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Combination of estradiol-17β and progesterone is required for synthesis of PGF2α in bovine endometrial explants

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Abstract

In cattle, luteolysis results from the pulsatile release of endometrial prostaglandin F2α (PGF2α). Estradiol-17β (E_2) and progesterone (P_4) are involved in the regulation of luteolysis, but their mechanisms of action are unclear. The overall objective of this experiment was to investigate the actions of E_2 and P_4 on the control of PGF2α synthesis. Crossbred beef cows were slaughtered on Day 17 of a synchronized estrous cycle. Endometrial explants were incubated for 1 h with culture medium alone and for additional 11 h in culture medium supplemented with either 0, 10^{-12} or 10^{-11} M E_2 (Experiment 1); 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} or 10^{-5} M P_4 (Experiment 2); or combinations of these concentrations (Experiment 3). Concentrations of PGF2α in culture medium were measured by a specific radioimmunoassay. Neither E_2 (Experiment 1) nor P_4 (Experiment 2) alone affected synthesis of PGF2α ($P > 0.1$). The combination of both steroids stimulated production of PGF2α in a concentration-dependent manner ($P < 0.01$). The greatest production of PGF2α was achieved when 10^{-11} M E_2 was combined with 10^{-8} or 10^{-7} M P_4. In conclusion, *in vitro* release of PGF2α was regulated by E_2 and P_4.

Keywords: cattle, sex steroids, endometrium, Prostaglandin F2α, luteolysis.

Introduction

In cattle, luteolysis is a physiological event that regulates duration of the estrous cycle. The role of prostaglandin F2α (PGF2α) as the main luteolysin is well established for ruminants (Poyser, 1995; McCracken et al., 1999). It is well accepted that oxytocin (OT) from neurohypophyseal and ovarian origins stimulates endometrial receptors to initiate luteolysis in sheep and cows (McCracken *et al*., 1984; Silvia and Raw, 1993). However, because blocking OT receptors with a specific antagonist did not prevent luteolysis from occurring, it was suggested that OT may not be determinant for luteolysis in cattle (Kotwica *et al*., 1997).

Progesterone (P_4) and estradiol-17β (E_2) regulate synthesis of endometrial PGF2α, but the mechanisms through which this effect is controlled are controversial. *In vivo*, administration of P_4 early during the estrous cycle hastened the onset of luteolysis (Garret *et al*., 1988). In contrast, higher P_4 concentrations during the cycle decreased basal- and OT-stimulated PGF2α secretion in the subsequent cycle (Shaham-Albalancy *et al*., 2001). *In vitro*, P_4 stimulated synthesis of endometrial PGF2α in ewes (Raw *et al*., 1995) and either stimulated (Asselin *et al*., 1996; Skarzynski *et al*., 1999) or had no effect (Mann, 2001) in cows. Furthermore, follicle removal reduced plasma E_2 concentrations and both delayed luteolysis and increased estrous cycle length in sheep and cattle (Karsch *et al*., 1970; Ireland *et al*., 1984; Villa-Godoy *et al*., 1985; Hughes *et al*., 1987). Injections of E_2 in cows on Days 13, 15, 17, or 19 of the estrous cycle stimulated release of 13, 14-dihydro-15-keto-PGF2α (PGFM; Thatcher *et al*., 1986; Larson *et al*., 1991; Castro e Paula, 2004). *In vitro*, E_2 alone stimulated synthesis of PGF2α in endometrial cells obtained early during the estrous cycle (Asselin *et al*., 1996) and in endometrial explants obtained from ovariectomized cows (Mann, 2001). Conversely, incubation of endometrial explants, obtained from heifers on Days 11 to 17 of the estrous cycle, with E_2 had no effect on synthesis of PGF2α (Skarzynski *et al*., 1999).

The overall objective of this study was to investigate the effects of P_4 and E_2 on *in vitro* PGF2α production from bovine endometrium harvested just prior to spontaneous luteolysis. The specific objective was to test the ability of E_2 (Experiment 1), P_4 (Experiment 2), or the combination of E_2 and P_4 (Experiment 3) to stimulate synthesis of endometrial PGF2α.

Materials and Methods

Tissue collection

Reproductive tracts were obtained from a slaughterhouse from cyclic, non-lactating, crossbred (*Bos Taurus* x *Bos indicus*) cows on Day 17 of a synchronized estrous cycle. Uteri were immediately placed on ice and taken to the laboratory within 15 min.

