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Equol and *para*-ethyl-phenol stimulate prostaglandin $F_{2\alpha}$ secretion in bovine corpus luteum: Intracellular mechanisms of action

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Abstract

Corpus luteum (CL) is a reproductive gland that plays a crucial endocrine role in the regulation of the estrous cycle, fertility, and pregnancy in cattle. The main function of CL is secretion of progesterone (P4), an important hormone for establishment a successful pregnancy, whereas prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 17β-estradiol (E_2) and testosterone (T) are implicated in the regulation of luteolysis. It has been shown that phytoestrogens may disrupt numerous reproductive functions on several levels of regulation and via different intracellular mechanisms. Using a cell–culture system of steroidogenic cells of the bovine CL, we determined effects of active phytoestrogen metabolites (equol and *para*-ethyl-phenol) on $PGF_{2\alpha}$, P4, and T synthesis in steroidogenic CL cells. Moreover, we examined the intracellular mechanisms of phytoestrogen metabolite actions. Phytoestrogen metabolites did not affect P4 production in steroidogenic CL cells. However, $PGF_{2\alpha}$ and T were significantly stimulated by metabolites of phytoestrogens in the bovine steroidogenic CL cells. To study the intracellular mechanism of endogenous E_2 and phytoestrogen metabolites action, steroidogenic cells were preincubated with a phospholipase C inhibitor (U73122), a protein kinase C inhibitor (staurosporine), an estrogen receptor antagonist (ICI) and a transcription inhibitor (actinomycin D) for 0.5 h, and then stimulated with *para*-ethyl-phenol, equol or E_2. Only U73122 and staurosporine totally reduced the stimulatory effect of E_2 on $PGF_{2\alpha}$ production by the cells. ICI and actinomycin D only partially reduced E_2 action on CL cells. In contrast, the stimulatory effect of phytoestrogen metabolites was totally inhibited by ICI and actinomycin D. Moreover, in contrast to E_2 action, phytoestrogen metabolites did not cause intracellular calcium mobilization in the cells. The present study demonstrated that phytoestrogen metabolites stimulate $PGF_{2\alpha}$ secretion in steroidogenic cells of the bovine CL via the estrogen receptor-dependent, genomic pathway.

Keywords: Phytoestrogens; $PGF_{2\alpha}$; Corpus luteum; Cattle

1. Introduction

Corpus luteum (CL) is a reproductive gland that plays a crucial endocrine role in the regulation of the estrous cycle, fertility, and pregnancy in cattle [1,2]. The main function of CL is secretion of progesterone (P4), an important hormone

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for establishment a successful pregnancy [1]. There are many various factors affecting CL development, maintenance and P4 secretion [2,3]. In this aspect prostaglandin (PG) $F_{2\alpha}$, 17β-estradiol (E_2) and testosterone (T) are implicated in the process of luteal regression in ruminants [3–5]. It has been proved that E_2, produced in CL [6], affects secretory function of cultured bovine luteal cells [7] and stimulates luteolytic $PGF_{2\alpha}$ synthesis [6]. In ruminants, endogenous estrogens are known to control the length of the estrous cycle by influencing PG synthesis (for review see [8,9]). For example, the removal of estrogens on Day 8 of the cycle by destroying ovarian follicles with X-irradiation in ewes, resulted in prolongation of the estrous cycle and lack of luteolysis [10]. On the other hand, administration of E_2 to heifers on Day 13 of the cycle initiated luteolysis by increasing the $PGF_{2\alpha}$ concentration [11]. We have recently shown that soy bean derived phytoestrogens influence in vivo $PGF_{2\alpha}$ secretion during the estrous cycle and early pregnancy in cattle [12]. Moreover, phytoestrogens and their active metabolites indirectly disrupt CL function by inhibiting LH- and PGE_2-stimulated P_4 secretion [13].

