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Original article

Effect of topical application of estradiol-17β and PGE_2 on PGE-binding sites in the porcine endometrium

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Summary — High- and low-affinity prostaglandin E_2 (PGE_2) binding sites were found on day 15 after estrus in the endometrium of cycling (Cy) and pregnant (Pr) gilts as well as gilts treated with intra-uterine Silastic beads containing estradiol-17β (E_2) alone or in combination with PGE_2 (E and PG gilts respectively) and inserted into the uterine lumen on day 10 of the cycle. The average apparent dissociation constants (K_d) and binding site concentrations (B_{max}) for the high- and low-affinity sites were respectively (mean ± SEM): 8.4 ± 0.7 pM and 3.28 ± 0.38 fmol/mg of protein and 5.3 ± 0.8 nM and 71 ± 9 fmol/mg of protein. Samples collected along the meso- and antimesometrial aspects did not differ ($P > 0.05$), although the low-affinity $B_{max}$was higher on the antimesometrial aspect for Pr and Cy gilts only. No difference in K_d ($P > 0.10$) was found between treatments for high-affinity binding sites. For the low-affinity binding sites, K_d was higher for Pr compared to PG and E but not to Cy gilts ($P < 0.05$). The high-affinity B_{max} was higher ($P < 0.05$) for PG, followed by E, Pr and Cy gilts (respectively: 5.50 ± 0.26; 4.19 ± 0.46; 1.78 ± 0.40; 1.64 ± 0.23 fmol/mg of protein), although Pr and Cy gilts were not different ($P > 0.05$). These results suggest that the localized presence of conceptuses in the uterus in early pregnancy does not markedly affect PGE binding sites but that intrauterine applications of Silastic beads containing E_2 and PGE_2 increase high-affinity B_{max} and decrease low-affinity K_d.

binding site / endometrium / estradiol-17β / PGE_2 / pig

Résumé — Effet d'applications locales d'œstradiol-17β et de prostaglandine E_2 sur les sites d'attachement dans l'endomètre chez le porc. *Des sites d'attachement de forte et faible affinité pour la prostaglandine E_2 (PGE_2) ont été évalués au jour 15 après l'œstrus dans l'endomètre de truies nullipares cycliques (Cy), gestantes (Pr) ou traitées avec des billes de «Silastic» contenant de l'œstradiol-17β (E_2) seul ou combiné à la PGE_2 (truies E et PG, respectivement) et insérées dans la lumière utérine au jour 10 du cycle. Les constantes de dissociation moyennes et les concentrations maximales moyennes étaient respectivement pour les sites de forte et faible affinité (moyenne ±*

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SEM) : 8,4 ± 0,7 pmol.l^{-1} et 3,28 ± 0,38 fmol/mg de protéines et 5,3 ± 0,8 nmol.l^{-1} et 71 ± 9 fmol/mg de protéines. Aucune différence (P > 0.05) n'a été observée entre des échantillons prélevés le long du mésomètre et ceux prélevés du côté opposé au mésomètre, bien que les concentrations maximales des sites de faible affinité aient été supérieures du côté opposé au mésomètre chez les truies Pr et Cy. Les constantes de dissociation des sites de forte affinité n'ont pas été affectées par les traitements (P > 0,10) mais, pour les sites de faible affinité, les constantes de dissociation étaient plus élevées pour les truies Pr que pour les truies PG et E, les truies Cy présentant des valeurs semblables à celles des truies Pr (P < 0,05). Les concentrations maximales des sites de forte affinité différaient (P < 0,05) entre les truies PG, E, Pr et Cy (respectivement : 5,50 ± 0,26; 4,19 ± 0,46; 1,78 ± 0,40; 1,64 ± 0,23 fmol/mg de protéines), quoique les valeurs obtenues pour les truies Pr et Cy ne différaient pas entre elles (P > 0,05). La présence localisée du conceptus en début de gestation n'affecte pas de façon marquée les sites d'attachement pour PGE_2, mais l'application intra-utérine de E_2 et de PGE_2 augmente les concentrations des sites de forte affinité et diminue les constantes de dissociation pour les sites de faible affinité.

