An Aldose Reductase with 20α-Hydroxysteroid Dehydrogenase Activity Is Most Likely the Enzyme Responsible for the Production of Prostaglandin F2α in the Bovine Endometrium*

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Prostaglandins are important regulators of reproductive function. In particular, prostaglandin F2α (PGF2α) is involved in labor and is the functional mediator of luteolysis to initiate a new estrous cycle in many species. These actions have been extensively studied in ruminants, but the enzymes involved are not clearly identified. Our objective was to identify which prostaglandin F synthase is involved and to study its regulation in the endometrium and in endometrial primary cell cultures. The expression of all previously known prostaglandin F synthases (PGFSs), two newly discovered PGFSs, and a 20α-hydroxysteroid dehydrogenase was studied by Northern blot and reverse transcription PCR. These analyses revealed that none of the known PGFS or the PGFS-like genes were significantly expressed in the endometrium. On the other hand, the 20α-hydroxysteroid dehydrogenase gene was strongly expressed in the endometrium at the time of luteolysis. The corresponding recombinant enzyme has a Km of 7 μM for PCH2 and a PGFS activity higher than the lung PGFS. This enzyme has two different activities with the ability to terminate the estrous cycle; it metabolizes progesterone and synthesizes PGF2α. Taken together, these data point to this newly identified enzyme as the functional endometrial PGFS.

Prostaglandins are local mediators acting through paracrine or autocrine mechanisms. Prostaglandins are produced from arachidonic acid liberated from phospholipid stores through the action of phospholipases. Arachidonic acid is then converted into prostaglandin H2 (PGH2), the common precursor of all subtypes of prostaglandins and because these prostaglandins share a common C5 precursor of all subtypes of prostaglandins and because these prostaglandins share a common C5

1 The abbreviations used are: PG, prostaglandin; PGHS, PGH synthase; PGFS, PGF synthase; 9K-PGR, 9-ketoprostaglandin reductase; DDBX, dihydrodiol dehydrogenase; RT, reverse transcriptase; HPLC, high pressure liquid chromatography; RACE, rapid amplification of cDNA ends.

PGHS: PGHS1 and PGHS2. These enzymes (also known as Cox-1 and Cox-2), which have been identified some 10 years ago, are still extensively studied. Because PGH2 is the common precursor of all subtypes of prostaglandins and because these prostaglandins isoforms cause different and even opposing actions, the pathways leading to their individual formation need to be identified.

Prostaglandin F2α (PGF2α) is involved in several physiological processes including pressure regulation in the eye (1), vasoconstriction (2), and renal filtration (3). It is associated 17–20 of the bovine estrous cycle, oxytocin initiates the release of large pulsatile waves of PGF2α, synthesized by epithelial cells of the endometrium (10). On days and the subsequent decrease in progesterone. Despite their primary involvement in the regulation of fertility, the mechanisms involved in the production of PGF2α at the cellular and molecular levels are poorly documented.

PGF2α can be produced from three distinct pathways (see Fig. 1). The most likely pathway to ensure selective production of PGF2α results from the production of PGF2α by a 9,11-endoperoxide reductase (now referred to as PGFS activity). Alternate pathways involve the reduction of PGD2 by a PGD2 11-ketoreductase or the reduction of PGE2 by a 9-ketoprostaglandin reductase (9K-PGR). Previous results obtained in vitro lead us to believe that the latter pathway could contribute to the production of PGF2α in cattle. In support of that hypothesis, we have identified a potential 9K-PGR in bovine endometrium (11).