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Subsequently, the endometrium was dissected, and explants (50 ± 5mg) were incubated in individual wells of 6-well plates at 37.5°C in humidified air, as described below.

Experimental design

Experiment 1. Endometrial explants from each cow (n = 3) were cultured for 1 h in 3 ml of HAM culture medium, which consisted of HAM-F10 (Sigma Chemical Co., N-6635, St. Louis, MO, USA) supplemented with 26.2 mM NaHCO3 (Fisher Chemical Co., BP-328-1, Fair Lawn, NJ, USA), 1 mM Hepes (Fisher Chemical Co., BP-310-00, Fair Lawn, NJ, USA), 5 mM D-(+)-Glucose (Sigma Chemical Co., St. Louis, G-6152, MO, USA), 10 ml/L of an antibiotic-antimycotic solution (Sigma Chemical Co., A5955, St. Louis, MO, USA), and titrated to a pH of 7.4. Then, 1 ml of HAM medium was collected and replaced by 1 ml of HAM medium supplemented with E_2 so that final concentrations in the wells were 0, 10^{-12}, or 10^{-11} M in triplicate, and explants were incubated for an additional 11 h period. Samples of the medium were collected and stored at -20° C until PGF2α concentrations were measured by radioimmunoassay.

Experiment 2. Endometrial explants from each cow (n = 4) were cultured in 3 ml of HAM culture medium for 1 h. Then, 1 ml of HAM medium was collected and replaced by 1 ml of HAM medium supplemented with P_4 (Sigma Chemical Co., St. Louis, P-0130, MO, USA) so that final concentrations in the wells were 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, or 10^{-5} M P_4 in triplicate, and explants were incubated for an additional 11 h period. Samples of the medium were collected and stored at -20° C until PGF2α concentrations were measured by radioimmunoassay.

Experiment 3. Endometrial explants from each cow (n = 3) were cultured in 3 ml of HAM culture medium for 1 h. Then, 1 ml of HAM medium was collected and replaced by 1 ml of HAM medium supplemented with E_2 and P_4 so that final concentrations in the wells were the following: 0, 10^{-12}, or 10^{-11} M E_2 (Sigma Chemical Co., St. Louis, E-8875, MO, USA); 0, 10^{-8}, or 10^{-7} M P_4 (Sigma Chemical Co., St. Louis, P-0130, MO, USA); or their respective combinations in triplicate. Explants were then incubated for an additional 11 h period. Samples of the medium were collected and stored at -20° C until PGF2α concentrations were measured by radioimmunoassay.

Radioimmunoassay

Concentrations of PGF2α in culture medium were measured by a tritium radioimmunoassay as described previously by Danet-Desnoyers *et al.* (1995). The anti-PGF2α antibody was a gift from Dr. William W. Thatcher (University of Florida, Gainesville, FL, USA). The intra-assay coefficients of variation (CV) were 6.4, 8.7, and 14.1%, and the interassay CVs were 1.74, 4.29, and 12.3%; both were determined from reference concentrations of 250, 1000, and 3500 pg/mL of PGF2α.

Statistical analyses

Concentrations of PGF2α in culture medium were analyzed by least squares ANOVA using the GLM procedure of SAS (SAS, 1998). The dependent variable was the concentration of PGF2α measured after 1 and 12 hours of culture. The assumption of normality or residuals was rejected in all three experiments (Shapiro-Wilk test, $P < 0.01$); therefore, data were transformed using the square root. Transformed data were suitable for ANOVA (Shapiro-Wilk test; $P > 0.01$). Independent variables were: animal and concentrations of E_2, P_4, and a combination of E_2 and P_4. For sake of clarity, data are presented as untransformed least square means ± SEM.

Results

Experiment 1

During the initial hour of incubation (medium alone), there was no effect of animal, E_2, or an animal by E_2 interaction ($P > 0.1$; data not shown) on synthesis of PGF2α by endometrial explants. Synthesis of PGF2α from experimental Hour 2 to 12 was not affected by E_2, animal, or an animal by E_2 interaction ($P > 0.1$; Fig. 1).

