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Prostaglandin F2α regulation of mRNA for activating protein 1 transcriptional factors in porcine corpora lutea (CL): lack of induction of *JUN* and *JUND* in CL without luteolytic capacity

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Abstract

Porcine corpora lutea (CL) develop sensitivity to regression by prostaglandin F2α (PGF2α), termed luteolytic capacity, about 13 d after estrus. We postulated that PGF2α regulation of activating protein 1 (AP-1) transcriptional factor expression underlies acquisition of luteolytic capacity. CL were collected from gilts on day 9 (estrous cycle) or day 17 (pseudopregnancy) before or after PGF2α treatment with mRNA measured for *FOS*, *FOSB*, *FOSL1, FOSL2*, *JUN*, *JUNB*, and *JUND* and the AP-1 target genes *CCL2* and *SERPINE1*. At 0.5 h after PGF2α, both day-9 and day-17 CL had increased ($P < 0.01$) mRNA for FOS (2,225% and 1,817%), *JUNB* (237% and 358%), and *FOSB* (1,060% and 925%). Intriguingly, at 0.5 h after PGF2α there was increased ($P < 0.01$) mRNA encoding *JUN* (1,099%) and *JUND* (300%) in day-17 but not day-9 CL. At 10 h after PGF2α there was elevated *FOSB* mRNA in day-17 (771%) but not day-9 CL and no PGF2α-induced change in *FOS*, *JUN, JUND*, and *JUNB* mRNA in day-9 or day-17 CL. Treatment with PGF2α increased mRNA for AP-1-responsive genes, *CCL2* at 0.5 h (202%) and *CCL2* and *SERPINE1* at 10 h (719% and 1,515%), only in day-17 CL. Thus, many of the fos family of transcription factors are dramatically induced by PGF2α in CL with or without luteolytic capacity. However, PGF only induced *JUN* and *JUND* expression in CL with luteolytic capacity, a finding that may be key for understanding the acquisition of luteolytic capacity, given that *JUN* is the only AP-1 family member with strong N-terminal *trans*-activation activity.

Keywords: Corpus luteum; Luteolysis; Ovary

1. Introduction

Luteolysis involves a decrease in progesterone (P4) production and induction of luteal cell death [1]. In most species, the early corpora lutea (CL) does not undergo luteolysis, even when challenged with the normal luteolysin, prostaglandin F2α (PGF2α) [2]. Acquisition of luteal sensitivity to PGF2α has been termed luteolytic capacity [3,4]. The pig CL acquires luteolytic capacity later in the luteal phase (day 12 to 13), well after CL have reached mature size and maximum hormonal secretion [2].

The mechanisms involved in acquisition of luteolytic capacity are largely undefined. Active receptors for PGF2α (FP receptors, *PTGFR*) are present on luteal cells well before acquisition of luteolytic capacity [5,6]. For example, PGF2α induces a decrease in *PTGFR* (FP re-

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ceptor) and *HSD3B1* (3-β-hydroxysteroid dehydrogenase) mRNA in CL that have or do not have luteolytic capacity [3,4]. Nevertheless, some gene expression pathways are only induced by PGF2α in CL with luteolytic capacity. For example, PGF2α differentially regulates pathways involved in production of PGF2α [3,4], P4 [2,7], endothelin-1 (*EDN1*) [8], chemokine C-C motif ligand 2 (*CCL2*; also known as monocyte chemoattractant protein-1 [9]), and estradiol biosynthesis and signaling [10]. Differential regulation of gene expression in CL with luteolytic capacity likely involves differences in activation of transcription factors and signaling pathways.

The activating protein-1 (AP-1) family of transcription factors contains characteristic basic leucine-zipper regions and includes *FOS*, *FOSB*, *FOSL1*, *FOSL2*, *JUN*, *JUNB*, and *JUND* proteins. Genes for AP-1 are immediate early genes that regulate a wide range of physiological responses such as cell death, inflammation, and proliferation [11]. Responses to induction of AP-1 are dependent on the specific gene promoter, cell type, and which AP-1 proteins are induced [12]. The Jun family members can homodimerize with other Jun proteins or heterodimerize with Fos proteins or with other basic leucine zipper–containing transcription factors such as activator transcription factor family members. In contrast, Fos members do not homodimerize but can only heterodimerize with Jun family members to form active transcription complexes.