Until now, a total of six PGFS have been identified. The first three were isolated in the cow: lung type prostaglandin F synthase (PGFS1) (12), lung type PGFS found in liver (PGFS2) (13), and liver type PGFS, also called dihydrodiol dehydrogenase 3 (DDBX) (14, 15). The three other PGFS were respectively isolated from humans (16), sheep (17), and Trypanosoma brucei (18). As a group, these enzymes belong to the aldoketoreductase family (19). The enzyme from Trypanosoma belongs to the AKR5A subfamily, whereas the others belong to the AKRJC.
family, which is also primarily associated with hydroxysteroid dehydrogenases. With the exception of the Trypanosoma enzyme, these enzymes also possess a PGD2 11-ketoreductase activity, thus giving them the ability to convert PGD2 into 9α,11β-PGF2α, an isomer of PGF2α (20). Bovine PGF2α possesses Km values of 120 μM for PGD2 and 10 μM for PGH2 (14). DBBX possesses Km values of 10 μM for PGD2 and 25 μM for PGH2 (14). The different bovine PGFS are closely related. The PGF2α and PGF2β enzymes are 95% identical, whereas DBBX is 86% identical to both PGFS1 and PGFS2.

9-Ketoprostaglandin reductase activity has been detected in the reproductive system of several species. In the rabbit, a 9K-PGR that possesses 20α-hydroxyandrosterone dehydrogenase activity accounts for 30% of soluble protein in the ovaries (21, 22). It is also present at a lesser extent in the corpus luteum. 9K-PGR activity has also been observed in the ovine endometrium and corpus luteum (23) and in the bovine placenta (24).

Recently, we have identified a potential 9K-PGR in bovine endometrium (11). The partial sequence of this putative enzyme showed 92% homology with bovine lung type prostaglandin F synthase. For simplicity, we will refer to this enzyme as PGFS1 for “prostaglandin F synthase-like 1.”

Studies on the regulation of PGFS1 have already been performed on cultured bovine endometrial cells (11, 25, 26). Treatments with hormones, oxytocin, and interferon τ had little influence on the level of PGFS1 mRNA expression (less than 50% variation), despite a large effect on prostaglandin production.

At parturition in the sheep, the level of PGFS1 mRNA did not vary in endometrium, myometrium, or placenta (17). Moreover, in all of these experiments, variations in the level of Cox-2 (prostaglandin H synthase 2) mRNA expression were observed. Thus, it was suggested that either Cox-2 alone was responsible for the increased production of PGF2α, or that the PGFS responsible for the production of PGF2α in the endometrium was a different, yet unidentified enzyme.

The objective of the present work was to identify the metabolic pathways and the enzymes involved in the formation of PGF2α in the endometrium. The measurement of mRNA levels in the bovine endometrium of the three known PGF2α-synthesizing enzymes (PGFS1, PGFS2, and DBBX) and three newly identified ones (PGFS1L, PGFS2L, and 20α-HSD) were done by Northern blotting and RT-PCR throughout the estrous cycle and in cultured endometrial cells. Recombinant proteins were produced and used to measure PGFS activity for each candidate enzyme.

**EXPERIMENTAL PROCEDURES**

**Materials**—Culture plates and Luria broth media were purchased from Becton Dickinson (Lincoln Park, NJ). RPMI 1640 was obtained from ICN Biomedicals (Aurora, OH). TRIZOL, fetal bovine serum, Moloney murine leukemia virus-RT, restriction enzymes, and buffer were purchased from Invitrogen. Oxytocin, hematoxylin (Mayer’s), 3-amino-9-ethyl-carbazol tablets, NADPH, and phenanthrenequinone were obtained from Sigma. Recombinant ovine interferon τ was kindly provided by Dr. Fuller W. Bazer. [3H]Arahanionic acid, [3H]PGF2α, [3H]PGD2, [3H]PGE2, the Ready-to-Go T-primed first strand kit, PCR enzymes (recombinant tag) and buffer, the T7 DNA polymerase sequencing kit, and the Ready-to-Go DNA labeling kit were purchased from Amersham Biosciences. The Ultrasphere C18 column was purchased from Beckman (Fullerton, CA). The TOPO TA-Cloning PCR2.1 cloning kit was acquired from Invitrogen. The oligonucleotides were synthesized with a DNA Synthesizer ABI-394 from Applied Biosystem Inc. Renaissance Western blot chemiluminescence reagent, [α-32P]UTP, [α-32P]dATP, and [γ-32P]ATP were bought from PerkinElmer Life Sciences. T7 RNA polymerase and RQ1 DNase were purchased from Promega (Madison, WI). Primers were obtained from Promega (Madison, WI). Primer extension was performed on a nylon membrane (Nytran Plus). Hybridization with the appropriate radiolabeled probe was performed overnight at 42 °C in a 50% formamide solution. The washes were done in 0.2% SSC at 65 °C, and the membranes were exposed to a Biomax film (Amersham Biosciences) at −80 °C until good signals were observed.