site d'attachement / endomètre / œstradiol-17β / PGE_2 / porc

INTRODUCTION

A number of observations support the hypothesis that prostaglandin E_2 (PGE_2) has a biological role in increasing endometrial vascular permeability and initiating the decidualization process at implantation in rodents and the rabbit (Kennedy, 1977, 1979, 1983, 1985; Evans and Kennedy, 1978; Hoffman *et al,* 1978; Kennedy and Armstrong, 1981; Phillips and Poyser, 1981). These studies involved the measurement of uterine PGE_2 concentrations, determining the effect of applying exogenous PGE_2 as well as preventing the synthesis of PGE_2. Another line of evidence for a role of PGE_2 came from observations that endometrial PGE binding site concentrations are increased at the time of decidualization and increased vascular permeability in the uterus of the rat (Kennedy *et al,* 1983).

Morphological evidence of increased vascular permeability at the time of porcine embryonic attachment has been reported (Keys and King, 1988) and the pig blastocyst synthesizes and secretes PGE_2 *in vitro* (Lewis and Waterman, 1983) at a corresponding developmental stage. These results suggest a role of PGE_2 in porcine early pregnancy that might be mediated by an increase in number and/or affinity of uterine PGE binding sites localized in regions where embryonic attachment occurs. A previous study (Kennedy *et al,* 1986) failed to show differences in PGE binding site concentrations between pregnant and non-pregnant uteri in pig, but endometrial samples were taken randomly throughout the uterus rather than at localized sites. Since the conceptus first attaches to the mesometrial aspect of the uterine horn, it is likely that any modification in number or affinity of binding sites would first occur there if they were actually caused by the presence of the embryo or by localized effects of its secretions. This study was therefore conducted to quantitate PGE binding sites during early gestation in pigs and to investigate possible roles of estrogens, which are produced in large amounts by the pre-attaching porcine conceptus (Perry *et al,* 1973), and PGE_2 in altering binding site populations in endometrial tissues collected from mesometrial and antimesometrial regions adjacent to or at a distance from sites of topical application.

MATERIAL AND METHODS

Animals and management

Sexually mature Yorkshire gilts of similar age (≈ 250-day-old) and weight (90–120 kg) were used in the experiments. Gilts were fed about 2.5 kg/day/animal of a 15% crude protein diet (corn–soybean) and water was always available from drinking nipples. Gilts were checked daily for estrus with a boar and were considered ready for experimentation after at least 2 normal-length estrous cycles (19–22 days). After assignment into experimental groups, the first day of standing estrus for each gilt was considered as day 0. Gilts allocated to the pregnant group were bred by natural service soon after estrus was detected and again 12 h later. The other gilts were not bred.

Twelve gilts were randomly assigned to one of the following treatments: cycling (Cy); pregnant (Pr); cycling with Silastic beads containing estradiol-17β (E_2) inserted *in utero* (E); cycling with Silastic beads containing E_2 + PGE_2 inserted *in utero* (PG). The Cy group was used as a control, the Pr group represented the full embryonic presence and secretions, the bead treatments were an attemp to mimick embryonic presence with selected secretory products. Beads containing only PGE_2 were not retained within the uterine lumen, so that treatment was not considered.