Soy phytoestrogens have been the subject of many reviews describing potential health benefits for human and animal body [14–16]. On the other hand, these substances also have some hazardous effects especially on pregnant animals fed with pasture rich in phytoestrogens [12,17,18]. Daidzein and genistein are two major phytoestrogens present in soy [19,20]. In ruminants, rumen microorganisms convert daidzein and genistein into their active metabolites—equol and *para*-ethyl-phenol, respectively [19]. There is increasing evidence that phytoestrogens disrupt reproductive process in various species, including humans [21,22], rats [23] and cows [17,24]. Phytoestrogens inhibit hypophyseal LH secretion in the rat [25]. Low LH level causes a decrease of progesterone (P4) production in CL which in turn leads to high abortion rate [26]. The decrease of pregnancy rate can also be attributed to phytoestrogen-dependent inhibition of endogenous estrogen production in the ovary leading to disturbances in follicle development [22,27]. Moreover, phytoestrogens acting as antagonists or/and agonists of endogenous estrogens may disrupt secretory function of bovine CL and uterus [13,28].

Phytoestrogens have the structural similarity to E_2. Therefore, we suppose that they elicit or selectively modulate genomic estrogenic responses by binding to both estrogen receptor (ER) α and β [29,30] as well as non-genomically by their influence on phospholipase (PL)C, protein kinase (PK)C activity, and/or intracellular calcium $[Ca^{2+}]_i$ mobilization [31–33]. Owing to the presence of estrogen receptors in the bovine CL [6,34], we presume that exogenous estrogens–phytoestrogens mimicking E_2 action influence luteal function. The aims of the present study were to determine: (1) whether phytoestrogen metabolites may directly influence secretory functions of the bovine CL via stimulating luteolytic factors such as $PGF_{2\alpha}$ and T; (2) what is the intracellular mechanism of phytoestrogen metabolite-dependent increase of $PGF_{2\alpha}$ synthesis in steroidogenic cells of the bovine CL; (3) whether phytoestrogen metabolites and E_2 cause intracellular $[Ca^{2+}]_i$ mobilization in steroidogenic cells of the bovine CL. In the present study, we selected two major metabolites of phytoestrogens—equol and *para*-ethyl-phenol, which have been identified in the serum of cows fed a diet rich in soy bean [12].

2. Materials and methods

2.1. Cell culture and experiments

Healthy, normally cycling Holstein/Polish Black and White (75/25%; respectively) cows (four to six lactation) at Day 17 of the estrous cycle were used for collection of the ovaries with CL. The animals were eliminated by the owner (Spółka Rolna "Wroblik" Sp. z o.o, Lidzbark Warminski, Poland) from the herd of dairy cows because of low milk production. The estrus was synchronized using implants of a progesterone analogue (Crestar, Intervet, Holland) with additional injection of an analogue of $PGF_{2\alpha}$ (cloprostenol; Bioestrophan, Biowet, Gorzow Wielkopolski, Poland), as recommended by the manufacturer for the estrus synchronization of multiparous cows and detailed previously [35]. Enzymatic dissociation of the luteal tissue and the culture of luteal cells were performed as previously described [3]. Cell viability was greater than 85% as assessed by trypan blue exclusion. The cell suspension contained about 20–25% of large luteal cells, 70–75% of small luteal cells and less then 5% endothelial cells or fibroblasts, but no erythrocytes [3]. The final pellet of steroidogenic cells was suspended in a culture medium, Dulbecco modified Eagle medium and Ham's F-12 medium (DMEM/Ham's F-12, 1:1 (v/v); Sigma–Aldrich, Inc., St. Louis, MO; #D8900) containing 10% calf serum (Gibco BRL, Grand Island, NY; #16170-078) and 20 μg/ml gentamicin (Gibco BRL; #15750-060). Dispersed luteal cells were seeded at 5.0×10^4 viable cells/ml in 48-well plates (Experiment 1 and 2; Costar, Cambridge, MA, USA; #150687) or 30 ml bottles (Experiment 3; Sarstedt, Numbrecht, Germany; #83.1813.300) and cultured at 37.5 °C