**RT-PCR Analysis**—Total RNA from specified sources was extracted with TRIZOL according to the manufacturer’s instructions. Reverse transcription was performed on 1 μg of RNA with Moloney murine leukemia virus-RT as described by the supplier’s protocol. PCR amplification of a given gene was performed with its corresponding oligonucleotides (Table I; oligonucleotides with names beginning with F) using bovine lung or liver cDNA. The resulting fragments were cloned with TOPO TA-Cloning pCR2.1 kit and sequenced as above. A 5′ RACE assay for PGFSL1 was performed with PCR (30 cycles, annealing at 55 °C on 50°C) on cDNA with the oligonucleotides RACE3prime and 35 (Table I). A ~700-bp fragment was obtained and cloned in pCR2.1 using the TOPO TA-Cloning pCR2.1 kit. Sequence analysis (using the T7 DNA polymerase sequencing kit) of several clones revealed a new putative enzyme homologous to the known bovine PGFS but different from the previously identified fragment of PGFSL1. This new putative PGFS was named “PGFSL2.” A second 3′ RACE assay for PGFSL1 was performed with liver cDNA. First, an asymmetric PCR (30 cycles, annealing at 55 °C) was done using oligonucleotide 55 followed by PCR (30 cycles, annealing at 55°C with oligonucleotides RACE3prime and 9KBOVs). The resulting 600-bp fragment was cloned and sequenced as previously described. This fragment corresponds to the 3′ end of PGFSL1. 5′ RACE of PGFSL2 was performed by PCR (30 cycles, annealing at 55°C on oxytocin-treated (6 h) bovine endometrial epithelial cell cDNA with oligonucleotides 36 and F1C7s (F1C11s and FDDBXs did not work). 5′ RACE of PGFSL1 was performed with PCR (30 cycles, annealing at 55°C on liver cDNA with the oligonucleotides 9KBOVs and FDBBXs (F1C11s worked but not F1C7).
amplified PCR products were sequenced and estimated to span over several introns by reference with corresponding human genes found in GenBank™.

RNase Protection Assay—Antisense riboprobes for AKR1B5 and β-actin were made by inserting amplified PCR fragments into TOPO pCR2.1 as described above. Plasmids containing AKR1B5 were digested with XcmI and transcribed with T7 RNA polymerase to yield a full-length RNA probe of 441 nucleotides comprising 339 nucleotides complementary (protected) to ARK1B5 mRNA. Plasmids containing AKR1B5 were digested with BglI and transcribed with T7 RNA polymerase to yield a full-length RNA probe of 242 nucleotides comprising 170 nucleotides complementary (protected) to ARK1B5 mRNA. The riboprobe synthesis and RNase protection assay were performed according to Pharmingen standard RPA procedure.

Enzymatic Activity—The full-length coding sequence of each gene was inserted in the NdeI restriction site of pET16B, and the His tag proteins were produced and purified as described by the manufacturer. Enzymatic activity was measured by monitoring NADPH degradation at 340 nm. The assays were performed in 1 ml of 50 mM Tris-HCl, pH 7.5, 100 µM NADPH with 10–100 µM of enzyme and variable concentrations of the tested compounds (17-hydroxyprogesterone, 17-hydroxyprogrenolone, phenanthrenequinone, and PGH2). Phenanthrenequinone was used as a universal AKR1C substrate to confirm the functionality of the enzymes. The production of PGF2α was confirmed by TLC using silica plates and enzyme-linked immunosorbent assay. Migration was performed in ethyl acetate:2,2,4-trimethylpentane:acetic acid (110:50:20) water-saturated solvent, and detection was achieved by spraying phosphomolybdic acid 10% (w/v) in methanol and cooking the plate at 120 °C for 10 min (30).

