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Amounts of an estrogen receptor β isoform increased in the theca of preovulatory follicles of sheep

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ABSTRACT

Determination of the specific roles of the estrogen receptor (ER) forms in reproductive processes of different species remains incomplete. In the present experiment, cellular localization and changes in relative amounts of the ERα and ERβ in late developing ovarian follicles, oviduct, and uterus were determined during the follicular phase of the estrous cycle in sheep. Ewes in mid-luteal phase were treated with prostaglandin $F_{2\alpha}$ (PG) to induce luteolysis and control the onset of the follicular phase. The oviducts, uterus, and the ovaries were collected at 0 (ewes not treated with PG), 4, 18, and 36 h after PG treatment (early, mid, and late follicular phase, respectively) and processed to evaluate the ERs using immunohistochemical (IHC) procedures. The ERα was localized to nuclei of granulosa cells of late developing follicles and most cells of the oviduct and uterus. The ERβ was detected only in ovarian follicles using two antibodies directed to different regions of the ERβ. Western immunoblotting demonstrated that the antibody directed against the N-terminal region of the ERβ detected one isoform (approximately 53 kDa) whereas the antibody directed against the C-terminus detected two ERβ isoforms (approximately 53 kDa and 59 kDa). Western and IHC results combined indicated presence of the 59 kDa ERβ in granulosa cells and the 53 kDa ERβ in both granulosa and theca cells. Relative amounts (immunostaining intensity) of the ERα increased ($P < .05$) in granulosa cells of preovulatory follicles and in the isthmian muscularis of the oviduct at the late follicular phase. Amounts of the ERα in the mucosal epithelium of the oviductal regions (isthmus, ampulla, and infundibulum), and in various uterine cell types (glandular and luminal epithelia, endometrial stromal cells, and myometrium) did not change ($P > .05$) throughout the follicular phase. A major increase (four-fold) in expression of the 53 kDa ERβ in the theca and a less pronounced increase in the granulosa occurred at the late follicular phase. The ERα is broadly expressed in reproductive organs of sheep and is upregulated only in few cell types during the late follicular phase. Immunoreactive ERβ was detected only in the ovary. Important estrogen actions in theca cells during preovulatory follicular development likely occur in association with a major increase in expression of an ERβ isoform.

1. Introduction

Estradiol-17β (estradiol) regulates follicular development, gonadotropin secretion, estrous behavior, and functions of all reproductive organs (Palter et al., 2001). Estradiol actions are mediated by at least two estrogen receptors (ERs); the ERα and ERβ, which based on their main mode of signaling are classified as ligand-activated

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transcription factors (Heldring et al., 2007). In addition to the two ER forms, a number of ER mRNA variants, most of them resulting from alternative splicing of the original transcripts, have been reported (Poola et al., 2002; Girault et al., 2004). Expression, regulation, and functions of the proteins encoded by the majority of ER mRNA variants remain to be elucidated (Deroo and Korach, 2006).

Determining the localization and changes in expression of the ERs is important for identifying cells responsive to estrogens and elucidating the roles of the ERs. The ERβ has been the predominant or only ER detected in granulosa cells of mice and rats (Fitzpatrick et al., 1999; Sar and Welsh, 1999), however, both the ERα and ERβ have been detected in granulosa cells of other species such as sheep and pigs (Tomanek et al., 1997; Cárdenas et al., 2001; Slomczynska et al., 2001). Expression of the ERs in the theca also differs among species; for example, the ERβ is expressed in sheep and pigs (Tomanek et al., 1997; Slomczynska et al., 2001) and the ERα in rodents and cattle (Fitzpatrick et al., 1999; Rosenfeld et al., 1999). In the uterus the ERα has been readily detected in various cell types, whereas the ERβ has been undetectable or significantly less abundant (Taylor and Al-Azzawi, 2000; Wang et al., 2000; Cárdenas and Pope, 2004). Similarly, in the oviduct the ERα has been observed in more cell types and in greater amounts than the ERβ (Wang et al., 2000; Okada et al., 2003; Ulbrich et al., 2003). The specific roles of the ERs in the reproductive processes, particularly in ovarian follicles where the ERs are co-expressed, are not well defined (Drummond and Fuller, 2010).

