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Estradiol-17β Is Produced in Bovine Corpus Luteum[1]

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ABSTRACT

The aim of this study was to investigate the expression of cytochrome P450 aromatase (aromatase) mRNA, its activity, and estradiol-17β (estradiol) secretion in bovine corpus luteum (CL) during the estrous cycle. Expression of aromatase mRNA was examined in CL at the early, mid, late, and regressed luteal stages by using a reverse transcription-polymerase chain reaction. Aromatase mRNA was detected in all luteal stages examined, although aromatase expression was significantly lower during the early and regressed luteal phases compared to the mid and late luteal phases. Moreover, cultured midluteal cells clearly converted exogenous [^{3}H]androstenedione into estradiol, and an aromatase inhibitor significantly inhibited this conversion. To characterize the local release of estradiol within the CL during the estrous cycle, an in vitro microdialysis system (MDS) of CL was conducted. Estradiol in MDS perfusate was confirmed by a reverse-phase high-performance liquid chromatography in combination with enzyme immunoassays. Basal release of estradiol from microdialyzed CL did not change during the estrous cycle. Additionally, when freshly prepared midluteal cells were exposed to estradiol (10^{-14} to 10^{-9} M), estradiol stimulated prostaglandin (PG) $F_{2\alpha}$ secretion ($P < 0.05$), although it did not affect progesterone and oxytocin secretion. The overall results indicate that estradiol is produced locally in bovine CL throughout the estrous cycle, and they suggest that estradiol plays a role in regulating $PGF_{2\alpha}$ production in CL as an autocrine/paracrine factor.

corpus luteum, corpus luteum function, estradiol, ovary, steroid hormones

INTRODUCTION

Estradiol-17β (estradiol), the most active estrogen in the ovary, is synthesized and secreted by granulosa cells in antral follicles, especially preovulatory dominant follicles [1]. The ''two cell type'' theory of estradiol production has been well demonstrated; that is, estradiol production by granulosa cells, which do not produce androgens (the precursor of estrogens), is supported by theca cells as androgen-producing cells in the preovulatory follicles. The rise in peripheral concentration of estradiol during luteal regression through estrus leads to initiation of the preovulatory LH surge in cattle [2]. On the other hand, concentrations of estradiol in both follicular fluid and peripheral blood decrease at 6 h after the LH surge [3]. Because the concentration of androstenedione in follicular fluid also decreases after the LH surge [4], the decrease in estradiol concentrations seems to be due to the acute decrease in androstenedione production by theca cells after the LH surge. Furthermore, the level of cytochrome P450 aromatase (aromatase), which converts androgens into estrogens, as well as of 17α-hydroxylase P450, which converts progestins into androgens, decreased at 20 h after the onset of estrus induced by prostaglandin (PG) $F_{2\alpha}$ injection in heifers [5].

The corpus luteum (CL) is a transient endocrine organ that plays a vital role in regulation of the estrous cycle, fertility, and maintenance of pregnancy. The primary function of the CL is to synthesize progesterone. Besides progesterone, the CL also produces a variety of other hormones. In the human [6], rat [7], and pig [8, 9], the CL is a source of estradiol. Furthermore, some evidence suggests that estradiol acts within the porcine [10] and human [11] CL as a potent autocrine and/or paracrine regulator. On the other hand, estradiol affects the function of bovine luteal cells [12] and microdialyzed bovine CL [13], and specific binding sites of estradiol are present in the bovine CL [14]. Thus, we hypothesized that bovine CL produces estradiol, and that luteal estradiol plays a role as a paracrine/autocrine regulator.

To examine the above hypothesis, we investigated in the present study 1) expression of aromatase mRNA, 2) aromatase activity in bovine CL, and 3) secretion of estradiol by microdialyzed CL. The effects of estradiol on progesterone, oxytocin, and $PGF_{2\alpha}$ output by cultured luteal cells were also studied.