Western Blot and Immunohistochemistry Assays—Rabbit immunizations were performed using 4 × 250 µg of purified recombinant AKR1B5 protein. Soluble endometrial proteins were obtained by homogenization of endometrial tissue in 20 mM Tris-HCL, 1 mM phenylmethylsulfonyl fluoride, followed by centrifugation at 13,000 × g for 10 min. Proteins in the supernatant were then quantified as described previously (31). Western blot was performed using 20 µg of protein/lane on a 10% polyacrylamide gel. The proteins were transferred onto a nitrocellulose membrane. A 1:5000 dilution of AKR1B5 antisemur and 1:10,000 dilution of goat anti-rabbit secondary antibody were used. The membranes were washed between antisera incubation with phosphate-buffered saline containing 0.05% Tween. Revelation was performed using the Renaissance kit, following the manufacturer’s instructions. Immunohistochemical staining was performed as described in the instructions manual of the Vectastain Kit (Paraffin section) using 1:750 dilution of AKR1B5 antisemur and hematoxylin as counterstain.

RESULTS

PGF2α Biosynthesis—We first determined whether PGF2α could be synthesized from PGH2, PGF2α or PGD2 (Fig. 1) in endometrial cells. Epithelial cells were treated with oxytocin, a treatment known to specifically increase the production of PGF2α (32) and supplied with [3H]arachidonic acid, [3H]PGF2α, or [3H]PGD2. 24 h later, the radioactive prostaglandins present in the supernatant were analyzed by HPLC (Fig. 2). As expected, [3H]PGF2α is the main prostaglandin produced when tritiated arachidonic acid is given to the cells. No radioactive PGF2α was produced when [3H]PGF2α or [3H]PGD2 were given
to the cells, indicating that no 9-ketoreductase or 11-ketoreductase activity was present in cultured endometrial epithelial cells. The absence of 11-ketoreductase activity was surprising because all known PGFS exhibit this activity. The product of PGD₂ reduction, 9α,11β-PGF₂α was shown to elute almost at the same position as PGF₂α (33). The unknown radioactive products appearing in 2B and 2C might be related to prostaglandin catabolism.

Identification of an Alternate PGFS—These results show that of a massive production of PGF₂α is present in the bovine endometrium in absence of any known PGFS. Because all of these PGFS belong to the AKR1C family and shared at least 80% homology, we have designed oligonucleotide probes in regions common to all AKR1C family members. Northern analysis of endometrial mRNA revealed that no AKR1C family member was expressed at times of high PGF₂α production (34). An alternate pathway to produce PGF₂α is through 9-ketoreductase conversion of PGE₂. A former candidate for PGF₂α production in the endometrium, the rabbit 9-ketoreductase, also exhibit 20α-HSD activity (21, 22). Therefore, we searched GenBank™ to find whether there was any aldoketoreductase with 20α-HSD activity that was known in cattle. Interestingly, we found an aldose reductase identified as AKR1B5, having only 45% homology with the PGFSs of the AKR1C family but expressing 20α-hydroxysteroid dehydrogenase activity (35). To investigate the potential role of this enzyme, we designed specific oligonucleotides and found that its gene was highly expressed during the estrous cycle and in endometrial cell cultures (data not shown) at times just preceding the expected production of PGF₂α. Northern blot, RT-PCR, and RNase protection analysis revealed that AKR1B5 gene was highly expressed in the endometrium from days 10 to 21 (Fig. 5). Panels A, B, and C illustrate the production of PGF₂α, PGE₂, and PGD₂, respectively, using different precursors and labeled with [³H]Arachidonic acid, [³H]PGE₂, and [³H]PGD₂, respectively.
A) PGFS 1 and PGFS 2

