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Prostaglandin (PTG) E and F receptors in the porcine corpus luteum; effect of tumor necrosis factor-α

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ABSTRACT

The porcine corpus luteum (CL) is NOT sensitive to the luteolytic effects of PGF-2α until days 12–13 of cycle. The control of "luteolytic sensitivity" (LS) of the pig CL to PGF-2α is unknown, but it is temporally associated with macrophage infiltration into the CL. Since macrophages are the predominant source of TNF-α in the porcine CL, in other studies we examined the effects of TNF-α on porcine luteal cells in culture and showed that TNF-α induces LS in vitro. In Experiment 1 of this study possible mechanisms involved in the control of LS were examined, and involved measurement of the protein levels of PTGER2/EP-2, and PTGER3/EP-3 in porcine CL collected before (days 7–10), versus after (day 13), the onset of the LS. In Experiment 2, an examination of potential mechanisms involved in the control of LS by TNF-α, was carried out in which the effects of TNF-α on mRNA and protein expression of EP-2, EP-3 and FP in cultured luteal cells, were examined. The results of Experiment 1 showed that PTGER-3/EP-3 (but not PTGER-2/EP-2) levels decreased in porcine CLs after (day 13) compared to before (day 7–10) LS. In Experiment 2, the data obtained showed that TNF-α decreased PTGER-3/EP-3 and increased PTGFR/FP protein (in EARLY stage CL). In conclusion, these studies suggest a role for PTGER-3/EP-3 in the acquisition of LS, and support the hypothesis that TNF-α from CL macrophages plays a critical role in the control of LS in the porcine CL, by increasing PTGFR/FP, and decreasing PTGER-3/EP-3 protein.

1. Introduction

It has been recognized for 40+ years that the porcine corpus luteum (CL) is "refractory" or "insensitive" to the luteolytic actions of PGF-2α for the first 12–13 days (d) of the estrous cycle (reviewed in Estill et al., 1993). However, our laboratory has previously shown that multiple injections of PGF-2α agonists caused premature luteolysis and reduced estrous cycle length by ~7 d (Estill et al., 1993; Estill et al., 1995; Gadsby et al., 1996), demonstrating that it is possible to induce the premature acquisition of "luteolytic sensitivity" to PGF-2α (LS), although the mechanism underlying this finding is unknown. Since PGF-2α treatment of cycling female pigs increased macrophage infiltration into the CL (Hehnke et al., 1994), one possible explanation for these findings is that macrophages play a role in the acquisition of LS. Macrophages infiltrate the porcine CL throughout the course of the natural estrous cycle (Zhao et al., 1998), confirming similar findings in multiple species (Bagavandoss et al., 1988, 1990; Bishop et al., 2015; Care et al., 2013; Duncan, 2000; Gaytán et al., 1998; Penny et al., 1999; Townson et al., 2002). Specifically in the pig, intra-luteal macrophage numbers increased ~4-fold between days 4–6 and days 7–12, and a further 2-fold (vs. day 7–12) increase was observed on days 14–18 of the estrous cycle (Zhao et al., 1998). The initial phase of elevated infiltration of macrophages into the CL coincides

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temporally with the development of LS (i.e. between days 7 and 13) during the normal estrous cycle (Gadsby et al., 2006). This lead us to hypothesize that CL macrophages, via their secretion of TNF-α (Zhao et al., 1998; *macrophages are the major source of TNF-α in the porcine CL*), may play a role in the development of LS in the normal estrous cycle (Chang et al., 2017; Gadsby et al., 2006), as well as in the premature acquisition of LS induced by PGF-2α, described above (Estill et al., 1993, 1995; Gadsby et al., 1996). Some support for this hypothesis was provided by other studies carried out in our laboratory showing that TNF-α sensitized porcine luteal cells to PGF-2α in culture (Gadsby et al., 2006).