MATERIALS AND METHODS

Collection of CL

Ovaries with CL were collected from Holstein cows at a local abattoir within 10–20 min after exsanguination. The stage of the estrous cycle was defined by macroscopic observation of the ovaries and uterus as described previously [15, 16]. Those CL were distinguished into early (Days 2–3 after ovulation), developing (Days 5–6), mid (Days 8–12), late (Days 15–17), and regressed (Days 19–21) luteal stages. After determination of these stages, CL were immediately separated from the ovaries, frozen rapidly in liquid nitrogen, and then stored at −80°C until processed for studies of gene expression. For experiments involving microdialysis or cell culture, the ovaries with CL were submerged in ice-cold physiological saline before being transported to the laboratory.

Isolation of RNA

Total RNA from bovine CL was isolated using the single-step method described by Chomczynski and Sacchi [17] with TRIzol reagent (Gibco BRL, Gaithersburg, MD) and spectroscopically quantified at 260 nm. Al-

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iquots were electrophoresed on a 1% (w/v) denaturing agarose gel to verify the quantity and quality of RNA by ethidium bromide staining.

Reverse Transcription-Polymerase Chain Reaction

Two micrograms of total RNA were used to generate single-strand cDNA in a 60-μl reaction mixture by use of hexanucleotides as primers according to the protocol for the M-MLV reverse transcriptase kit (Promega, Madison, WI). Conditions for enzymatic amplification were optimized for each polymerase chain reaction (PCR) on a gradient cycler (Eppendorf, Hamburg, Germany) as follows: The PCR for aromatase contained 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.6 μM each primer, and 0.5 U of thermostable Taq DNA polymerase (Boehringer, Mannheim, Germany) to 2.5 μl of cDNA (final volume, 25 μl). Ubiquitin PCR was performed under the same conditions as those for aromatase, but a higher concentration of primer (1.0 μM) was used. Samples for aromatase were amplified for 25 cycles (one single denaturation step of 94°C for 2 min, each cycle at 94°C for 30 sec and 62°C for 45 sec, and afterward, one additional elongation step of 72°C for 2 min). Samples for the housekeeping gene *ubiquitin* were amplified for 22 cycles (one single denaturation step of 94°C for 2 min; each cycle at 94°C for 45 sec, 55°C for 45 sec, and 72°C for 45 sec; and afterward, one additional elongation step at 72°C for 2 min). To determine the optimal quantity of reverse transcript needed for PCR and to verify that the cDNA product depended on the mRNA transcript used for the template, varying quantities of transcriptase were used in the PCR reaction. The reverse transcription (RT) product from 2.5 μl was in the linear range for this amount and produced a visible band. To exclude any possible amplification of genomic DNA, all experiments included reactions in which the RT enzyme or cDNA template was omitted. As a negative control, water was used instead of RNA for the RT-PCR to exclude any contamination from buffers and tubes.

The primers encoding the bovine sequences were designed by using the EMBL database or were used as described elsewhere and commercially synthesized (Amersham-Pharmacia, Freiburg, Germany). The primers used were as follows: aromatase forward 5′-AAGCCTTAGAGGATGATGTC-3′ and reverse 5′-GGTCTCGTCTGGATGCAAGG-3′ (326 base pairs [bp]) [18], and ubiquitin forward 5′-ATGCAGATCTTTGTGAAGAC-3′ and reverse 5′-CTTCTGGATGTTGTAGTC-3′ (189 and 417 bp, respectively) [19].

Aliquots of the PCR reaction products (5 μl) were added to 1 μl of bromphenol blue glycerin and fractionated by electrophoresis through a 1.5% agarose gel containing ethidium bromide in a constant 60-V field. To determine the length of the products, a DNA Mass Ladder (Gibco BRL) was used. The ethidium bromide-stained gel was evaluated by a video documentation system (Amersham-Pharmacia). Band intensities were analyzed by computerized densitometry using the Image Master 1D program (Amersham-Pharmacia). This method allowed only a relative quantification. Confirmation of the PCR product identity was obtained by cDNA subcloning into a transcription vector (pCR-Script; Stratagene, La Jolla, CA) and subjecting them to commercial DNA sequencing (TopLab, Munich, Germany).