B) DDBX

C) PGFSL-1

D) PGFSL-2

Fig. 3. Northern blot analysis of PGFS (1 and 2), DDBX, PGFSL1, and PGFSL2 throughout the estrous cycle in the endometrium. Lanes 1, days 1–4; lanes 2, days 5–8; lanes 3, days 9–12; lanes 4, days 13–15; lanes 5, days 16–18; lanes 6, days 19–21. Lu, lung; Li, liver; Ep, epithelial cells (treated with oxytocin for 6 h); St, stromal cells. Exposure was for one week. 18 S ribosomal RNA is shown as loading controls.

A, B, and D of Fig. 5 represent three distinct sets of samples, and slight variations in expression may depend on individual variations. The identity of the amplified products was confirmed by sequence analysis. RNase protection assay confirmed unequivocally that AKR1B5 is expressed in the endometrium and modulated throughout the estrous cycle. We cloned and expressed the recombinant protein to generate antibodies needed to characterize this enzyme. Western blot analysis showed that the protein followed a similar but slightly delayed pattern of expression (Fig. 6, B and C). Fig. 6A indicates that the AKR1B5 antibody is specific to AKR1B5 or at least to the AKR1B family because it can also recognize its human counterpart AKR1B1 (data not shown). A faint signal was observed for some AKR1C recombinant proteins, but we had to put 50 times more AKR1C protein to obtain a signal equivalent to that of AKR1B5. We evaluated the amount of AKR1B5 protein present in our sample by comparing its signal against a standard curve of AKR1B5 recombinant protein (Fig. 6D). Samples from days 4–8 contain about 20 ng (0.2% of the proteins present in the sample) of AKR1B5, and those from days 16–18 contain 200 ng (2%). Thus, if any member of the AKR1C family was responsible for this signal, it would have to constitute 100% of the protein in the sample.

**DISCUSSION**

Despite the preeminent role of PGF$_{2\alpha}$ in reproductive function in mammals, there has been no formal identification of the
biosynthetic enzyme(s) responsible for its selective production. The bovine endometrium is the main source of PGF$_{2\alpha}$ at the time of luteolysis. The results obtained in vitro in our laboratory suggested that PGF$_{2\alpha}$ could be produced at least in part through conversion of PGD$_2$ and/or PGE$_2$. This possibility is supported by the ability of cultured epithelial cells to generate PGF$_{2\alpha}$, PGE$_2$, and PGD$_2$. HPLC analysis of prostaglandin metabolism (Fig. 2), however, showed no detectable 9-ketoreductase or 11-ketoreductase activity in control or oxytocin-treated epithelial cells. These two activities were not observed in stromal cells either (data not shown). Therefore, in these cells, PGF$_{2\alpha}$ production appears to derive mainly by direct PGH$_2$ reduction (Fig. 1). This result was surprising because all PGFS are known to possess 11-ketoreductase activity. This was the first indication that in the bovine endometrium, PGF$_{2\alpha}$ is produced by a PGFS activity distinct from what was observed in other tissues. Some may argue that the phenomenon observed in cell culture may vary from what occurs in vivo. This is unlikely, because the large increase in PGF$_{2\alpha}$ production occurring in vivo in response to oxytocin at the time of luteolysis can be reproduced in vitro (32).

As an alternate pathway to generate PGF$_{2\alpha}$, we were able to complete the previously published partial putative 9K-PGR sequence (PGFS1L1) (11). In the process, we were fortunate to find PGFSL2, another gene highly related to PGFS1. With DDBX and PGFS2, the AKR1C family now counts five members in the bovine species. This situation is similar to the humans where PGFS (AKR1C3) belongs to a group of four highly homologous enzymes of the same family (AKR1C1, AKR1C2, AKR1C3, and AKR1C4) (19). Because all the members of this family are highly homologous, the use of Northern blots and RT-PCR may be misleading, and results must be interpreted with caution.

