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Influence of brain plasmalogen changes on gonadotropin secretion from the cultured bovine anterior pituitary cells

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ABSTRACT

We recently discovered that the orphan G-protein–coupled receptor (GPR) 61 colocalized with GnRH receptors (GnRHRs) on the surface of most of bovine gonadotrophs. A recent study suggested that ethanolamine plasmalogen (PI) is a ligand for GPR61 in mouse neuroblastoma. Therefore, this study evaluated the hypothesis that PI alters LH and FSH secretions from the cultured bovine anterior pituitary (AP) cells. We prepared bovine AP cells from postpubertal heifers (26 mo old) and cultured the cells for 3.5 d. We treated the cells with increasing concentrations (0, 5, 50, 500, 5,000, 50,000, or 500,000 pg/mL) of phosphoethanolamine PI (PEPI) extracted from the bovine brain, or L-α-lysophosphatidylethanolamine PI (LEPI) extracted from the bovine brain, for 5 min before either no treatment or GnRH stimulation. The medium samples were harvested 2 h after culture for LH and FSH assays. Phosphoethanolamine PI (50–500 pg/mL) stimulated ($P < 0.05$) the basal secretion of FSH but not LH. Phosphoethanolamine PI at 50 pg/mL also enhanced ($P < 0.05$) GnRH-induced FSH secretion. However, higher doses (500–500,000 pg/mL) of PEPI suppressed GnRH-induced FSH secretion. Moreover, 50 to 500,000 pg/mL PEPI suppressed GnRH-induced LH secretion. None of the tested concentrations of LEPI showed any effect on basal or GnRH-induced LH or FSH secretion. Pretreatment with contraction of Sma and Mad pathway inhibitors suppressed FSH secretion induced by PEPI, whereas the extracellular signal-regulated kinase pathway inhibitor blocked the PEPI-induced suppression of GnRH-stimulated LH secretion. Therefore, PEPI, but not LEPI, extracted from the bovine brain, alters FSH and LH secretion from the cultured AP cells. Further studies are required to decide whether PEPI binds to GPR61 and whether PEPI plays an important role in the control of gonadotropin secretion from gonadotrophs.

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1. Introduction

Gonadotrophs in the anterior pituitary (AP) secrete gonadotropins, LH and FSH, to regulate reproductive functions in animals. Gonadotropin secretion is controlled primarily by GnRH via the GnRH receptor (GnRHR), a G-protein–coupled receptor (GPCR) expressed at the gonadotroph surface. Blood concentration of LH increases in heifers within 30 min and peaks a few hours after intramuscular injection of GnRH [1]. Gonadotropin-releasing hormone receptors are present in gonadotroph plasma membrane lipid rafts [2–4], which facilitate signaling by allowing colocalization of membrane receptors and their downstream signaling components [5,6].

We recently found that an orphan GPCR gene, *GPR61*, is expressed in the AP of heifers [7]. Subsequently, we found that GPR61 colocalizes with GnRHR on the plasma membrane of a majority of bovine gonadotrophs [8]. G-protein–coupled receptor 61s express at a low level ($P < 0.05$) in the AP during the early-luteal phase [8] when pulsatile LH secretion is still relatively frequent [9], as compared to the preovulatory or midluteal or late-luteal phase. G-protein–coupled receptor 61 is widely expressed in the brain, including in the hypothalamus and pituitary [10,11].

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Although its function and ligand are unknown, a recent study [12] suggested that ethanolamine plasmalogen (PI) may be a ligand for GPR61 in mouse neuroblastoma-derived cells.

We previously clarified the relationship between GnRH concentration and induced LH secretion from cultured bovine gonadotrophs [3]. Luteinizing hormone secretion was stimulated by increasing amounts of GnRH, with a peak at 0.1 nM or 1 nM GnRH. However, excess GnRH (greater than 1 nM) suppressed LH secretion from the bovine gonadotrophs. It is unclear whether GPR61 colocalization with GnRHR in the plasma membrane of gonadotrophs can alter the GnRH-induced secretion of gonadotropins.

It is important to clarify the cytoplasmic signaling pathway downstream of GPR61. G-protein–coupled receptor 61 stimulates extracellular signal-regulated kinase (ERK) signaling in mouse neuroblastoma-derived cells [12]. An inhibitor of the ERK pathway, U0126, inhibits estradiol-induced suppression of GnRH-stimulated LH secretion in bovine gonadotrophs [13–15]. Therefore, ERK may be a pathway by which PI alters GnRH-induced LH secretion in bovine gonadotrophs. The contraction of Sma and Mad (SMAD) pathways, especially SMAD3, are important pathways that connect GnRHR activation and FSH synthesis in mice gonadotrophs [16]. Therefore, SMAD pathways may have an important role in controlling FSH secretion in bovine gonadotrophs. In this study, we tested the hypothesis that PI can alter gonadotropin secretion. In addition, we evaluated the contribution of the ERK and SMAD pathways to an effect of PI on gonadotropin secretion from the bovine AP.