Experiment 2

During initial hour of incubation (medium alone), mean production of PGF2α by endometrial explants varied among animals ($P < 0.01$). However, there was no effect of P_4 or P_4 by animal interaction ($P \geq 0.1$; data not shown). Concentrations of P_4 did not affect production of PGF2α from experimental Hour 2 to 12. Mean PGF2α production varied among animals (effect of animal; $P < 0.01$; Fig. 3) but was similar for different concentrations of P_4 (no animal by treatment interaction; $P > 0.1$).

Experiment 3

There were no effects of animal, E_2, P_4, or their interactions on production of PGF2α during initial hour of incubation ($P > 0.1$; data not shown). There was a E_2 by P_4 concentration interaction that affected release of PGF2α by endometrial explants in culture from experimental Hour 2 to 12 ($P < 0.01$; Fig. 4). In the absence of E_2, production of PGF2α was similar, regardless of the concentration of P_4 used. When 10^{-12} M E_2 was used, it stimulated production of PGF2α in the absence of P_4 but not in the presence of either 10^{-8} or 10^{-7} M P_4. In the absence of P_4, treatment of explants with 10^{-11} M E_2 had no effect on production of PGF2α. However, the largest magnitude of PGF2α release was observed when 10^{-11} M E_2 was associated with 10^{-8} or 10^{-7} M P_4.

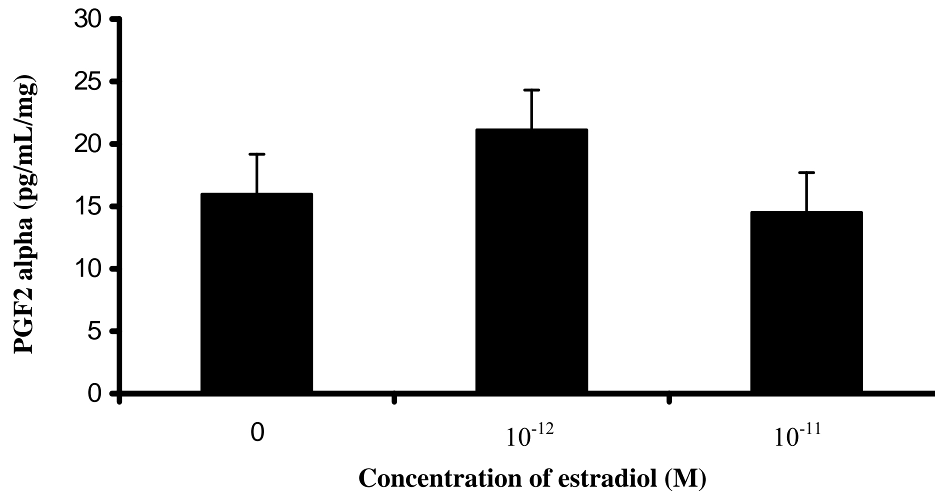

Figure 1. Concentrations of PGF2α synthesized by endometrial explants treated with 0, 10^{-12} or 10^{-11} M E_2 for 11 h. Untransformed data (LSMeans ±SEM).

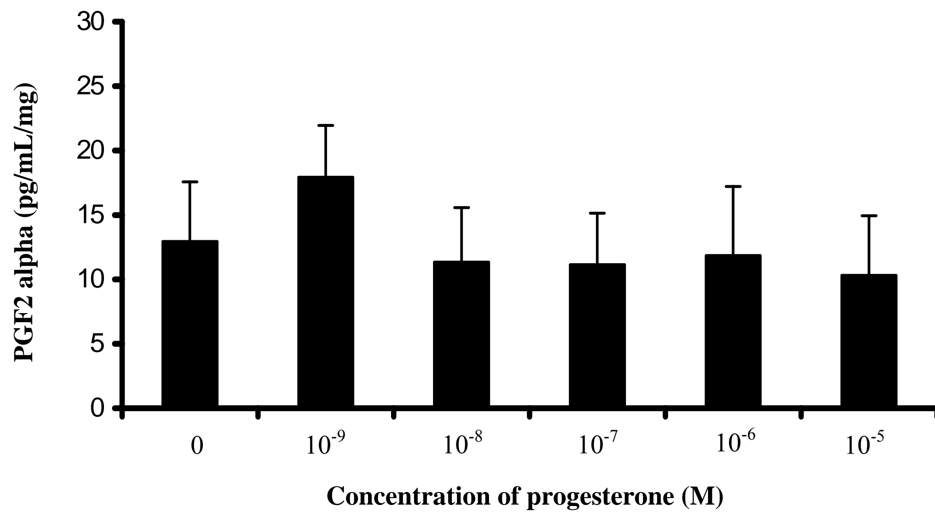

Figure 2. Concentrations of PGF2α synthesized by endometrial explants treated with 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} or 10^{-5} M P_4 for 11 h. Untransformed data (LSMeans ±SEM).