Some genes of the AKR1C family were expressed but only at the beginning of the cycle. No signal was visible at other periods of the cycle or in cultured cells. By RT-PCR we observed slightly different results. PGFS1, PGFS2, and PGFS1L1 were no longer detectable at the beginning of the cycle, whereas DDBX expression was visible in cultured epithelial cells, and PGFSL2 was expressed all along the estrous cycle and in cultured cells. The detection of a positive signal for PGFSL2 can be explained by the greater sensitivity of RT-PCR analysis. We observed no signal for PGFS1, PGFS2, and PGFS1L1 by PCR at the beginning of the cycle. These results, contrasting with Northern blot analysis, can be related to probe cross-hybridiza-

**Fig. 5. Expression of AKR1B5 gene throughout the estrous cycle.** Total RNA was extracted from endometrial scrapings collected at different days of the estrous cycle as described under “Experimental Procedures.” A, Northern blot analysis with 15 g of total RNA/lane. B, RT-PCR. C, relative expression of AKR1B5 gene (means ± S.D.) present in four sets of samples evaluated by PCR. D, RNase protection assay. Signals at 411 and 242 represent the full-length probe for AKR1B5 and β-actin, respectively. Those at 339 and 170 correspond to the portion of the probes protected by AKR1B5 and β-actin mRNA, respectively. Each assay was performed with 1 g of total RNA. NS, nonspecific control where yeast RNA was used. The sequence reaction to the left was used as a ladder. E, relative expression of AKR1B5 over β-actin is presented as a ratio of density of bands observed at 339 and 170. Throughout the figure, D followed by number represents the day of the estrous cycle. 18 S RNA and β-actin were used as loading controls.

**Fig. 6. Expression of AKR1B5 protein throughout the estrous cycle as evaluated by Western blot analysis.** A, the specificity of the AKR1B5 antibody was evaluated with different recombinant protein of the AKR1C family (60 ng of protein/lane). B and C represent two different series of proteins extracted from endometrial scrapings taken at different days of the estrous cycle as described under “Experimental Procedures” (10 μg/lane). D, titration of AKR1B5. 10 μg of endometrial protein from days 4–8 or 16–18 were compared with 10 and 100 ng of purified recombinant AKR1B5. LC, Coomassie Blue staining used as loading controls.
gation. Indeed, the PGFS1 and PGFSL1 cDNA probes may have hybridized with the DDBX and/or PGFSL2 mRNA. This hypothesis is highly probable because they share long stretches of common nucleic acid sequences; PGFS1 and PGFSL2 have only one mismatch between position 490 and 587. The same is true for PGFSL1 and PGFSL2 between positions 824 and 916 and so on. It is likely that the PGFS cDNA probe bound to the PGFSL2 mRNA present on the membrane, even under the high stringency conditions used. Moreover, higher expression of DDBX (and PGFSL2) at the beginning of the cycle may be related to the presence of leukocytes invading the endometrium at that moment. The endometrium, collected by scraping, is likely to contain some leukocytes that may express this gene at a high level.

The present results appear to be in contradiction with those previously published. Xiao et al. (25, 26) observed variation of the PGFS1 messenger in cultured cells in response to oxytocin, interferon τ, and steroid treatments. These experiments were conducted by Northern blot analysis with 25 μg of total RNA (compared with 15 μg in the present study) under low stringency conditions (2× SSC at 55°C). Under these conditions, the PGFS1 cDNA probe may have cross-hybridized with any of the five members of the AKR1C family. We have also detected PGFS1 and PGFSL2 combined with the lack of PGFS cDNA probes may have hybridized with the absence of mRNA for the known PGFS and the lack of actual enzyme levels would have to be even higher. Moreover, the absence of mRNA for the known PGFS and the lack of 11-ketoreductase activity in endometrial cells despite a large production of PGF2α eliminates these enzymes as functional PGFS in the bovine endometrium. Similarly, the low expression of PGFSL1 and PGFSL2 combined with the lack of PGFS activity of their recombinant proteins indicate that neither of these enzymes is involved in PGF2α formation in the endometrium. These enzymes may, however, be involved in steroid metabolism because it is the case for most enzymes of the AKR1C family.