Beads were prepared according to the method outlined by Pope *et al* (1982) with some modifications. A paste of Silastic glue (3 ml; 1.5 g/ml; Dow Corning Corp, Corning, NY, USA) was mixed with either 2 g of E_2, 2 g of E_2 + 10 mg of PGE_2 or 2 g of cholesterol (all from Sigma Chemical Co, St Louis, MO, USA) with a small volume of diethyl ether added as a solvent. Cholesterol beads were used only in experiment 2 as a control, since it has been shown that uterine secretions and uterine morphological changes with these beads are similar to those of cycling gilts (Keys, 1987). Cholesterol was added to the vehicle to obtain beads that had similar density, shape, size and texture as those containing E_2. After drying for 0.5 h, the slurry was placed into a syringe and extruded into long ridges from which beads with a diameter of approximately 2 mm were formed by hand. The weight of the beads (mean ± SEM) was 190 ± 3, 199 ± 2, 221 ± 3 mg/10 beads, for E_2, E_2 + PGE_2 and cholesterol beads, respectively. The calculated hormonal content of the beads was approximately 65 mg of estrogens or cholesterol/10 beads and 300 mg of PGE_2/10 beads. Two days before surgery, beads were incubated at 4 °C for 24 h in 5 ml of barrow plasma, rinsed 3 times with Dulbecco's phosphate buffered saline (PBS, pH 7.4; Gibco Laboratories, Grand Island, NY) and blotted dry. Beads were kept for 10 min in a 70% ethanol solution and washed 3 times in bacteriostatic water immediately before uterine insertion. Release rates *in vitro* (at 37 °C in 3 ml of PBS, pH 7.4, in triplicate), from day 1–6 of incubation, decreased rapidly from 17.0 ± 6.0 to 0.4 ± 0.1 μg of E_2/day/10 beads and 4.2 ± 1.3 to 0.8 ± 0.2 μg of PGE_2/day/10 beads. Therefore, incubation in barrow plasma before using the beads did not prevent an initial peak release, especially for E_2.

Beads were inserted into the uterine lumen by midventral laparotomy on day 10. Anesthesia was induced with methohexitone and maintained by a mixture of halothane, nitrous oxide and oxygen. To allow an even repartition of the beads along the uterine horn, 2 incisions were made in each horn, one at the tip (near the oviduct) and the other near the middle of the horn, and 5 beads were introduced into the uterine lumen through each incision. Antibiotics and analgesic were administered after surgery. This surgical procedure has been frequently used in our laboratories and has been shown not to affect cycle length *per se*. All gilts were slaughtered on day 15 by electrical stunning and exsanguination.

PGE binding site experiment

Uteri were removed immediately after exsanguination was completed, placed on ice and taken to the laboratory. Uterine horns were dissected from the mesometrium, cut open longitudinally along the antimesometrial aspect and sections of 20–30 cm long were pinned on a large dissection tray with the endometrium side up. Specimens from females in the pregnant group (n = 3) were kept only if normally developing blastocysts were found within the uterine lumen. Specimens from the bead-treated gilts (n = 3) were kept only if the beads had spaced themselves

along the mesometrial side of each horn and were surrounded by endometrial folds. Uteri were held by the mesometrium and manipulated as little as possible to reduce any synthesis of prostaglandins during the preparation process. Endometrial strips (≈ 2 cm wide) were taken directly along the mesometrium (meso) and from sites parallel but at least 3 cm distal to the mesometrium (anti). Approximately 5 g of tissue from each strip were taken and prepared for the PGE binding site assay (Kennedy *et al*, 1986). Therefore for each gilt one sample came from the meso and one sample from the anti. Because of the large quantity of tissue needed for the assay, it was difficult to take samples exclusively underneath the beads. Sampling was therefore done underneath the beads but also at adjacent sites within 1 cm of a bead along the mesometrial aspect.

