Domestic Animal Endocrinology 58 (2017) 39–52

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

# Effect of fish oil on lateral mobility of prostaglandin $F_{2\alpha}$ (FP) receptors and spatial distribution of lipid microdomains in bovine luteal cell plasma membrane in vitro

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ARTICLE INFO

*Article history:*
Received 9 May 2016
Received in revised form 29 July 2016
Accepted 3 August 2016

*Keywords:*
Microdomain
Fish oil
Prostaglandin $F_{2\alpha}$ FP receptor
Single particle tracking
Corpus luteum

ABSTRACT

Lipid microdomains are ordered regions on the plasma membrane of cells, rich in cholesterol and sphingolipids, ranging in size from 10 to 200 nm in diameter. These lipid-ordered domains may serve as platforms to facilitate colocalization of intracellular signaling proteins during agonist-induced signal transduction. It is hypothesized that fish oil will disrupt the lipid microdomains, increasing spatial distribution of these lipid-ordered domains and lateral mobility of the prostaglandin (PG) $F_{2\alpha}$ (FP) receptors in bovine luteal cells. The objectives of this study were to examine the effects of fish oil on (1) the spatial distribution of lipid microdomains, (2) lateral mobility of FP receptors, and (3) lateral mobility of FP receptors in the presence of $PGF_{2\alpha}$ on the plasma membrane of bovine luteal cells in vitro. Bovine ovaries were obtained from a local abattoir and corpora lutea were digested using collagenase. In experiment 1, lipid microdomains were labeled using cholera toxin subunit B Alexa Fluor 555. Domains were detected as distinct patches on the plasma membrane of mixed luteal cells. Fish oil treatment decreased fluorescent intensity in a dose-dependent manner ($P < 0.01$). In experiment 2, single particle tracking was used to examine the effects of fish oil treatment on lateral mobility of FP receptors. Fish oil treatment increased microdiffusion and macrodiffusion coefficients of FP receptors as compared to control cells ($P < 0.05$). In addition, compartment diameters of domains were larger, and residence times were reduced for receptors in fish oil–treated cells ($P < 0.05$). In experiment 3, single particle tracking was used to determine the effects of $PGF_{2\alpha}$ on lateral mobility of FP receptors and influence of fish oil treatment. Lateral mobility of receptors was decreased within 5 min following the addition of ligand for control cells ($P < 0.05$). However, lateral mobility of receptors was unaffected by addition of ligand for fish oil–treated cells ($P > 0.10$). The data presented provide strong evidence that fish oil causes a disruption in lipid microdomains and affects lateral mobility of FP receptors in the absence and presence of $PGF_{2\alpha}$.



## 1. Introduction

Prostaglandin (PG) $F_{2\alpha}$ is the endogenous luteolysin in domestic ruminants [1–4]. It is secreted in a series of pulses late in the estrous cycle from the uterus, causing regression of the corpus luteum (CL) [5–7]. In nonpregnant cows, $PGF_{2\alpha}$ binds to the $PGF_{2\alpha}$ (FP) receptor which is a seven-helix, G-protein–coupled, membrane-bound receptor located on luteal cells [8–10]. The binding of $PGF_{2\alpha}$ to its receptor initiates the phosphatidylinositol-phospholipase C intracellular signaling pathway that leads to the inhibition of progesterone synthesis and induction of apoptosis within the CL [11]. However, the interactions of

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http://dx.doi.org/10.1016/j.domaniend.2016.08.001

ligand-bound receptors and –associated heterotrimeric G-proteins that lead to activation of phospholipase C in bovine luteal cells are largely unknown.

The plasma membrane of cells is composed of a lipid bilayer containing cholesterol, sphingolipids, and glycerophospholipids [12–14]. The lipids of the bilayer are not homogenous but rather segregated into microdomains [15,16]. Lipid microdomains are regions on the plasma membrane of cells rich in cholesterol and sphingolipids, ranging in size from 10 to 200 nm in diameter [17,18]. Membrane-bound receptors, including G-protein–coupled receptors, have been reported to be associated with lipid microdomains in both ligand-bound and -unbound states [19]. Moreover, heterotrimeric G-protein alpha subunits have been reported to reside in lipid microdomains as reviewed in depth by Chini et al, [20]. The cellular functions of these domains are still being resolved but may allow for the colocalization of receptors with its associated heterotrimeric G-protein, leading to the activation of downstream signaling. Thus, disruption of lipid-lipid, lipid-protein, or protein-protein structure may cause alteration in downstream signaling.

