is positive for LPA$_2$ and LPA$_4$. Scale bars, 100 μm (left column).

Effects of ATX/LPA system on the ovulated COC in vivo

Given the massive evidence for the presence of LPA synthetic and signaling system in the just ovulated COCs and the microenvironment for fertilization and early development, we investigated the impact of ATX/LPA system on ovulated COCs in immature rats that were induced to ovulate but not mated. Ovulated eggs in the ampulla were intact and still mostly surrounded

Table 1. *In vivo* effects of reagents on the rates of ova denuded and unfragmented

	Rate (denuded/total)	Rate (unfragmented/total)	No. of ovary
Intact	62.2 (163/262)	19.5 (51/262)	10
Vehicle	67.0 (148/221)	74.6 (165/221)	10
LPA	87.0[a] (604/695)	82.4[a] (573/695)	27
S32826	75.7 (199/263)	63.5[a] (167/263)	10
NRS	71.5 (392/548)	65.3 (358/548)	21
Anti-ATX	52.3[b] (391/748)	71.0 (531/748)	29

Gonadotropins-primed immature rats were locally treated with reagents at hCG19h. Reagents included vehicle (saline), LPA (10 μM), S32826 (1 mM), anti-ATX or normal rabbit sera (NRS) (both 1:50 dilution), and a 50 μl of each solution was injected into one bursa. At hCG43h, ova in the ampulla were microscopically counted and examined whether they were denuded of cumulus cells and fragmented. Rates (%) of denuded and unfragmented ova were evaluated with Tukey's WSD test. Numbers of total ova and ovaries examined were shown. $P<0.05$ a) vs vehicle, b) vs NRS.

Table 2. Effect of LPA on the rate of cleavage of embryos

	Rate (cleaved/total)	No. of ovary
Intact	78.9 (30/38) [a]	6
Vehicle	75.0 (33/44) [a]	6
LPA	94.2 (81/86) [b]	6

Adult females induced to ovulate with eCG/hCG and mated with a male were treated locally with LPA or saline. Thirty-three hours later, the eggs in the ampulla were examined to be fertilized and cleaved or not. Values of cleaved/total eggs were analyzed by Tukey's WSD test. a) vs. b) $P<0.05$.

by cumulus oophri at hCG24h (Supplementary Fig. 1A) but became denuded and degenerated with appearance of fragmentation (Supplementary Fig. 1B) at hCG43h. The rate of ova denudation in intact rats was 62.2% at hCG43h (Table 1). The value was similar in the vehicle-treated control group but was significantly higher in LPA treatment group. S32826 treatment was without a significant effect, but anti-ATX serum treatment significantly reduced the rate of denuded ova compared to that of normal serum treatment.

The ova viability in the absence of mating was also determined with an index of occurrence of fragmentation. In intact animals, the rate of unfragmented ova decreased to 19.5% by hCG43h (Table 1). Vehicle treatment increased the rate up to 74.6%. Compared with that value, the rate of viable ova was significantly increased by LPA treatment and decreased by S32826 treatment. There was no significant difference between non-specific serum and anti-ATX serum treatments.

Effects of LPA on fertilized eggs in vivo

To further investigate the influence of LPA on fertilized eggs *in vivo*, the effect of local LPA administration was tested in adult mated rats. In intact rats given only gonadotropins and mated, 78.9% of eggs were cleaved (30 cleaved and 8 non-cleaved eggs) at hCG52h (Table 2). The cleavage rate of vehicle-treated group was 75.0% with no significant difference from that of intact females. Local LPA addition produced 94.2% of the cleavage rate (81 cleaved and 5 non-cleaved ova), being significantly higher than the control group ($P<0.05$). In the similar and preliminary experiment with a shorter period of co-housing with male (5 hr, from hCG5h to hCG10h), where the copulation occurrence was less successful, LPA treatment also increased the rate of cleaved eggs significantly (91.5 versus 82.3% in vehicle-treated group).