Endometrial samples were minced with scissors and homogenized in ice-cold buffer (10 mM tris–HCl, pH 7.0; 250 mM sucrose; 1 mM $CaCl_2$; 10 mM monothioglycerol; 10 μM indomethacin). The homogenate was centrifuged at 700 *g* for 10 min at 4 °C and the supernatant recovered and centrifuged at 10 000 *g* for 45 min at 4 °C to obtain the partially purified membrane fraction. The pellet was resuspended in phosphate–sucrose buffer (0.1 M phosphate, pH 6.0; 0.1 M sucrose; 10 mM monothioglycerol; 10 μM indomethacin) at a protein concentration of approximately 1.0 mg/ml (0.75 to 2.00). The protein of the membrane preparation was determined using bovine serum albumin as a standard (Lowry *et al*, 1951). Membrane preparations were incubated with [^{3}H]PGE_2 ({5,6,8,11,14-^{3}H(N)} prostaglandin E_2 (New England Nuclear, Boston, MA), 165 Ci/mM) at concentrations ranging from 4.9×10^{-12} M to 4×10^{-8} M (serial dilutions with 14 concentrations) with or without 5×10^{-6} M PGE_2 to determine total and non-specific binding, respectively. Tubes without the membrane preparation (blanks) were prepared and handled similarly. Incubation was carried out overnight at 4 °C and the reaction volume was 0.4 ml. The bound fraction was separated from the unbound by filtration (pore size: 0.45 μm; cellulose filter; Millipore Corp, Bedford, MA).

The size of individual PGE binding site assays required considerable time between determining radioactivity in the first and last vial, and evaporation was a problem. Radioactivity in the later samples was thus adjusted for evaporation of scintillation fluid. The last series of the 14 concentrations for non-specific binding values that did not present a drop in the counts compared to previously counted series was used as a reference. Regression curves were thereafter obtained to evaluate the decrease in value for each concentration in the series according to the time the series was counted after the reference series (R^2 of the curves varied from 0.738–0.947). These regression curves were used to correct the counts of the corresponding membrane preparations for the specific binding values. Corrected values were used for the rest of the analysis. The corrected binding data were plotted according to the graphical representation of Scatchard (1949). The apparent dissociation constant (K_d) and binding site concentration (B_{max}) were estimated by computer program (Munson and Rodbard, 1980).

Data were analysed with the SAS GLM procedure (SAS, 1987) using a split-plot design in which treatments were the main plots (using the gilt mean square as the error term) and sampling sites inside the uterus were the subplots (Snedecor and Cochran, 1980). All data presented homogeneity of variance among treatments as assessed by Burn–Foster's test (Anderson and McLean, 1974). Means for the effect of treatments (main plots) were compared using Duncan's multiple range test (Snedecor and Cochran, 1980), with a level of significance of $P < 0.05$. The effect of sampling position inside the uterus (meso *vs* anti) was evaluated directly from the analysis of variance.

Uterine flushing experiment

To study the effect of the beads on the uterine environment, 12 gilts treated similarly to those in the previous experiment were randomly assigned to one of the following treatments: E; PG; cycling with Silastic beads containing cholesterol inserted *in utero* (C), as a control. Beads were inserted into the uterine lumen by midventral laparotomy on day 10. All gilts were slaughtered on day 16 by electrical stunning and exsanguination. Uteri were removed and taken to the laboratory within 10 min. One horn was dissected from the mesometrium and flushed immediately with 20 ml of phosphate buffered sa-

line (PBS; pH 7.4). The flushing was done slowly while keeping the manipulation of the horn to a minimum. The quantity of fluid recovered was measured and an aliquot centrifuged, filtered and stored frozen at –20 °C until assayed for estrone alone (E_1), E_1 + E_1 sulfate (total E_1), progesterone (P_4), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), PGE_2 and total proteins.

Hormonal and protein assays

Previously validated RIAs were used to determine the concentration of E_1 and total E_1 (Raeside and Rosskopf, 1980), E_2 (Guilbault *et al*, 1988), P_4 (King *et al*, 1985), $PGF_{2\alpha}$ and PGE_2 (Kennedy, 1979), with performance criteria as indicated in table I. All uterine flushing samples were done in the same assay so no inter-assay coefficient of variation (CV) was calculated. Since the E_1 antiserum used had the same affinity for E_1 sulfate and E_1, the assay done without extraction gave a combined value for both hormones (total E_1 = E_1 sulfate + E_1). The concentration of E_1 sulfate alone was obtained by the difference between total E_1 and E_1, and negative calculated E_1 sulfate values were considered to be zero. For all the hormonal assays, reported values were corrected according to extraction efficiency.