Luteal Cell Culture and Aromatase Activity

Midluteal cells were prepared as described previously [20] and then adjusted to 3×10^5 viable cells per 1 ml of culture medium, which consisted of a 1:1 (v:v) mixture of Dulbecco modified Eagle medium and Ham F-12 (DMEM/F-12; D8900; Sigma Chemical Co., St. Louis, MO) supplemented with 10% calf serum (C6278; Sigma) and 20 μg/ml of gentamicin (Gibco BRL; 15750-060). Two milliliters of cell suspension were incubated in glass culture tubes (12 × 75 mm) in a shaking water bath at 37.5°C. The cultured medium was continuously gassed with 5% CO_2 in air. Thirty minutes after the start of culture, the cells were exposed to varying concentrations of estradiol (10^{-14} to 10^{-9} M; E2758; Sigma). After an additional 2 h of incubation, 1 ml of conditioned media was collected in tubes with 10 μl of a stabilizer (0.3 M EDTA, 1% aspirin [A2093; Sigma], pH 7.3) and stored at −30°C until assayed for progesterone, oxytocin, and $PGF_{2\alpha}$.

Aromatase activity was measured by the tritiated water method [21, 22]. Briefly, midluteal cells were seeded at 5×10^5 viable cells/ml in 24-well cluster dishes (Costar, Cambridge, MA) and cultured in DMEM/F-12 supplemented with 10% steroid-free calf serum (C1969; Sigma) and 20 μg/ml of gentamicin in a humidified atmosphere of 5% CO_2 in air at 37.5°C. Twenty-four hours after the start of culture, culture media were replaced by semi-serum-free fresh medium, DMEM/F-12 containing 0.1% BSA, 0.5 mM ascorbic acid, 5 ng/ml of sodium selenite, 5 μg/ml of transferrin, 2 μg/ml of insulin, and 20 μg/ml of gentamicin. The cells were simultaneously exposed to [1β,2β-^{3}H]androstenedione (10^{-7} M; New England Nuclear, Boston, MA) and the aromatase-inhibitor YM511 (kindly donated by Yamanouchi Pharmaceutical Co., Ltd., Ibaraki, Japan) [22]. After an additional 4 h of incubation, conditioned media were collected in glass culture tubes. After 200 μl of 20% trichloroacetic acid and 1 ml of 5% charcoal were added, the mixture was gently shaken at 37°C in air for 30 min to remove residual steroids. The mixture was then centrifuged (1000 × g), and the radioactivity in the aliquot of the supernatant was determined by a liquid scintillation spectrometer (LSC-5100; Aloka, Tokyo).

Microdialysis System In Vitro

The microdialysis system (MDS) of the bovine CL in vitro has been previously described in detail [23]. In brief, CL of the early luteal stage having diameters of 0.8–1.0 cm were isolated from the ovary and divided into halves. The surrounding stromal tissue of each piece was then removed. Two capillary dialysis membranes (cut-off molecular size, 1000 kDa; diameter, 0.2 mm; length, 5 mm; Fresenius SPS 900 Hollow Fibers; Fresenius AG, St. Wendel, Germany) with each end glued to a 4.5-cm-long piece of Silastic tubing (i.d., 0.3 mm) were implanted into each half of the CL with a 5-mm distance between capillaries. On the other hand, each CL of mid or late luteal stage was cut to a 15-mm cube, divided into four pieces, and then penetrated by a 10-mm-long dialysis capillary. Thus, four membranes were implanted in each CL. For infusion, one end of the tube was connected to a multiple-line peristaltic pump (IPC-24; Ismatec SA, Zürich, Switzerland), and the other was routed to a multiple-line fraction collector. The prepared luteal pieces were then placed in organ culture chambers (modified 2070 Tube; Falcon, Franklin Lakes, NJ) that were filled with 50 ml of M199 (M5017; Sigma) supplemented with 25 mM Hepes, 0.5% BSA, 60 μg/ml of penicillin, 100 μg/ml of streptomycin, 56 μg/ml of ascorbic acid (Wako Chemical Co., Osaka, Japan; 013-12061), and 2 μg/ml of amphotericin B (A9528; Sigma) at pH 7.4 that were maintained in a water bath at 38°C. The luteal pieces were perfused with Ringer solution at a flow rate of 3.0 ml/h throughout the experiments. To estimate the basal release of estradiol, fractions of the perfusate after a 3-h preperfusion were collected for 2 h (6 ml/fraction). The collected samples were stored at −30°C until hormone determination.