Degree of unsaturation present in long-chain fatty acids determines fluidity of biological membranes. It has been reported in bovine platelet cells that cis-unsaturated fatty acids greatly increase fluidity [21]. In addition, inclusion of marine fish oil into the diet has been reported to alter lipid dynamics within the plasma membrane of many cell types. Studies have shown that fish oil supplementation alters membrane fluidity in ovine oocytes [22], murine macrophages [23], and primate erythrocytes [24] which may influence lateral mobility of membrane-bound receptors. Other studies have shown that omega-3 fatty acids, predominantly eicosapentaenoic and docosahexaenoic acid, both present in triglycerides found in fish oil, incorporate into plasma membranes of T-cells which have an effect on the fatty acid composition and morphology of the lipid microdomains [25,26]. This suggests that the incorporation of omega-3 fatty acids from fish oil could also affect the composition of lipid microdomains of bovine luteal cells which may lead to the alteration of lateral mobility of membrane-bound receptors. It is hypothesized that fish oil will alter lipid microdomains leading to an increase in spatial distribution of these lipid-ordered domains resulting in an increase lateral mobility of FP receptors in bovine luteal cells. The objectives of this study were to determine the effects of fish oil on (1) the spatial distribution of lipid microdomains, (2) lateral mobility of FP receptors, and (3) lateral mobility of FP receptors in the presence of $PGF_{2\alpha}$ on the plasma membrane in bovine luteal cells in vitro.

## 2. Methods

### 2.1. Tissue collection, cell preparation, and cell culture

Bovine ovaries containing a CL were collected at a local abattoir and transported to the laboratory at the University of Northern Colorado in 1× sterile PBS. Age of the CL was determined by gross morphological differences as previously described by Miyamoto et al [27]. Mature CLs were used for all experiments in the present study. The ovaries were then immersed in 70% ethanol to destroy any microorganisms that may be present from time of collection.

Using sterile techniques under a laminar flow hood, the CL was removed from the ovary and placed into a 60-$mm^2$ Petri dish containing ice-cold $Ca^{+2}/Mg^{+2}$-free Hank's-balanced salt solution (HBSS, pH 7.34). The CL was dissected free of connective tissue and cut into approximately 1-$mm^3$ fragments. Approximately 1 g of tissue was placed into T-25 culture flasks containing 5-mL dissociation medium (HBSS supplemented with 2000 units of collagenase type 1 and 0.1% BSA) and incubated in a water bath at 37°C with agitation for 45 min. Following incubation, the supernatant was removed and transferred to a sterile 15-mL culture tube. Cells were then washed 3× with sterile PBS, resuspended in 10 mL of culture medium (Ham's F12 supplemented with fetal bovine serum [5%], insulin [5 μg/mL], transferrin [5 μg/mL], sodium selenate [5 ng/mL], 100 unit per mL penicillin, 0.1-mg/mL streptomycin, and 0.25-mg/mL amphotericin B [pH 7.34]), and placed on ice. Fresh dissociation medium was added to the remaining undigested tissue and incubated with agitation for an additional 45 min. The remaining cells were collected, washed 2× with sterile PBS, and combined with the previous sample. After the final wash, cells were resuspended in 10 mL of culture medium.

Viability of cells was determined using trypan blue and cell concentration was estimated using a hemocytometer. Only preparations with a cell population of greater than 85% viability were used for each experiment. Cell cultures were maintained at 37°C in an atmosphere of 95% humidified air and 5% $CO_2$.

### 2.2. Fish oil preparation for in vitro culture

A commercial fish oil was used for all the experiments (Pharmavite, Mission Hills, CA, USA). Lipids in fish oil were prebound to BSA before the addition to culture. In brief, fish oil was added to culture medium containing 33-mg/mL fatty acid free BSA at the appropriate dose for each experiment as described by Mattos et al [28] Control medium was prepared using the same conditions as fish oil treatment, without the addition of fish oil.

#### 2.2.1. Experiment 1: effects of fish oil on spatial distribution of lipid microdomains

Mixed luteal cell cultures were prepared from 4 CL and plated in 35-mm glass bottom culture dishes at $5 \times 10^4$ cells/dish (n = 15 dishes/CL). Cells were incubated overnight at 37°C in an atmosphere of 95% humidified air and 5% $CO_2$ to allow adhesion to glass cover slips. Culture medium was removed and cells were treated with 0, 0.0003, 0.003, 0.03, or 0.3% (vol/vol) fish oil (n = 3 dishes/treatment) for 72 h to allow incorporation of fatty acids into membranes. Additional dishes (n = 6 dishes/CL) were incubated in 0% (vol/vol) fish oil to serve as positive and negative controls.

#### 2.2.2. Lipid microdomain labeling and visualization

Lipid microdomains were labeled using a commercially available kit per manufacture's protocol (Vybrant Lipid Raft Labeling kit; Invitrogen, Carlsbad, CA, USA). In brief, cells were washed 3× with 1-mL PBS. After the final wash, 1-mL ice-cold culture medium was added to each dish containing