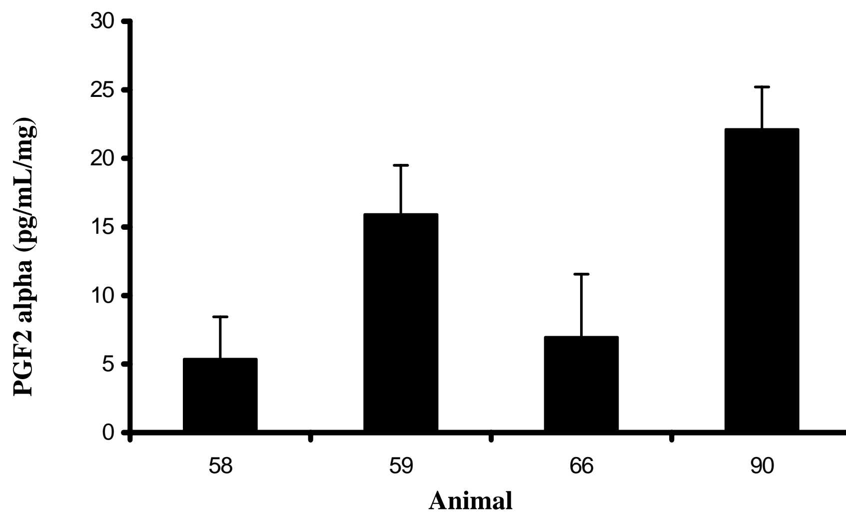

Figure 3. Average concentration of PGF2α synthesized by endometrial explants cultured for 12 h from individual animals. Untransformed data (LSMeans ±SEM).

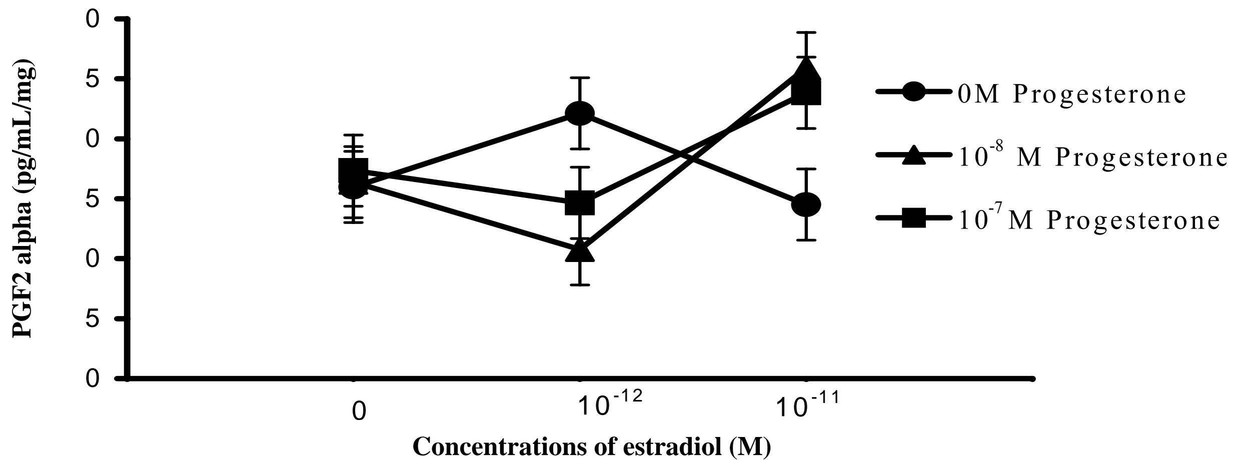

Figure 4. Concentrations of PGF2α synthesized by endometrial explants treated with 0, 10^{-8} or 10^{-7} M P_4, 0, 10^{-12} or 10^{-11} M E_2 and associations for 11 h. Untransformed data.