Hormone Determination

Measurement of estradiol in perfusate samples was performed using an enzyme immunoassay (EIA) as described previously [24] but with several modifications. The volume of perfusate samples was adjusted with Ringer solution to 6 ml. These fractions were adjusted to pH 3.5 with acetic acid and extracted using diethyl ether as described previously [25]. The residue was dissolved in 120 μl of assay buffer (40 mM PBS, 0.1% BSA, pH 7.2). The samples were concentrated 30- to 50-fold as a result of the process. The recovery rate for estradiol used in this extraction method was 75%. Cross-reactivities of the antiestradiol serum against 17β-diol-6-one 6-carboxymethyloxime-BSA, which were validated by comparing the inhibition binding of peroxidase-labeled estradiol to antiserum, were as follows: estradiol, 100%; estrone, 0.7%; estradiol-17α, 0.9%; estriol, <0.1%; testosterone, <0.25%; and progesterone, 0.017%. The estradiol standard curve ranged from 2.0 to 2000 pg/ml, and the effective dose for 50% inhibition (ED_{50}) of the assay was 120 pg/ml. The intra- and interassay coefficients of variation were, on average, 5.9% and 9.2%, respectively.

The concentrations of progesterone in the conditioned medium were determined directly with an EIA as described previously [26]. Antiprogesterone serum (OK-1) was used at a final dilution of 1:200 000 (v:v). The progesterone standard curve ranged from 0.39 to 100 ng/ml, and the ED_{50} of the assay was 2.3 ng/ml. The intra- and interassay coefficients of variation were 6.2% and 10.6%, respectively.

The concentrations of oxytocin in the conditioned medium were also determined directly with an EIA as described previously [27]. Antioxytocin serum (R-1; donated by Dr. G. Kotwica of the University of Agriculture and Technology, Olsztyn, Poland) was used at a final dilution of 1:50 000. The oxytocin standard curve ranged from 3.91 to 1000 pg/ml, and the ED_{50} of the assay was 60.9 pg/ml. The intra- and interassay coefficients of variation were 10.2% and 12.2%, respectively.

The concentrations of $PGF_{2\alpha}$ in the conditioned medium were also determined directly with an EIA as described previously [28]. The anti-$PGF_{2\alpha}$ serum (donated by Dr. S. Ito of Kansai Medical University, Osaka, Japan) was used at a final dilution of 1:40 000. The $PGF_{2\alpha}$ standard curve ranged from 15.625 to 4000 pg/ml, and the ED_{50} of the assay was 217.7

pg/ml. The intra- and interassay coefficients of variation were 6.1% and 3.3%, respectively.

Quantification of Estrogens in the MDS Perfusate by High-Performance Liquid Chromatography and EIA

The 4-ml fractions (Ringer) from in vitro MDS of early CL were extracted with 12 ml of diethyl ether (pH 3.5). After evaporation, they were dissolved in PBS (pH 7.5). After determination of estradiol concentration, they were pooled (80 μl each, n = 40), resulting in 3.2 ml in PBS (pH 7.5). This pooled sample was again extracted with 12 ml of diethyl ether (pH 3.5), evaporated, and re-extracted with petrol ether (pH 7.5). After evaporation, the sample was dissolved in 167 μl of 100% methanol. The total calculated estradiol was 1990 pg in 167 μl of methanol.

The methanol was evaporated at 60°C in a centrifugal evaporator (Univapo 100 H; Uni Equip, Martinsried, Germany) under reduced pressure (3 HPa; Unijet II; Uni Equip), and the residue was redissolved in 2 ml of 20 mM Tris-acetate (pH 7.2)/acetonitrile in an 80:20 (v/v) mixture for high-performance liquid chromatography (HPLC). The sample (100 μl) was then applied to a RP18-column (ProntoSIL 120-5-C18, 5.0 μm; NC-04, 250 × 4.0 mm; Bischoff, Leonberg, Germany) by an autosampler (LC 507e; Beckman, Munich, Germany) and eluted with acetonitrile/20 mM Tris-acetate (pH 7.2) at a 42:58 (v/v) mixture at 25°C (column thermostat, Jetstream Plus; Beckman) at a flow rate of 1 ml/min (pump, LC 125; Beckman). Fractions of 250 μl were collected after HPLC separation (fraction collector, FRAC-100; Pharmacia, Uppsala, Sweden), evaporated, and redissolved in 1 ml of 40% methanol each.