Although cellular localization of the ERs in reproductive organs has been described in some detail, information on changes in ER expression during the estrous cycle of the majority of species is limited. We hypothesize that changes in ER expression occur in reproductive organs which probably contribute to regulation of estrogen actions. Estradiol is important during the follicular phase of the estrous cycle when its secretion increases influencing a number of reproductive processes. In sheep, relative amounts of the ERα have been determined in the uterus during the follicular phase (Cherny et al., 1991; Spencer and Bazer, 1995), but little is known about the fluctuations in amounts of the ERs in late developing follicles and the oviduct. Late developing follicles undergo rapid growth, ovulation and luteinization, processes that might be partially regulated by estrogens. In addition, late developing follicles are a major source of estradiol and are constantly exposed to high concentrations of this hormone. Estradiol actions under these conditions might be regulated by changes in elements of the estrogen signaling pathways including expression of the ERs. The oviduct has three regions (isthmus, ampulla, and infundibulum) with different roles in the processes of ova pickup, transport of gametes, fertilization and transport of embryos (Jansen, 1984; Abe, 1996). It is not clear if differences exist in expression of the ERs among the oviductal regions that would relate to differences in functions.

The objectives of the present experiment were to determine the localization and relative changes in amounts of immunoreactive ERs in late developing follicles, oviduct, and uterus during the follicular phase of the estrous cycle in sheep.

2. Materials and methods

Crossbred ewes (Hampshire by Targhee) received two injections (i.m.), nine days apart, of 20 mg of $PGF_{2\alpha}$ (PG, Lutalyse, Pfizer, New York, NY) to control regression of corpora lutea (CL) and onset of the follicular phase of the estrous cycle. Before the second PG injection, ewes were randomly assigned to be evaluated at 0 (no PG treatment), 4, 18 or 36 h post-PG administration ($n = 4$ per group). In a previous experiment conducted in our laboratory using ewes of the same genetic background, plasma progesterone decreased to less than 1 ng/ml within 4 h, and onset of estrus occurred in average 34 h after PG treatment (Wiley et al., 1997). Based on these results, it was estimated that the period of evaluation from 0 to 36 h after PG treatment in the present experiment comprised the entire follicular phase.

Ewes were euthanized at the different evaluation times to obtain the reproductive organs. Proper response to PG treatment for induction of the follicular phase was verified by observation of signs of regression of corpora lutea (changes in size and color) and size of ovarian follicles. The ovaries, a cross section (approximately 1 cm^2) of the uterine wall (non-caruncular area from the miduterine horn), and portions of approximately 2 cm in length of oviductal isthmus (proximal to the uterine–tubal junction), ampulla (mid-region wider than the isthmus) and infundibulum (funnel-shaped end) were collected from each ewe. Tissues were fixed in 4% paraformaldehyde for 18 h at 4 °C, dehydrated in a series of ethanol solutions and then embedded in paraffin following standard procedures. Experimental protocols involving animals were approved by the Animal Care and Use Committee of the College of Food Agriculture and Environmental Sciences.

2.1. Immunohistochemistry

The ERs were detected in the different tissues of each ewe ($n = 4$ per time-point) by immunohistochemistry (IHC) using a Vectastain ABC kit for peroxidase (Vector Laboratories Inc., Burlingame, CA). The ER antibodies and corresponding immunizing peptides were purchased from Affinity BioReagents (Golden, CO). The ERα PA1-309 polyclonal antibody was raised against a peptide corresponding to amino acids 21–32 of the human ERα. This peptide sequence is completely conserved in several species including sheep. Two polyclonal antibodies: PA1–311 and PA1–313 were used for detection of the ERβ. The PA1–311 was raised against residues 55–70 of the rat ERβ (15 out of 16 homologies with sheep), and the PA-313 against a 19 amino acid sequence of the human ERβ C-terminus (13 out of 19 homologies with sheep).

The IHC procedure has been previously described (Cárdenas and Pope, 2004) and was applied with few modifications. Briefly, deparaffinized tissue sections (7 μm) were incubated in 0.01 M sodium citrate, pH 6.0, for 30 min at 95 °C (antigen retrieval), allowed to cool to 38 °C, rinsed in PBS (pH 7.3) at room temperature, treated with 0.3%