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Lysophosphatidic receptor, LPA_3, is positively and negatively regulated by progesterone and estrogen in the mouse uterus

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Abstract

Reciprocal interactions between blastocysts and receptive uteri are essential for successful implantation. This process is regulated by the timely interplay of two ovarian hormones, progesterone and estrogen. However, the molecular targets of these hormones are largely unknown. We showed recently that a small bioactive lysophospholipid, lysophosphatidic acid, plays a pivotal role in the establishment of implantation via its cellular receptor, LPA_3. Here we demonstrate that LPA_3 expression is positively and negatively regulated by steroid hormones in mouse uteri. The LPA_3 mRNA level in the uteri increased during early pseudopregnancy, peaking around 3.5 days post coitus (3.5 d.p.c.), then, decreased to the basal level on 4.5 d.p.c. LPA_3 expression remained at a low level in ovariectomized mice, and administration of progesterone to ovariectomized mice up-regulated LPA_3 mRNA expression. In addition, simultaneous administration of estrogen counteracted the effect of progesterone. These results show that progesterone and estrogen cooperatively regulate LPA_3 expression, thereby contributing to the receptivity of uteri during early pregnancy.
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Keywords: Implantation; LPA_3; Lysophosphatidic acid; Progesterone; Estrogen

Introduction

Implantation is the process by which embryos make a close physical and physiological contact with the maternal endometrium for the establishment of pregnancy (Norwitz et al., 2001; Paria et al., 2002). In mice, implantation occurs in the evening of 3.5 days post coitus (3.5 d.p.c.) and is preceded by embryo spacing, uterine edema and luminal closure resulting in an intimate apposition of the blastocyst with the uterine luminal epithelium (Carson et al., 2000). Around 3.5 d.p.c., the endometrium acquires the ability to implant the developing embryo within a specific time window, termed the "receptive phase". During this period, the endometrium undergoes pronounced structural and functional changes induced by the ovarian steroids, estrogen and progesterone, which prepare it to be receptive to invasion by the embryo (Dey et al., 2004). Progesterone acts on the uterus and influences numerous aspects of uterine physiology including the differentiation of the endometrium and the development of the placenta. Estrogen drives the proliferation of the endometrium and regulates reproductive behavior. In mice, the progesterone level in blood is low from 0.5–2.5 d.p.c. but gradually increases after that reaching to the maximum level from 3.5–5.5 d.p.c. (McCormack and Greenwald, 1974). On 3.5 d.p.c., a transient surge of estrogen occurs. As a result, the two hormones initiate uterine epithelial cell differentiation to the receptive state in a well-coordinated fashion (Carson et al., 2000). A critical balance of estrogen and progesterone is, thus, necessary for successful embryo implantation but the precise mechanism by which they regulate implantation is unknown.

Abbreviations: LPA, lysophosphatidic acid; GPCR, G-protein-coupled receptor; d.p.c., days post coitus.

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Lysophosphatidic acid (1- or 2-acyl-lysophosphatidic acid; LPA) is a simple phospholipid that mediates multiple cellular processes, including platelet aggregation, smooth muscle contraction, cell proliferation, and cytoskeletal reorganization (e.g., generation of actin stress fibers and inhibition of neurite outgrowth) (Contos et al., 2000b; Moolenaar, 1999). Recent identification and genetic manipulations of G-protein-coupled receptors specific to LPA (LPA_{1-4}) have provided mechanistic and functional insights into their diverse roles in biological processes (An et al., 1998; Bandoh et al., 1999; Contos et al., 2000a, 2002; Gardell et al., 2006; Hecht et al., 1996; Noguchi et al., 2003). For example, targeted disruption of lpa_1 and lpa_2 has demonstrated their physiological and pathological functions in neural development, neuropathic pain and diarrhea (Contos et al., 2000a; Inoue et al., 2004; Li et al., 2005).