The total protein content of uterine flushing samples was determined in one assay by the Bradford protein assay kit (Bio-Rad Laboratories, Mississauga, Ontario). The intra-assay CV was 3.5%.

Hormonal and protein data from uterine flushings were analyzed as a completely randomnized design. All values were expressed and analyzed on a total recovery basis by multiplying the concentrations of hormones and proteins per ml by the volume of fluid recovered after flushing the uterine horn. All estrogen data required transformation to natural logarithm. The effect of treatments was divided using orthogonal contrasts (C *vs* E + PG; E *vs* PG) and original data means are reported.

Table I. Assay details for various hormonal measurements performed on uterine flushings collected from one uterine horn of gilts treated with Silastic beads containing either cholesterol, estradiol-17β or estradiol-17β + prostaglandin E_2 and inserted in the uterine lumen on day 10 post-estrus. Flushing was done immediately after slaughter (day 16 post-estrus) with 20 ml Dulbecco's phosphate buffered saline.

Hormone	*Sample size (µl)*	*Sensitivity (pg)*	*EE (%)*	*CV (%)*
P_4	50	80	85.2	3.8
E_2	500	5.0	93.1	6.7
E_1	100	2.0	92.2	6.8
Total E_1	100	2.0	*	8.1
$PGF_{2\alpha}$	200	52	95.0	9.2
PGE_2	200	56	83.9	6.7

EE: extraction efficiency; CV: intra-assay coefficient of variation (all samples were assayed in one assay); P_4, E_2, E_1 and total E_1: progesterone, estradiol-17β, estrone and estrone + estrone sulfate respectively; $PGF_{2\alpha}$ and PGE_2: prostaglandin $F_{2\alpha}$ and prostaglandin E_2 respectively; * the assay was performed directly on uterine flushing.

RESULTS

Reproductive tract and Silastic beads

Pregnant gilts and gilts treated with beads containing E_2 had well vascularized corpora lutea (CL) on days 15 and 16, compared to the small and pale CL of gilts treated with cholesterol beads and cycling gilts. Generally the uteri of pregnant gilts and gilts treated with beads containing E_2 were more turgid and paler than the uteri of gilts treated with cholesterol beads and cycling gilts. Beads had moved down the uterine horns in all gilts but cholesterol beads were free inside the uterine lumen, whereas E_2 and E_2 + PGE_2 beads were tightly "embedded" in epithelial folds, along the mesometrial aspect of the uterine horns. Beads containing E_2 were still all present in the uterine lumen and spaced over all

the uterine length. Clusters of 2 or 3 beads were sometimes found. No obvious difference in the spacing of the beads was detected between E and PG treatments in both experiments.

PGE binding sites

Low-affinity and high-affinity binding sites were found. An effect of treatment was found for high-affinity binding site concentrations (B_{max1}: $P < 0.001$) but not for apparent dissociation constants (K_{d1}: $P > 0.10$: table II). B_{max1} was higher in the endometrium of PG followed by E gilts. Pr and Cy gilts had lower B_{max1} than gilts that received Silastic beads but did not differ from one another ($P > 0.05$).

In contrast, for low-affinity sites an effect of treatment was found for apparent dissociation constants (K_{d2}: $P < 0.031$) but not for binding site concentrations (B_{max2}: $P > 0.10$; table II). Pr gilts had K_{d2} values similar to Cy gilts but higher than E and PG gilts, whereas K_{d2} values of Cy, P and PG gilts did not differ ($P < 0.05$).

The sampling position inside the uterus did not have an influence ($P > 0.10$) on either K_{d1}, B_{max1} or K_{d2}, but B_{max2} was higher ($P < 0.035$) overall in samples taken from the antimesometrial compared to the mesometrial aspect. This effect was observed mainly for Pr and Cy treatment groups.

Table II. Specific [^{3}H] PGE_2 binding (mean ± SEM) by porcine endometrial membrane preparations: summary of analysis of Scatchard plots.