The content of estradiol in 20 μl of each fraction was analyzed in duplicate by an EIA [29] using specific standards for each. Cross-reactivities of the antiserum against 3,17β-hydoxy-1,3,5[10]-estratrien 17-hemisuccinate-BSA used for HPLC/EIA were as follows: estradiol, 100%; estrone, 100%; estradiol-17α, 66%; ethinyl estradiol, 14%; estriol, 1.5%, progesterone, <0.1%; and testosterone, <1%. The analytes were identified by retention time (11.4 min for estradiol, 14.3 min for estradiol-17α, and 17.7 min for estrone) and the specific antigen-antibody reaction. Calibration curves of the EIA were prepared in 40% methanol. The working interval ranged from 0.15 pg (80% displacement of labeled antigen) to 7.2 pg (20% displacement of labeled antigen) of estradiol per 20 μl, from 0.48 to 9.7 pg of estradiol-17α per 20 μl, and from 0.32 to 5.4 pg of estrone per 20 μl.

Statistical Analysis

The data are shown as the mean ± SEM of values obtained from separate experiments. The statistical significance of differences in mRNA expression of aromatase and estradiol concentrations in CL during the estrous cycle and in aromatase activity in luteal cells and the concentrations of progesterone, oxytocin, and $PGF_{2\alpha}$ in the conditioned media between the control and treated groups was assessed by an analysis of variance followed by the Fisher protected least significant difference (PLSD) procedure as a multiple-comparison test. For the statistical analyses of differences in the concentrations of progesterone, oxytocin, and $PGF_{2\alpha}$, the relative percentages of the control were used.

RESULTS

Expression of mRNA for Aromatase

Specific transcripts for aromatase were detected in bovine CL. The PCR product showed 100% homology to the known bovine gene after sequencing. To confirm the integrity of the mRNA templates and RT-PCR protocol, the housekeeping gene ubiquitin was examined in all samples. A representative sample for the ubiquitin-specific RT-PCR product is shown in Figure 1a. The relative signal intensities for PCR products specific for aromatase were assessed after correction based on the ubiquitin signal intensities. The ubiquitin was stably expressed in the bovine CL during the estrous cycle. A representative example for aromatase RT-PCR is shown in Figure 1b. Clear differences were found during the estrous cycle. The results of the densitometric analysis of aromatase mRNA are demonstrated in Figure 1c. The mRNA expression in the mid and late luteal stages was higher than in the early and regressed luteal stages ($P < 0.05$).