DISCUSSION

Main findings of the current histological and *in vivo* functional studies with a rat model are as follows, 1) COCs appeared to lack LPA-producing potency but to have multiple signaling receptors both in oocytes and cumulus cells, 2) the epithelium and thus the cavity of oviductal ampulla were very likely the sites of LPA production, and 3) LPA supplementation (and ATX inhibition) affected denudation of cumulus cells and viability of pre-fertilized eggs and the initial cleavage of the fertilized eggs. These results suggest the oviductal cavity as ATX- and LPA-rich environment that may affect the just entered COCs to fertilize and develop optimally. Multiple positive actions *in vivo* of LPA on the peri-fertilization stage of eggs are newly suggested in addition to previously established potential roles in the later stages of embryo development of nervous, vasculature, and other systems [23, 27].

A significant immunoreaction of ATX, together with co-existing PLA$_2$ enzymes in oviductal epithelia, suggests rat oviductal cavity as a site of LPA production and/or secretion. The existence of LPA producing machinery is partly supported by very recent and detailed studies with a bovine model by Woclawek-Potocka and Yamamoto's groups [29, 37]. They demonstrated expression of mRNAs for ATX, group IVA PLA$_2$, and PLA$_1\alpha$ in epithelial and stromal cells and LPA content in oviductal tissues. In addition, pre- and post-ovulatory COCs are currently found to be the probable site of LPA action with oocytes positive for LPA$_4$ and LPA$_2$ and cumulus cells positive for LPA$_2$ and LPA$_3$. This is consistent in large part with previous findings on mRNA expression by bovine COCs [6], in which oocytes expressed LPA$_2$ and LPA$_4$, while cumulus cells did LPA$_2$ and LPA$_3$.

Subsequent functional studies have demonstrated possible actions of LPA and ATX on COCs ovulated, located and fertilized in the oviductal ampulla. Relating with the current evidence *in vivo*, several previous studies *in vitro* have reported on LPA's effects on pre- and post-ovulatory COCs, including cumulus expansion, maturation, fertilization, and developmental competence of oocytes and embryos. Cumulus expansion involving accumulation of hyaluronic acid is thought to occur within preovulatory follicles in response to ovulatory stimuli, and this process in a bovine COC model was shown to be unaffected by LPA supplementation *in vitro* [4]. We found that rat eggs just ovulated and remaining in the ampulla were still virtually surrounded

by cumulus oophri and that a bolus local administration of LPA increased ova denudation of cumulus cells (87.0 versus 67.0% in control). Information on possible direct action of LPA on cumulus cells is very limited [10], but it is possibly involving LPA_2 and/or LPA_3. The mechanism and significance of egg denudation within the oviduct are still obscure. Fertilization can occur before partial and complete denudation *in vivo* and *in vitro*.

We further showed that ova fragmentation, implying degeneration and decreased quality, was prevented by vehicle treatment (unfragmented, 74.6%) compared to that of intact animals *in vivo* (19.5%) at hCG43h and that LPA administration was significantly more effective (82.4%) than vehicle control. Up-regulated viability by the vehicle treatment is surprising since the vehicle treatment was thought to decrease the concentration of endogenous LPA and/or ATX if they had any effects. The reason is quite unknown but may be possibly due to any osmotic stimulus of injected vehicle or any surgical-treatment-associated systemic change. The positive impact of LPA addition on viability of non-fertilized ova is likely supported by anti-apoptotic potency of LPA on bovine COCs [4] and porcine blastocysts [38], where mRNA expressions of growth/proliferation (GDF9 and cyclin B1)- and apoptosis (CASP6, CASP3, BCL2L and BAX)-related genes were found to be regulated. Our results with immature, non-copulated females imply that LPA might promote both denudation and viability of rat ova in the peri-fertilization stage. It should also be noted that ATX inhibition by antibody or a chemical inhibitor produced responses opposite to those by LPA administration. These results mean that ATX *in situ* would exert physiological effects on ovulated COCs probably via LPA production. For different effect(s) of antibody-mediated neutralization and pharmacological inhibition, we also do not have reasonable explanation(s) at present. It may be partly related to the different chemical natures between antibody and a drug, such as molecular size, diffusion potency in hyaluronic acid-rich COC microenvironment, and unknown capturing substances(s) affecting distribution.