Treatment[1]	n	*Sampling*[2]	*High affinity binding site*		*Low-affinity binding site*	
			Apparent dissociation constant (pM) K_{d1}	*Binding site (fmol/mg protein)* B_{max1}	*Apparent dissociation constant (nM)* K_{d2}	*Binding site (fmol/mg protein)* B_{max2}
Cy	3	M	8.9 ± 1.1	1.83 ± 0.16	4.1 ± 0.6	24 ± 5
		A	7.0 ± 3.1	1.45 ± 0.46	6.7 ± 1.1	54 ± 22
Pr	3	M	9.4 ± 0.6	2.12 ± 0.83	6.3 ± 1.9	56 ± 13
		A	6.2 ± 2.3	1.43 ± 0.07	10.3 ± 4.9	104 ± 35
E	3	M	10.7 ± 0.7	4.25 ± 0.87	3.1 ± 0.8	56 ± 17
		A	10.0 ± 1.1	4.14 ± 0.55	3.9 ± 1.0	66 ± 4
PG	3	M	7.1 ± 2.9	5.72 ± 0.28	4.6 ± 1.3	108 ± 25
		A	7.7 ± 2.3	5.28 ± 0.45	3.5 ± 0.3	103 ± 30
Level of significance ($P <$ value)						
Main effect of treatment			0.528	0.001*	0.031**	0.2089
Sampling site			0.625	0.362	0.581	0.035
Treatment x sampling site			0.861	0.951	0.807	0.212

[1] Cy: cycling gilts, day 15; Pr: pregnant gilts, day 15; E: cycling gilts, day 15, with estradiol-17β (E_2) Silastic beads inserted into the uterine lumen on day 10; PG: cycling gilts, day 15, with E_2 + prostaglandin E_2 Silastic beads inserted in the uterine lumen on day 10; [2] M, A: Mesometrial aspects, antimesometrial aspects of the uterine horn. Means for treatments, compared with Duncan's multiple range test, were as follows: *PG 5.50 ± 0.26^a; E 4.19 ± 0.46^b; Pr 1.78 ± 0.40^c; Cy 1.64 ± 0.23^c. ** Pr 8.3 ± 2.5^a; Cy 5.4 ± 0.8^{ab}; PG 4.1 ± 0.6^b; E 3.5 ± 0.4^b (means with different superscripts differ; $P < 0.05$).

Uterine flushing

Volumes of uterine flushing recovered from an initial 20 ml of PBS varied from 14 to 18 ml, and were smaller ($P < 0.003$) for E and PG compared to C gilts (table III). No treatment effect was found for total P_4 and E_1 recovered in uterine flushing. There was more total E_1 ($P < 0.004$), E_1 sulfate ($P < 0.002$) and E_2 ($P < 0.096$) in uterine flushing of E and PG gilts than C gilts. However, no significant difference ($P > 0.10$) was found between E and PG gilts for all steroids measured. Quantities of both $PGF_{2\alpha}$ and PGE_2 recovered in uterine flushing did not differ significantly ($P > 0.05$) but both prostaglandins seemed to be found in larger amounts in uterine flushing of E and PG compared to C gilts. Contrary to what was expected, the total recovery of PGE_2 was not higher in PG compared to E gilts. A tendency for higher quantities of total proteins recovered from uterine flushing of E and PG compared to C gilts was observed. However, overall results did not differ significantly among treatments ($P > 0.10$).

DISCUSSION

Endometrial PGE binding capability has been described previously in the pig (Kennedy *et al*, 1986) as being temperature- and pH-dependent as well as reversible. Binding sites were shown to be most likely proteinaceous in nature and they did not seem to distinguish PGE_1 from PGE_2. Furthermore, curvilinear Scatchard plots indicated that 2 populations of binding sites were present. This last observation was confirmed in the present study with apparent dissociation constants and binding site concentrations similar to those found previously.