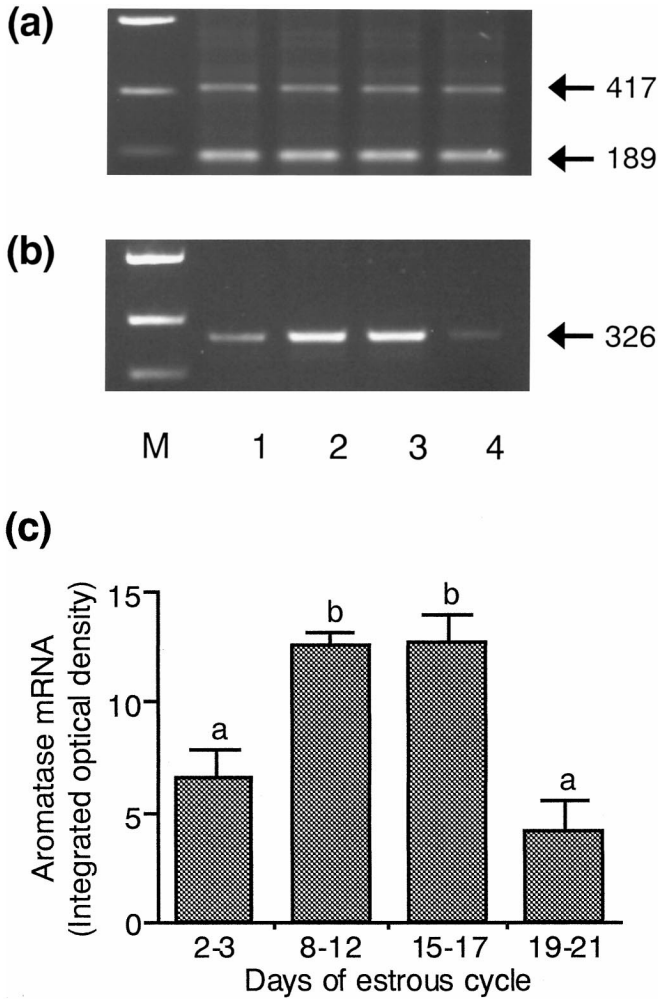

FIG. 1. Representative sample of specific RT-PCR products for **a**) ubiquitin (mean ± SEM, 189 + 417 bp) and **b**) aromatase (326 bp) in bovine corpus luteum tissue during different luteal phases separated by agarose gel electrophoresis. Lanes: M, DNA mass ladder (200, 400, and 800 bp); 1, early luteal phase (Days 2–3); 2, mid luteal phase (Days 8–12); 3, late luteal phase (Days 15–17), and 4, regression (Days 19–21). **c**) Relative levels of aromatase mRNA (RT-PCR, 25 cycles, arbitrary units) in bovine CL tissue during the estrous cycle (early, mid, late, and regression). Results represent mean ± SEM from four CL/stage. Different letters indicate significant differences ($P < 0.05$).

Aromatase Activity

When the luteal cells were incubated with [^{3}H]androstenedione, the cells aromatized it into estradiol and produced [^{3}H]water. The aromatase-inhibitor YM511 suppressed the production of [^{3}H]water by the cells in a dose-dependent manner (Fig. 2).

Local Release of Estradiol from Microdialyzed CL

Basal release of estradiol from microdialyzed CL is shown in Table 1. No significant difference was found in the concentrations of estradiol among three luteal stages.

Effects of Estradiol on Secretory Function of CL

Estradiol stimulated $PGF_{2\alpha}$ secretion by cultured bovine luteal cells ($P < 0.05$), although it did not affect progesterone and oxytocin secretion (Fig. 3).

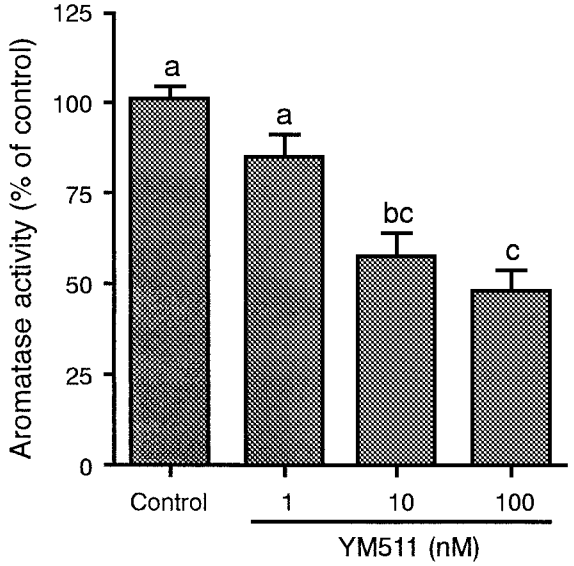

FIG. 2. Inhibition of aromatase activities in bovine luteal cells by an aromatase inhibitor (YM511; mean ± SEM, n = 6). Luteal cells were incubated for 4 h with [1,2-^{3}H]androstenedione in the presence or absence of YM511. All values are expressed as a percentage of the control value. Different letters indicate significant differences ($P < 0.05$) as determined by analysis of variance followed by Fisher PLSD as a multiple-comparison test.

Characterization of Estrogen Activity in the MDS Perfusate

Using a combination of HPLC and EIA, estradiol, estradiol-17α, and estrone could be separated and quantified in the MDS perfusate. The concentration for estradiol was 10.4 ng/ml. In contrast, the concentrations of estradiol-17α (<3.87 ng/ml) and estrone (<1.07 ng/ml) were less than the sensitivity of the assay. Thus, the determined estrogen concentration by the above EIA in the MDS perfusate was mostly estradiol.