Our recent studies on LPA_3 knockout mice have revealed that LPA signaling mediated by LPA_3 has a great influence on the female reproductive system, especially on embryo implantation (Ye et al., 2005). Targeted deletion of LPA_3 in mice resulted in significantly reduced litter size, which could be attributed to delayed implantation and altered embryo spacing. We also demonstrated that LPA_3 expression in uterus is strictly regulated during early pregnancy. Within the uterus, LPA_3 mRNA increased on 2.5 d.p.c., peaking around 3.5 d.p.c., peri-implantation period, then returned to the basal levels on 4.5 d.p.c. through the end of pregnancy (Ye et al., 2005). Furthermore we showed that LPA_3 expression was up-regulated during postnatal development, and was regulated under the control of estrous cycle in adult uterus (Ye et al., 2005). These results, combined with the stringent regulation of the uterine implantation by ovarian steroid hormones, prompted us to test whether LPA_3 expression is under the control of these hormones. In this study, we examined the effect of progesterone and estrogen on LPA_3 expression in the uteri. The result raises the possibility that progesterone and estrogen contribute to define uterine receptivity by modulating LPA_3-mediated signaling.

Materials and methods

Animals, ovariectomy and steroid treatments

Adult BL/6J females (SLC, Shizuoka, Japan) were mated with fertile or vasectomized males to induce pregnancy or pseudopregnancy (day 0.5 = vaginal plug), respectively. Mice on days 1–5 were killed between 11:00–13:00 h. For steroid treatments, mice (8–10 weeks) were ovariectomized and, 2 weeks later, were injected subcutaneously with progesterone (4-Pregnen-3,20-dione) (Sigma, St. Louis, MO), estrogen (1,3,5 [10]-estratriene-3,17β-diol) (Sigma, St. Louis, MO) or a combination of progesterone and estrogen. Mice were killed at various times after the hormone injections, and their tissues were collected for RNA extraction.

Quantitative RT-PCR

Total RNA from tissue or cell was extracted using ISOGEN (Nippongene, Toyama, Japan) and reverse-transcribed using SuperScript First-strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). Oligonucleotide primers for PCR were designed using Primer Express Software (Applied Biosystems, Foster City, CA). The sequences of the oligonucleotides used in PCR reaction were described previously (Hama et al., 2004). PCR reactions were performed using an ABI Prism 7000 Sequence Detection System (Applied Biosystems). The transcript number of mouse GAPDH was quantified, and each sample was normalized on the basis of GAPDH content.

Cell line cultures

Human endometrial carcinoma KLE cells and RL95-2 cells were purchased from the American Type Culture Collection (ATCC; Rockville, MD). Cells were grown in plastic flasks in 5% CO_2 in air at 37 °C. KLE cells were seeded in a 1:1 mixture of Dulbecco's modified Eagles medium and Ham's F12 medium (DMEM/F12) (Invitrogen) containing 1.2 g/L sodium bicarbonate, 2.5 mM L-glutamine, 15 mM HEPES, 0.5 mM sodium pyruvate and 10% fetal bovine serum. RL95-2 cells were cultured in a DMEM/F12 supplemented with 10 mM HEPES, 2.0 g/L sodium bicarbonate, 0.005 mg/ml insulin and 10% fetal bovine serum.

Primary endometrial epithelial cell cultures

Mice were killed by cervical dislocation between 11:00–13:00 h on day 3.5. Endometrial cells were prepared under sterile conditions. Uteri from individual mice were excised, trimmed, slit lengthwise, and weighed before incubation in 0.5% trypsin (Sigma), 2.5% pancreatin (Sigma), in PBS for 45 min at 4 °C and then for 45 min at 37 °C. DMEM supplemented with 10% fetal bovine serum (DMEM-FCS) was added to neutralize proteases, and uteri were gently agitated by repeated pipetting to release the epithelium. Cells obtained from uterine horns were seeded in DMEM-FCS and incubated in 5% CO_2 in air at 37 °C.

Statistical analysis

Results are expressed as means ± SE. The data were analyzed by Student's t test with differences with a P value < 0.05 being considered significant.

Results

LPA_3 expression in uterus during early pseudopregnancy

To determine whether any embryonic signal contributes to LPA_3 expression, we examined the LPA_3 mRNA levels in pseudopregnant mice, which were derived by mating females to vasectomized males. The level of LPA_3 mRNA increased on 2.5 d.p.c., peaked on 3.5 d.p.c. and declined to the basal level on 4.5 d.p.c (Fig. 1). This temporal profile of LPA_3 expression in the uterus of pseudopregnant mice was essentially similar to that observed in normal pregnant mice (Ye et al., 2005), suggesting

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