The concept of mutual antagonism between PGE (has stimulatory/luteotropic effects on CL function) and PGF-2α (has inhibitory/luteolytic effects on CL function) in determining CL function/regression has been reported in multiple species (Arosh et al., 2004a,b; Diaz et al., 2000, 2002; Fujino et al., 2004; Lee et al., 2012; Niswender et al., 2007; Waclawik et al., 2008; Weems et al., 2006; Zerani et al., 2007), and specifically in the pig, was illustrated by studies showing that PGE counteracted the luteolytic effects of PGF-2α, in the indomethacin-treated pig (Akinlosotu et al., 1988). Furthermore, measurements of PGE and PGF-2α receptors in the porcine CL, have shown that while total luteal cell PGE receptor binding significantly decreased (~ 2-fold) between day 9 and 14 of the cycle (Feng and Almond, 1999), PTGFR/FP receptor concentrations increased dramatically around the same time (day-13–14; Gadsby et al., 1990, 1993). PGE's actions on cells occur via different PGE receptor (PTGER/EP) sub-types (PTGER/EP-1, 2, 3 and 4) that involve distinct intracellular signal transduction pathways; EP-1 and 3 involve Ca^{2+} and Ca^{2+} mediated signaling, while EP-2 and 4 involve cAMP and its signaling pathways (An et al., 1994; Arosh et al., 2004b; Narumiya et al., 1999; Sugimoto and Narumiya, 2007). Several PGE receptor subtypes have been identified in the porcine (EP2, EP4; Waclawik et al., 2010), ovine and bovine (EP-1, 2, 3 and 4; Anderson et al., 1999; Arosh et al., 2004b; Lee et al., 2012) CL. Waclawik et al. demonstrated that while EP-2/EP-4 mRNA expression did not vary between days 9 and 14 of the estrous cycle, EP-2 protein increased, while EP-4 decreased, on day 14, vs. earlier stages (Waclawik et al., 2010). In contrast, PGF-2α acts via a single (FP) receptor (Sugimoto et al., 1997), and although two isoforms of this receptor have been identified at the protein level (FPA – full length; FPB – with a truncated C-terminus; Fujino et al., 2004), FPA is believed to be the major mediator of the luteolytic actions of PGF-2α (Sugimoto et al., 1997). As reported above, studies carried out in our laboratory have shown that both FP mRNA expression (days 10–15 of cycle (Boonyaprakob et al., 2003) and PGF-2α receptor binding (i.e. protein; day 13–14 of the cycle (Gadsby et al., 1990, 1993) in porcine CL, increased around the time of the onset of LS during the estrous cycle, suggesting that increased FP levels may play a role in the acquisition of LS in the pig (Boonyaprakob et al., 2003; Gadsby et al., 1990, 1993).

Taking into consideration the aforementioned background on PGE/EP and PGF/FP receptors in the porcine CL, in this study we proposed to examine the potential role that EP receptors play in the CL with regard to the control of LS in Objective/Experiment (1) which was designed to measure the abundance (protein levels) of EP-2 and EP-3 (PTGER2 and 3) in porcine CL collected before (days 7–10) versus after (day 13) the onset of the LS during the natural estrous cycle. Secondly, we proposed to investigate our hypothesis that macrophages (via TNF-α secretion) play a role in regulating LS, by carrying out Objective/Experiment (2), which was designed to examine the effects of TNF-α on the expression (mRNA) and abundance (protein) of EP-2, EP-3 (PTGER2 and 3) and FP (PTGFR) in cultured luteal cells (Experiment 2).

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals and CL collection

Regularly cycling gilts (Sus scrofa, White Landrace x Blackline hybrid) were obtained from the herd of the NCSU Swine Educational Unit. Animals were checked daily for standing estrus with a mature boar. The first day of estrus was designated as day 0. Animals were subjected to surgical ovariectomy on days 7–10 (i.e. ***before*** the onset of luteolytic sensitivity; LS) and 13 (i.e. **after** the onset of LS; n = 5 per stage), for an examination of the EP-2 and EP-3 receptor protein levels in CL by Western blotting, as described previously (Zorrilla et al., 2009, 2010, 2013). Ovaries were collected by surgical ovariectomy via mid-ventral laparotomy (Chang et al., 2017; Zorrilla et al., 2009, 2010, 2013), CL were dissected from ovarian tissue and were snap frozen in liquid nitrogen and then stored at −80 C until use for Western blotting. All animal use protocols were approved by the NCSU Institutional Animal Care and Use Committee (IACUC).

2.1.2. Protein extraction and analysis by Western blot – whole CL

CL protein extraction and Western blotting were performed using methods those reported previously (Chang et al., 2017; Zorrilla et al., 2009, 2010, 2013). In brief, total proteins were extracted from CL by homogenization in a "lysis buffer" containing 1% Triton-X, 0.5% NP-40, 1% P8340 protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) and 1% Halt Phosphatase inhibitor (Thermo-Fisher, Waltham, MA), on ice, followed by sonication for 10–15 s. Samples were then centrifuged at 14,000*g* to remove cellular debris, the supernatants collected and frozen at −20 C prior to Western blot analysis. Protein concentrations were determined using the Micro-BCA™ protein assay (Pierce, Rockford, IL) according to manufacturer's instructions. Between 15–40 μg of CL protein lysates were boiled (95 C for 5 min.) in Laemmli buffer, loaded on to 4–12% Bis-Tris gels (Invitrogen, Carlsbad, CA) and subjected to electrophoresis at 180v for 50 min. Following electrophoresis, proteins were transferred to PVDF membrane (Pall Company, Pensacola, FL) using a Turbo-Blot trans-blot system (Bio-Rad, Hercules, CA). Blots were subsequently blocked in 5% (w/v) milk dissolved in tris-buffered saline – Tween (TBST) and then incubated with primary antibody; both steps for 1 h at room temperature with rocking. Primary antibodies used were PTGER2 – EP-2 (1:200; rabbit polyclonal primary, Cat. No. 101750, Cayman Chemical, Ann Arbor, MI), PTGER3 – EP-3 (1:200; rabbit polyclonal primary, Cat. No. 101760, Cayman), PTGFR – FP (1:200; rabbit polyclonal

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