DISCUSSION

The present study clearly demonstrated the mRNA expression of aromatase and the activity of this enzyme in bovine CL. In addition, we confirmed the estradiol is produced using in vitro-microdialyzed CL. Furthermore, a reversed-phase HPLC analysis in combination with EIAs confirmed the estradiol in MDS perfusate. These findings indicate that bovine CL has an ability to produce estradiol. The concentrations of estradiol in peripheral blood during the luteal phase are low and fluctuate with the emergence of follicular waves [30, 31]. Because bovine CL produces very little androgen [32], however, it seems that the source of estradiol in peripheral blood during the luteal phase is not CL, and that the luteal estradiol does not play a systemic role in cattle. Although to our knowledge the physiological significance of luteal estradiol as an endocrine factor has not been determined in any mammal, luteal estradiol has been well demonstrated as a paracrine and/or autocrine regulator in pig [10] and human CL [11]. Because estrogen receptor is present in bovine CL [14], we assume that luteal estradiol may play a role as a local regulator in bovine CL.

It is well demonstrated that the level of aromatase mRNA in granulosa cells decreased at 20 h after the onset of estrus induced by $PGF_{2\alpha}$ injection in heifers [5]. Furthermore, that study cited unpublished data that aromatase mRNA was not present in bovine CL. The discrepancy between those and the present results is due to differences in the methods for analysis of mRNA expression. Those authors observed the mRNA expression of steroidogenic enzymes in bovine ovary by Northern blot analysis. On the other hand, the present RT-PCR results demonstrated that the aromatase mRNA was expressed throughout the luteal phase and was higher in the mid and late luteal stages than

TABLE 1. Basal release of estradiol from microdialysed bovine CL.

Luteal phase	Days after ovulation	No. of CL tested	Estradiol (pg/ml)[a]
Early	4–5	16	14.0 ± 1.0
Mid	8–12	10	15.2 ± 1.0
Late	15–17	14	12.1 ± 0.7

[a] Mean ± SEM.