Table III. Hormonal and protein total recoveries (mean ± SEM) in uterine flushings from one uterine horn of gilts treated with Silastic beads containing either cholesterol (C), estradiol-17β (E) or estradiol-17β + prostaglandin E_2 (PG) and inserted in the uterine lumen on day 10 post-estrus. Flushing was done immediately after slaughter (day 16 post-estrus) with 20 ml Dulbecco's phosphate buffered saline.

Variable	*C*	*E*	*PG*	*C* vs *E + PG* α	*E* vs *PG* α
n	4	4	4		
Volume recovered (ml)	17.5 ± 0.5	15.5 ± 0.5	14.8 ± 0.5	0.003	0.310
P_4 (ng)	13.5 ± 5.8	21.0 ± 4.2	20.4 ± 2.8	0.218	0.927
Total E_1 (ng)	1.5 ± 0.6	42.4 ± 19.6	12.6 ± 4.5	0.004	0.304
E_2 (ng)	2.7 ± 1.2	14.0 ± 5.7	4.9 ± 1.4	0.096	0.432
E_1S (ng)	0.0 ± 0.0	12.0 ± 6.0	5.5 ± 2.0	0.002	0.349
$PGF_{2\alpha}$ (ng)	39 ± 33	486 ± 230	195 ± 126	0.141	0.210
PGF_2 (ng)	137 ± 88	176 ± 82	156 ± 94	0.478	0.359
Proteins (mg)	27 ± 10	45 ± 28	45 ± 17	0.482	0.997

α: level of significance ($P <$ value) for the orthogonal comparisons; P_4, Total E_1, E_2, E_1, E_1S: progesterone, estrone + estrone sulfate, estradiol-17β, estrone and estrone suflate respectively (a natural logarithm transformation of the data was used for analysis of variance, except for progesterone); $PGF_{2\alpha}$ and PGE_2: prostaglandin $F_{2\alpha}$ and prostaglandin E_2 respectively.

Curvilinear Scatchard plots have been generally reported for PGE binding capacity in other species (Rao, 1973; Dazord *et al,* 1974; Okamura and Terayama, 1977; Crankshaw *et al,* 1979; Tepperman and Soper, 1981; Asboth *et al,* 1985; Hofmann *et al,* 1985) but linear plots were also reported (Robertson *et al,* 1980; Robertson and Little, 1983; Asboth *et al,* 1985). Apparent dissociation constants for the high-affinity binding sites in these studies were in nM instead of pM as reported here and in the previous study with pig (Kennedy *et al,* 1986), indicating a much higher affinity for pig endometrial sites. The apparent dissociation constant for the high-affinity binding sites was near the lowest concentration of [^{3}H]PGE_2 utilized (4.9 pM) for the assay, where the error on the estimate is higher than in the middle part of the curve. Therefore, the results should be interpreted with caution. Moreover, as mentioned previously (Kennedy *et al,* 1986), the physiological significance of these high-affinity sites is doubful.

Previous studies (Kennedy *et al,* 1986) as well as the present one did not show any difference ($P > 0.05$) in either K_{d1} and B_{max1} between endometrial samples taken from gilts on day 15 of pregnancy or the cycle. Endometrial tissues were collected from specific locations along the mesometrial and antimesometrial aspects of the horn in the present study compared to a random sampling in the previous one (Kennedy *et al,* 1986) in an attempt to identify a more localized response of endometrial PGE binding sites, which did not occur.

Placement of Silastic beads impregnated with E_2 inside the uterine lumen did not affect the apparent dissociation constant but markedly increased the high-affinity binding site concentrations compared to cycling and pregnant animals. E_2 most likely stimulated the production or exteriorization of binding sites in the endometrium. PGE_2 seems to have an effect on its own binding sites since more high-affinity binding sites were obtained per mg of protein in PG compared to E gilts, suggesting that PGE_2 released from the beads into the uterine lumen might contribute to increase B_{max1}, either by itself or by potentiating the E_2 effect.