Discussion

In endometrial explants of cows on Day 17 of the estrous cycle, the combination of E_2 and P_4 stimulated synthesis of PGF2α in a dose dependent manner. However, production of PGF2α was not stimulated by either E_2 or P_4 alone. Skarzynski *et al.* (1999) also verified that E_2 was only able to stimulate synthesis of PGF2α when endometrial explants received exogenous P_4 or were co-incubated with luteal cells. It is suggested that a coordinated action of both steroids is required for PGF2α synthesis by the endometrium in the peri-luteolysis period in the cow.

It is well accepted that pulsatile release of PGF2α at luteolysis is dependent on stimulation of OT receptors in the endometrium (McCracken *et al*., 1999; Goff, 2004). Numbers and activity of OT receptors increase gradually starting during the mid-cycle, and the increase is modulated by E_2 and P_4. In the present experiment, production of PGF2α was independent of OT because this peptide was never added to the culture medium. Thus, in addition to stimulating OT receptors, sex steroids may induce synthesis of PGF2α through alternative mechanisms. For example, E_2 and P_4 could regulate synthesis and activity of intracellular enzymes involved in PGF2α synthesis.

The main substrate for synthesis of PGF2α is arachidonic acid. Therefore, synthesis of PGF2α is dependent on enzymes that increase availability of arachidonic acid and enzymes that increase its conversion to PGF2α. Binelli *et al.* (2004) reported that E_2 stimulated release of PGF2α in endometrial explants treated with a calcium ionophore. Because intracellular enzymes involved in PGF2α synthesis, such as protein kinase C and phospholipase A_2, are Calcium dependent, it is possible that E_2 stimulates activity of such enzymes to result in synthesis and release of PGF2α. On the other hand, P_4 was able to stimulate synthesis of the enzyme cyclooxygenase-2 (converts arachidonic acid to prostaglandin H_2; Goff, 2004; Kombé *et al*., 2003).

In vitro treatment of endometrium with E_2 alone resulted in responses ranging from inhibition to stimulation of PGF2α synthesis (Asselin *et al*., 1996; Xiao *et al*., 1998; Skarzynski *et al*., 1999; Mann, 2001; Castro e Paula *et al*., 2002; Kombé *et al*., 2003; Bertan *et al*., 2004; Cunha *et al*., 2004). These differences were probably due to different experimental conditions, such as concentrations of E_2 (10^{-12} to 10^{-7} M), duration of incubation (12 to 96 h), and the sources of endometrium used (ovariectomized cows or intact cows from Days 1 to 3 and 11 to 19 of the estrous cycle). In the present experiment, concentrations of E_2 were in the physiologic range and incubations were for 12 h, which was enough time to detect an increase in concentrations of PGFM after injections of E_2 in cows (Thatcher *et al*., 1986). Only endometrium from cows on Day 17 of the estrous cycle was used in that experiment. The choice of the day of the cycle was important because production of PGF2α during the estrous cycle is dependent on a P_4-primed uterus. Moreover, concentrations of endometrial steroid receptors vary throughout the estrous cycle, which probably modulate responses to such steroids. Therefore, it is proposed that experimental conditions used in the present experiment were adequate to study mechanisms regulating production of PGF2α in the periluteolytic period of the estrous cycle. Thus, lack of response to E_2 was probably due to the absence of other important factors, such as P_4.

In the present experiment, P_4 by itself was unable to stimulate synthesis of PGF2α. This was similar to findings reported by other authors (Mann, 2001; Kombé *et al*., 2003). Similar to E_2, P_4 probably needs to interact with other factors in order to stimulate PGF2α release.

It is important to recognize the limitations of an *in vitro* culture system when trying to make assumptions

about physiologic processes occurring *in vivo*. Data from the present work indicate that the endometrium responded to sex steroids acutely by producing PGF2α. It is possible that cellular and molecular mechanisms observed *in vitro* also operate *in vivo* and are involved in luteolysis.

In conclusion, production of endometrial PGF2α is under a concentration-dependent regulation of P_4 and E_2. Because it was possible to stimulate PGF2α production in the absence of OT, mechanisms dependent on other factors may exist and participate in the control of luteolysis in cattle. Further studies will be necessary for the elucidation of such mechanisms.

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