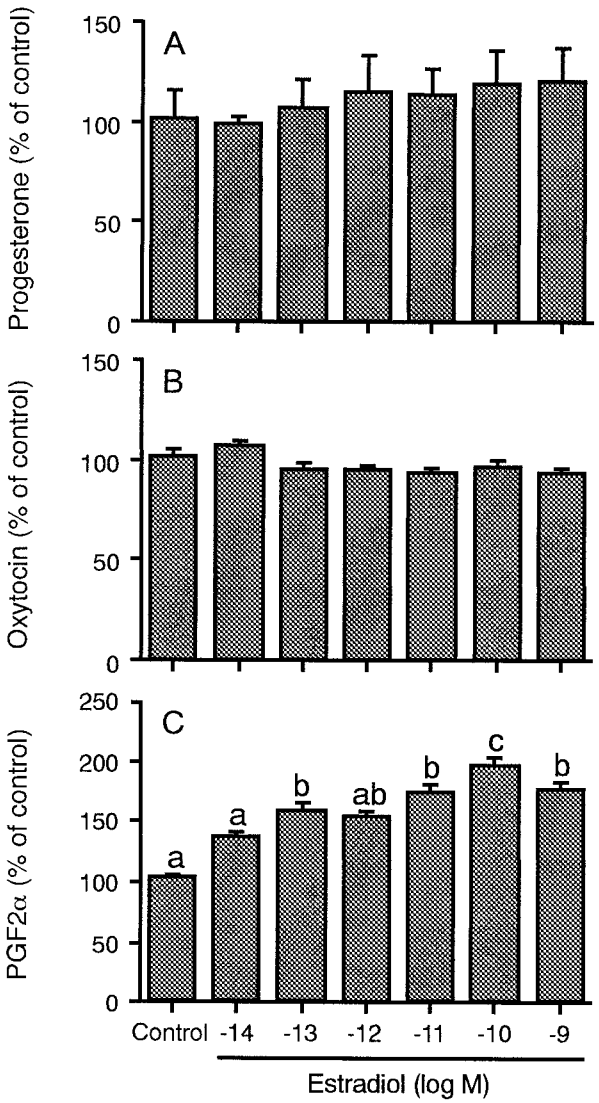

FIG. 3. Effects of estradiol on **A**) progesterone, **B**) oxytocin, and **C**) $PGF_{2\alpha}$ secretion by cultured bovine luteal cells from the midluteal stage of the estrous cycle (mean ± SEM, n = 3). Freshly prepared luteal cells were exposed to estradiol for 2 h. All values are expressed as a percentage of the control value. The concentrations of progesterone, oxytocin, and $PGF_{2\alpha}$ in the controls were 245 ± 63 ng/ml, 159 ± 33 pg/ml, and 52 ± 11 pg/ml, respectively. Different letters indicate significant differences ($P < 0.05$), as determined by analysis of variance followed by Fisher PLSD as a multiple-comparison test.

in the early and regressed luteal stages. That RT-PCR is more sensitive than Northern blot analysis is generally accepted. It is interesting to note that the increase in the mRNA expression observed in the present study was accompanied by CL development. However, the estradiol levels in microdialyzed CL were constant throughout the luteal stages. Thus, further studies are needed to understand the mechanisms regulating estradiol synthesis in bovine CL and, especially, to clarify the stage-dependent changes in the transcription and translation levels of aromatase mRNA, activities of the enzyme, and amount of precursors needed to synthesize estradiol.

Possible actions of estradiol on cultured luteal cells were also examined in the present study. Although estradiol showed no effect on progesterone and oxytocin secretion, $PGF_{2\alpha}$ production in isolated bovine midstage luteal cells was stimulated by estradiol in a dose-dependent manner. The expected concentrations of estradiol in bovine CL might be approximately 10^{-9} M, because the diffusion capacity of the microdialysis capillary membrane is approximately 1% [23]. Thus, the concentrations of estradiol (10^{-13} to 10^{-9} M) that maximally stimulated $PGF_{2\alpha}$ secretion in the present study are readily comparable with the expected concentration of estradiol in bovine CL and with the affinity of cytosolic estradiol receptors in bovine CL ($K_d = 2.7 \times 10^{-9}$ M) reported previously [14]. These findings suggest that luteal estradiol enhances $PGF_{2\alpha}$ production, and that the effect is mediated by estrogen receptors, as demonstrated previously in bovine CL [14]. The present results are also consistent with those of a previous study [12] that showed estradiol stimulated $PGF_{2\alpha}$ secretion by bovine luteal cells obtained from CL on Days 8, 14, and 18 of the estrous cycle. On the other hand, we could not explain how the lower concentrations of estradiol (10^{-13} to 10^{-12} M) stimulated $PGF_{2\alpha}$ secretion. In our previous study, however, estradiol at a very low concentration (10^{-15} M) stimulated oxytocin release from microdialyzed bovine CL [13]. Based on the present and previous studies [13], it could be speculated that other binding sites for estradiol exist that have a sensitivity much higher than previously reported [14]. An earlier study by Kimball and Hansel [14] demonstrated only cytosolic receptors for estrogen. However, to our knowledge, no data are yet available for nuclear estrogen receptors in bovine CL. Further studies are needed to clarify these points.

It has also been documented that $PGF_{2\alpha}$ stimulates progesterone secretion by microdialyzed bovine CL [33] or cultured bovine luteal cells [34]. Therefore, we expected that estradiol could indirectly stimulate progesterone production in bovine CL via estradiol-induced $PGF_{2\alpha}$ in a paracrine and/or autocrine fashion. However, an increase of progesterone was not observed in the present study. The culture time in the present study may have been too short to show the effect of estradiol-induced $PGF_{2\alpha}$. On the other hand, although estradiol showed no effect on oxytocin secretion in the present study, oxytocin secretion from the in vitro-microdialyzed bovine CL was clearly stimulated by estradiol only during a specific stage (Days 5–7) in a previous study [13]. These contradictory results might be due to differences in the luteal stages used. Alternatively, they might be due to differences in the experimental systems used; that is, the cell-to-cell contact is maintained in an in vitro-microdialyzed system [13], but not in the isolated luteal cells used during the present study.

In conclusion, the overall results indicate that estradiol is produced by bovine CL and support the hypothesis that estradiol plays a role as a paracrine and/or autocrine regulator of $PGF_{2\alpha}$ production in bovine CL.

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