The AP-1 proteins have been localized within the pig CL [13]. Also, treatment with PGF2α was found to induce AP-1 proteins in pig CL [14] and in bovine luteal cells via a protein kinase C–dependent mitogen-activated protein kinase pathway [15]. One indication that AP-1 proteins may be differentially regulated in CL without luteolytic capacity is that some AP-1-regulated genes are regulated differently in CL with or without luteolytic capacity. For example, PGF2α increases *CYP19A1* [10] and *CCL2* mRNA [9] and decreases *STAR* mRNA [7] only in CL with luteolytic capacity. All 3 of these genes (*CYP19A1*, *STAR*, and *CCL2*) are regulated by AP-1 transcriptional complexes [16−20]. Therefore, this study was undertaken to determine whether AP-1 transcription factors are differentially regulated by PGF2α in porcine CL before and after acquisition of luteolytic capacity. We hypothesized that PGF2α would induce AP-1 transcription factors only after acquisition of luteolytic capacity. Alternatively, specific AP-1 transcription factors may be differentially regulated, potentially providing insight into the underlying transcriptional mechanisms associated with acquisition of luteolytic capacity.

2. Materials and methods

2.1. Chemicals and reagents

Cloprostenol was purchased from Bayer Corp. (Shawnee Mission, KS, USA), ketamine was from Fort Dodge Animal Health (Fort Dodge, IA, USA), and xylazine was from Phoenix Pharmaceuticals (St. Joseph, MO, USA). T7 RNA polymerase, Taq polymerase, reverse transcriptase, dNTPs, RNasin, and DNase I were purchased from Promega (Madison, WI, USA). Molecular weight markers were from GIBCO/BRL (Gaithersburg, MD, USA). Magnetight oligo(dt) beads were from Novagen (Madison, WI, USA). Unless otherwise specified, other chemicals and reagents used in these studies were purchased from Sigma (St. Louis, MO, USA).

2.2. Animals

Crossbred gilts (Cambrough × Line 19), 6 to 8 mo of age, were obtained from the university herd or purchased from Pig Improvement Company (Franklin, KY, USA). Animals were kept in individual pens with free access to water and were fed a maintenance diet of corn and soybean meal. For all studies, animals were checked daily for standing estrus with a mature boar. The first day of estrus was designated as day 0. Pseudopregnancy was induced in some gilts with daily injections of estradiol benzoate (2 mg intramuscularly [i.m.]) on d 11 to 15. On the day ovaries were collected, anesthesia was induced with i.m. injection of ketamine (15 mg/kg) and xylazine (0.3 mg/kg). Gilts were intubated and surgical plane of anesthesia was maintained with halothane. Ovaries were collected via midventral laparotomy and CL were dissected away from the ovarian stroma and either frozen in liquid nitrogen or transported to the laboratory in cold medium (M199, 100 IU/mL penicillin, 10 mg/mL streptomycin, 0.1% bovine serum albumin) for further processing. The Research Animal Resource Center Committee of the College of Agricultural and Life Sciences at the University of Wisconsin–Madison approved all procedures performed on animals.

2.3. Experiment I

This experiment examined the acute (0.5 h) in vivo regulation of mRNA for *FOS*, *FOSB*, *FOSL1*, *FOSL2*, *JUN*, *JUNB*, *JUND*, *SERPINE1*, and *CCL2* by PGF2α. On day 9 after estrus (n = 4) or day 17 of pseudopregnancy (n = 4), gilts were anesthetized and 1 ovary was collected (control CL). Following removal of the control ovary, 500 μg of PGF2α (cloprostenol i.m.) was given and the other ovary was collected 0.5 h later