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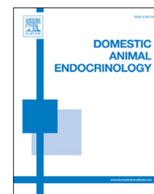

Prostaglandin synthesis by the porcine corpus luteum: effect of tumor necrosis factor-α

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ABSTRACT

The porcine corpus luteum (CL) displays delayed sensitivity to PGF-2α (luteolytic sensitivity, [LS]) until days 12 to 13 of cycle. The control of LS is unknown, but it is temporally associated with macrophage (which secrete tumor necrosis factor-α; TNF-α) infiltration into the CL. Other studies showed that TNF-α induces LS in vitro and that prostaglandins (PGs) may be involved in this mechanism. In experiment 1, PGF-2α and PGE secretion by luteal cells (LCs) was measured on days 4 to 14 of the estrous cycle, and the expression of PTGFS/AKR1B1 and PTGES/mPGES-1, determined by Western blot, before (day 7) vs after (day 13) the onset of LS. Results showed that the PGF-2α:PGE ratio increased significantly ($P < 0.05$) from day 4 to 13–14, and PTGFS/AKR1B1 and PTGES/mPGES-1 were significantly increased ($P < 0.05$) on day 13 (vs day 7). In experiment 2, LCs were collected from porcine CL at early (~days 4–6) or mid (~days 7–12) stages of the estrous cycle and cultured with 0, 0.1, 1, or 10 ng/mL TNF-α. Results showed that TNF-α significantly increased ($P < 0.05$) messenger RNA (mRNA) expression of cyclooxygenase (COX)-2 and mPGES-1 but not AKR1B1. TNF-α had no significant effects on AKR1B1 or mPGES protein abundance. TNF-α significantly increased ($P < 0.05$) PGE-2 but had no effect on PGF-2α secretion or on the PGF-2α:PGE2 ratio. In conclusion, although TNF-α increased COX2 and mPGES-1 mRNA, and PGE-2 secretion in vitro, it did not increase the PGF-2α:PGE2 ratio. Studies are currently directed toward exploring other pathways (eg, FP receptor signaling) by which TNF-α induces LS in the porcine CL.

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1. Introduction

It has been recognized for 40 yr or more that the porcine corpus luteum (CL) is “refractory” or “insensitive” to the luteolytic actions of PGF-2α for the first 12 to 13 d of the estrous cycle [1]. However, we have demonstrated that multiple injections of PGF-2α agonists induced premature luteolysis and reduced estrous cycle length by ~7 d [1,2], indicating that the premature acquisition of “luteolytic sensitivity” (LS) to PGF-2α could be induced by this approach. Since PGF-2α treatment of cycling female pigs was shown to increase macrophage infiltration into the CL [3], one possible explanation for these findings is that macrophages play a role in the acquisition of LS. Macrophages infiltrate the porcine CL throughout the estrous cycle [4] in confirmation of similar observations in other species [5–12]. Specifically in the pig, intraluteal macrophage numbers increased ~4-fold between days 4 to 6 and days 7 to 12 of the estrous cycle [4], which coincides temporally with the development of LS during the normal estrous cycle [13]. Since porcine CL macrophages produce tumor necrosis factor-α (TNF-α) [4], we hypothesized that macrophage-derived TNF-α may play a role in the premature acquisition of LS by PGF-2α, described above [1,2] and provided some support for this hypothesis by showing that TNF-α “sensitized” porcine luteal cells (LCs) to PGF-2αs in culture [13]. It is known that TNF-α increases luteal PGF-2α synthesis by bovine LCs [14], and preliminary data (not

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shown) using inhibitors of prostaglandin (PG) synthesis suggested that PGs may be involved in the TNF-α–induced "sensitization" pathway in porcine LCs. It has been shown in several species, that during the early luteal phase, luteal PG secretion favors the luteotropic PGs, eg, PGE-2 (or PGI2), but in the mid-late luteal phase, the synthesis of the luteolytic PG, PGF-2α increases, resulting in an increased intraluteal PGF-2α:PGE ratio [15–19]. The intraluteal PGF-2α:PPGE-2 ratio is thought to play an important role in determining the luteolytic response of the CL to PGF-2α [15–20]. Thus, we hypothesized that TNF-α acts to induce LS by increasing PGF-2α vs PGE secretion by, and thus the PGF-2α:PGE ratio in, the porcine CL.