2. Materials and methods

2.1. Pituitary cell culture and analysis of the effects of PI on LH and FSH secretions

All experiments were performed according to the Guiding Principles for the Care and Use of Experimental Animals in the Field of Physiological Sciences (Physiological Society of Japan) and were approved by the Committee on Animal Experiments of the School of Veterinary Medicine, Yamaguchi University.

We obtained APs from postpubertal (26 mo of age, $n = 10$) Japanese Black heifers at a local abattoir, using a previously described method [3]. The heifers were in the middle-luteal phase, that is, 8 to 12 d after ovulation, as determined by macroscopic examination of the ovaries and uterus [17]; the APs show the highest LH, FSH, and GnRHR concentrations in this phase [18]. Enzymatic dispersal of the AP cells was performed using a previously described method [19], and confirmation of cell viability of greater than 90% was determined via Trypan blue exclusion. Total cell yield was $19.7 \times 10^6 \pm 0.8 \times 10^6$ cells per pituitary gland. The dispersed cells were then suspended in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA) containing 1× nonessential amino acids (Thermo Fisher Scientific), 100 U/mL penicillin, 50 µg/mL streptomycin, 10% horse serum (Thermo Fisher Scientific), and 2.5% fetal bovine serum (Thermo Fisher Scientific). After the cells (2.5×10^5 cells/mL, total 0.3 mL) had been plated in 48-well culture plates (Sumitomo Bakelite, Tokyo, Japan), they were maintained at 37°C in a humidified atmosphere of 5% CO_2 for 82 h. Each experiment was repeated 10 times with each of the 10 different pituitary glands, using 4 wells per treatment. We supplied recombinant human activin A (final concentration, 10 ng/mL; R&D systems, Minneapolis, MN) to stimulate FSH synthesis at 24 h before the tests. Mature bovine activin A (National Center for Biotechnology Information reference sequence of bovine activin A is NP_776788.1) and ovine activin A (NP_001009458.1) have 100% sequence homology with that of humans (CAA40805.1), and 24-h culture with the same concentration of recombinant human activin A stimulates FSH expression in cultured ovine AP cells [20].

To evaluate the effect of PI in the absence of GnRH, the initial medium was replaced with 295 µL of DMEM containing 0.1% BSA and 10 ng/mL activin A and incubated for 2 h. Treatment was performed by adding 5 µL of DMEM alone or 5 µL of DMEM containing various concentrations (final concentrations of 0, 5, 50, 500, 5,000, 50,000, or 500,000 pg/mL) of either phosphoethanolamine PI (PEPI) extracted from the bovine brain (Larordan fine chemicals, Solna, Sweden; purity is more than 98%), or L-α-lysophosphatidylethanolamine PI (LEPI) extracted from the bovine brain (Olbracht Serdary Research Laboratories, Toronto, ON, Canada; purity is more than 98%). According to the manufacturer, the sn-1 position of PEPI is occupied by either a stearoyl or an oleoyl (ratio is 1:1.27), and the sn-2 position is occupied by various polyunsaturated fatty acids. The sn-1 position of LEPI is occupied by an oleoyl, but the sn-2 position is not occupied by any fatty acid. The head group at position sn-3 of both PEPI and LEPI is ethanolamine. After incubation for another 2 h, the medium from each well was collected for RIA of the LH and FSH levels. The physiological concentrations of PI in ruminant blood were not reported when this study was conducted. However, a previous study used 500,000 pg/mL of PEPI for the mouse neuroblastoma-derived cells [12]. The blood concentrations of all the well-known hormones are in the order of pg/mL (eg, estradiol) or ng/mL (eg, progesterone, cortisol, LH, growth hormone, leptin, insulin, and IGF1), respectively, in bovines [21–23]. Therefore, we used the aforementioned PI concentrations in this study.

To evaluate the effect of PI in the presence of GnRH, the initial medium was replaced with 290 µL of DMEM containing 0.1% BSA and 10 ng/mL activin A and incubated at 37°C for 2 h. Pretreatment was performed by adding 5 µL of DMEM alone or 5 µL of DMEM containing various concentrations (final concentration, 0, 5, 50, 500, 5,000, 50,000, or 500,000 pg/mL) of PEPI or LEPI. The cells were incubated while gently shaking for 5 min, and then, cells were treated with 5 µL of 6 nM GnRH (Peptide Institute Inc, Osaka, Japan) dissolved in DMEM for 2 h to stimulate LH and FSH secretion. The final concentration of GnRH was 0.1 nM in all treatments [3] except the "control." Control wells were treated with 5 µL of DMEM but were not incubated with GnRH. "Gonadotropin-releasing hormone" wells were pretreated with 5 µL of DMEM for 5 min and were then incubated with GnRH for 2 h. After incubation for 2 h, the medium from each well was collected for LH and FSH RIAs.

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