The absence of expression of the known PGFS lead us to search for an alternate enzyme as described under “Results.” We were fortunate that the AKR1B5 candidate qualified as the PGFS of the bovine endometrium. First, its mRNA was expressed in endometrial tissues and cells in relation with the ability of these sources to produce PGF2α. Second, the recombinant AKR1B5 protein did not display any 9-ketoreductase activity but a strong PGFS activity. The Km of AKR1B5 for PGH2 is half that of the recombinant lung type PGFS (14), and its specific activity is twice as high. The 20α-HSD activity of our recombinant protein was present as expected and was comparable with the purified native enzyme (35).

Gene expression of AKR1B5 peaks around day 12, and at the same moment, progesterone reaches its highest systemic concentration, suggesting a link between the two. AKR1B5 may be directly or indirectly up-regulated by progesterone. Because AKR1B5 will also inactivate progesterone, at least locally, by transforming it into 20α-hydroxyprogesterone, it may down-regulate its own expression. Moreover, local metabolism of progesterone may have a physiological role in the endometrium. In ovariectomized sheep, progesterone withdrawal causes a build-up in oxytocin receptors (37). In intact animals,
it is thought that progesterone down-regulates its own receptor, therefore creating a situation similar to progesterone withdrawal (9). However, Robinson et al. (38) recently observed that the up-regulation of oxytocin receptors, occurring around days 15–16, in luminal epithelial cells was not dependent on a prior change in progesterone or estradiol α receptors. Additionally, Bogacki et al. (39) demonstrated that oxytocin was unable to bind to its own receptor in the presence of progesterone. This can constitute a major problem to initiate PGF2α production and luteolysis, because when the initial oxytocin bursts occurs, the organism is flooded with progesterone. It is possible that AKB15 reduces local progesterone concentration, allowing oxytocin to act on the endometrium.

We propose the following model incorporating AKB15 in the estrous cycle. First, ovulation marks the beginning of estrous cycle and the corpus luteum grows to produce progesterone. Progesterone secretion peaks between days 12 and 18, and concomitantly AKB15 expression rises in the endometrium. At some point, there is enough AKB15 protein to locally shift the influx:degradation balance toward degradation of progesterone, hence decreasing the local progesterone concentration. Then the oxytocin receptor concentration increases. Around day 18, the first wave of oxytocin occurs, and interaction with its receptor is possible because the local concentration of progesterone has been reduced. This interaction leads to activation of phospholipases (40, 41) and subsequently to an increase in PGH2 concentration. PGH2 is then transformed by AKB15 into PGF2α. After several waves of PGF2α, the corpus luteum is destroyed, abolishing the production of progesterone. The reproductive system is then ready for a new cycle.

The ability to combine two converging functions, inactivation of progesterone and generation of PGF2α, makes AKB15 a multifunctional enzyme with complementary action in the endometrium. Moreover, as an aldose reductase, this enzyme can also reduce benzaldehyde, glyceraldehyde, glucose, and several other carbonyl-containing compounds. This enzyme can be found in a wide variety of tissues. In humans a corresponding enzyme, AKB1, is involved in some complications associated with diabetes such as eye disease (cataracts) and nephropathy. It is believed that these complications are caused by sorbitol accumulation followed reduction of glucose. Because PGF2α is also involved in these diseases, the possibility that AKB15 may act as a PGF synthase in those organs is worth investigating.

In summary, we have found that AKB1 family members (to which all the currently known PGFS belong) are not expressed in the bovine endometrium. Instead, an aldose reductase known for its 20α-HSD activity, AKB15, is a likely candidate enzyme for controlling the sufficient and timely production of PGF2α in the bovine endometrium. This is the first time that a member of the AKB1 family has been associated with PGFS activity and also the first report of such an enzyme being expressed in the endometrium of any species.

REFERENCES

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