Prostaglandins are derived from arachidonic acid, which is released from cellular phospholipids via phospholipase A2 [21]. Arachidonic acid is then converted to PGH2, the common precursor of the PGs, via cyclooxygenase (COX, PGG/H synthase or PTGS2). COX-1 is the constitutive form of this enzyme, whereas COX-2 is the inducible form and subject to control by hormones, cytokines (including TNF-α), and growth factors [22]. The final PG product of this pathway depends on the activities of the terminal synthases (*S*) (ie, PTGE*S*→PGE-2; PTGF*S*→PGF-2α; and PTGD*S*→PGD-2) [22]. A novel PTGFS subtype of the aldose-keto-reductase (AKR) family, AKR1B1, was identified in CL of ruminants and localized to large LCs [15,18,23]. Although AKR1B1 has not previously been reported in the porcine CL, it does contain a different PTGFS (AKR1C3), the levels of which increased from days 2 to 4 to days 14 to 15 of the cycle [17]. The porcine CL also expresses PTGES (microsomal = mPGES-1), the levels of which also increased from early to late cycle [17]. However, the PGF-2α:PGE-2 (CL content of PGs) ratio (indicating the functional activity of these enzymes), appeared to increase on days 9 to 15 vs days 2 to 8, coincident with the acquisition of LS [17].

Thus, the objectives of this study were to (1) examine the abundance (protein levels) of mPGES (PTGES1) and AKR1B1 (PTGFS) in, and the secretion of PGE and PGF-2α by, CL or LCs collected during the normal estrous cycle (experiment 1) and (2) to examine the effects of TNF-α on the expression (messenger RNA [mRNA]) and abundance (protein) of mPGES-1 and AKR1B1 in, and the secretion of PGE-2 and PGF-2α by, LCs in culture (experiment 2).

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals and CL collection

Regularly cycling gilts (*Sus scrofa*, White Landrace × Blackline hybrid) were obtained from the herd of the North Carolina State University (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 4, 7 to 8, 10, and 13 to 14 (n = 5–8 per day; PG secretion by LCs in vitro) or on days 7 and 13 (n = 4 and 6, per day respectively; PG synthetic enzyme protein levels in CL by Western blotting), as described previously [24–26]. Before surgery, animals were anesthetized with an intra-venous injection of ketamine (2.2 mg/kg; Webster Veterinary Supply, Sterling, MA, USA) and xylazine (0.4 mg/kg; Webster), and a surgical plane of anesthesia was subsequently maintained with halothane (Webster) and an oxygen/nitrous oxide mixture. Ovaries were collected via mid-ventral laparotomy, and CL were dissected from ovarian tissue. Corpora lutea were either subjected to dissociation and culture (in vitro studies), or snap frozen in liquid nitrogen and then stored at −80°C until further use (Western blotting). All animal use protocols were approved by the NCSU Institutional Animal Care and Use Committee.

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

CL protein extraction and Western blotting were performed using similar protocols to those previously reported [24–26]. In brief, total proteins were extracted from CL by homogenization in a "lysis buffer" (10-mM Tris-HCl, 100-mM NaCl, 1-mM EGTA, and 1-mM EDTA) containing 1% Triton-X, 0.5% NP-40, 1% P8340 protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO, USA) and 1% Halt Phosphatase inhibitor (Thermo-Fisher, Waltham, MA, USA), 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 before Western blot analysis. Protein concentrations were determined using the Micro-BCA protein assay (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Between 15 and 40 μg of CL, protein lysates were boiled (95°C for 5 min) in Laemmli buffer and loaded on to 4% to 12% Bis-Tris gels (Invitrogen, Carlsbad, CA, USA) and subjected to electrophoresis at 180v for 50 min. After electrophoresis, proteins were transferred to PVDF membrane (Pall Company, Pensacola, FL, USA) using a Turbo-Blot trans-blot system (Bio-Rad, Hercules, CA, USA). Blots were subsequently blocked in 5% (wt/vol) milk dissolved in tris buffered saline (TBS)-Tween and then incubated with primary antibody, both steps for 1h at room temperature with rocking. Primary antibodies used were PGFS-AKR1B1 (1:2,000; rabbit polyclonal, from Dr Michel Fortier, Laval University, Quebec, or rabbit polyclonal primary, cat. no. ab71405, Abcam Cambridge, MA, USA), mPGES-1 (1:250; rabbit polyclonal primary, cat. no. 160140l, Cayman Chemical, Ann Arbor, MI, USA) and β-actin (1:2,000; mouse monoclonal primary, cat. no. A2228, Sigma–Aldrich). Membranes were washed in TBS-Tween, followed by incubation in secondary antibody (1:1,000–1:2,000; goat anti-rabbit, cat. no. sc-2004 or goat anti-mouse, cat. no. sc-2005, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 to 2 h at room temperature, and washed again with TBS-Tween. Chemiluminescent reagent (SuperSignal West Pico, Thermo-Fisher) was added for 5 min, protein bands were visualized using a Bio-Rad, Chemi-Doc (Bio-Rad) gel-imaging system, and bands were quantified with Image-Lab software (Bio-Rad). The intensity (determined by densitometry) of chemiluminescent-specific protein bands were normalized to that obtained with the β-actin antibody, and the data expressed as a ratio [24–26].

2.1.3. Corpus luteum dissociation and LC culture

CLs were dissected from the ovaries, minced (on ice), and subjected to dissociation with 0.1% collagenase, 0.1% hyaluronidase, 0.06% DNase in calcium and magnesium-free

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