Following the study on non-fertilized eggs in immature, non-copulated rats, we further tested LPA's effect with adult mated animals. At hCG19h, most but not all eggs must have been fertilized, and thus the effect of LPA administration should have been in large part on just fertilized embryo (and nearby cumulus cells) and in very minor part on fertilization process. The rate of cleaved embryo was significantly increased by LPA challenge *in vivo*, suggesting that LPA might promote initial embryo development from one- to two-cell stage. Several studies *in vitro* have been available on the effect of LPA supplementation (at the concentration of 10 or 30 μM) on fertilized embryos. Some studies [12, 38] showed LPA's significant effect on promoting early embryo cleavage, but others [5, 13] did not. Importantly, LPA seems effective on the later stages of development (blastocyst formation by peri-implantation period) in several animal species (mice, [12, 13, 20]; sheep, [19]; pigs [38]; cow, [31]).

Besides the fertility processes that we focused on, oocyte maturation has also been demonstrated to be stimulated by LPA *in vitro*. Supplemented LPA promoted nuclear and cytoplasmic maturation of immature oocytes in mice [14], golden hamster [10], pigs [38] and cows [4]. These extensive studies have been conducted based on the original demonstration by Tokumura *et al.* [30] of a significant amount of lysoPLD activity and 10–25 μM level LPA in follicular fluids obtained from human patients for *in vitro* fertilization. In addition, very recent reports have demonstrated the unique molecular species composition of LPA in human follicular fluid [33] and follicular type-dependent presence of LPA in follicular fluid and preferential expression of ATX in theca cells rather than granulosa cells and COCs in bovine follicles [28]. Our previous and current observation, however, found little expression of ATX protein in any type of follicles [22], preovulatory follicles, and postovulatory COCs. Whether ATX/LPA system is physiologically significant *in vivo* in oocyte maturation, cumulus expansion, and follicle rupture in rodent model needs further study to demonstrate the existence of ATX and an amount of LPA within the preovulatory follicles. Nevertheless, it is notable that LPA has a potential promise in application to human and domestic animal reproductive technology, such as *in vitro* maturation, *in vitro* fertilization, and *in vitro* development of pre-implantation embryos.

Just as the uterus has extensively been shown as the potential targets of LPA's action [7–9, 32, 36], the oviduct is also the one in rats. Probable localization of all receptors examined in epithelial cells suggests role(s) of LPA signaling in some exocrine/endocrine activity relating to supportive function of gametes and their fertilization. Also, the presence of LPA_3, LPA_1 and LPA_2 in smooth muscle cells suggests a possible role for LPA signaling in oviduct contraction and eggs transport. A very small size of this tissue of rodents limits its functional and *in vitro* investigation. In the current microscopic observation, we failed to find any convincing differences in the location and the spreading extent of the eggs within the ampulla, which might have reflected oviductal transporting activity *in vivo*. A previous report showed that LPA addition promoted ova transport in murine isolated oviducts *in vitro* [15]. Another recent report has shown a stimulating effect of LPA on prostaglandins production by stromal cells of bovine oviducts [36]. Functional roles of ATX/LPA system in oviduct regulation would require further study with large animal models.

In conclusion, our results strongly suggest that rat ova just ovulated and entering the oviductal ampulla would respond to ATX-synthesizing LPA with increased denudation of cumulus cells and viability and, in the case of fertilization, with increased first cleavage of embryos. Although molecular and cellular mechanisms remain largely elusive, our novel evidence with a rat model *in vivo* further supports for multiple positive effects of LPA around fertilization in mammalian species.

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