The low-affinity binding sites may represent more biologically significant receptors for E-series prostaglandins, since estimates of their apparent dissociation constants were similar to those reported for other tissues and species (*ie* in nM instead of pM). Apparent dissociation constants for the low affinity sites were similar to those found previously in the endometrium of gilts on day 15 of the cycle or pregnancy (Kennedy *et al,* 1986), but binding site concentrations were lower and more in the range of what had been reported for gilts at the beginning of the cycle. Mean values for low-affinity binding site dissociation constants were larger in pregnant than bead-treated gilts and tended to be larger for pregnant compared to cycling gilts. The opposite tendency had been reported previously (Kennedy *et al,* 1986). However, in both studies the difference between cycling and pregnant gilts was not significant. Taken together, these results suggest a positive overall effect of topical application of E_2 on the endometrium, with Silastic beads, for high-affinity PGE binding site concentrations as well as a negative effect on the low-affinity PGE binding site dissociation constants.

Higher amounts of estrogens were recovered in uterine flushings of pregnant gilts compared to cycling gilts on days 13–15 post-estrus (Geisert *et al,* 1982; Keys, 1987). The same was observed with beads containing E_2 and inserted inside the uterine lumen compared to cycling gilts or gilts with cholesterol beads, in this and in a

previous study (Keys, 1987). Thus, E_2 could be an important factor influencing both PGE high- and low-affinity binding sites. E_2 has a stimulatory effect on the formation of its own receptors and of progesterone receptors in rodent endometrium (de Brux, 1981). A similar role of E_2 and PGE binding sites is possible, although apparently not documented. In rats, the number of endometrial PGE binding sites is progesterone-dependent (Kennedy *et al,* 1983), and binding site concentrations in the endometrium can be considerably reduced with RU 486 (a progesterone antagonist) injections on the first day post-estrus (Martel *et al,* 1989). In contrast, numbers of PGE binding sites in human endometrium are at their highest during the proliferative phase when progesterone is low but estrogen concentrations are high (Hofmann *et al,* 1985). High-affinity binding site concentrations are also elevated at estrus in pigs when peripheral estrogen concentrations are elevated (Kennedy *et al,* 1986), supporting a stimulatory effect of E_2 of intra-uterine origin on high-affinity PGE binding site concentrations.

PGE_2 released by the beads could have a stimulatory effect on the formation and/or exteriorization of the PGE binding sites. However, PGE_2 has been shown to down-regulate its own receptors in rat liver and adipocytes (Robertson *et al,* 1980; Robertson and Little, 1983) and if the same effect occurred in the pig uteri, PGE_2 released from the beads should reduce the high- and low-affinity binding site concentrations. Pig blastocysts have been shown to release PGE_2 *in vitro* (Lewis and Waterman, 1983) and higher intra-uterine concentrations of PGE_2 were observed in pregnant compared to cycling gilts (Geisert *et al,* 1982; Keys, 1987). Also, Silastic beads containing E_2 increased the PGE_2 concentrations in the uterine flushing compared to cycling gilts or gilts treated with Silastic beads containing cholesterol (Keys, 1987). If PGE_2 from the beads had a down-regulating effect in the pig endometrium, PGE_2 of blastocyst origin or secreted in the uterine lumen by the endometrium under the influence of E_2 beads, should have had the same effect. Therefore, results of the present study do not support a down-regulating effect of PGE_2 of intra-uterine origin. Uterine flushing concentrations of PGE_2 on day 16 post-estrus were not higher with E_2 and particularly E_2 + PGE_2 beads compared to cholesterol beads inserted in the uterine lumen on day 10, and these results could explain why PGE_2 did not have a down-regulating effect on the PGE binding sites in the pig endometrium.

Previous experiments have shown that treatments with Silastic beads containing E_2 prevented the drop in progesterone concentrations in the utero-ovarian vein plasma until at least day 16 of the cycle, whereas cholesterol beads did not (Laforest and King, 1992). These results indicate that E_2 treatments maintain luteal function until at least days 15–16 and are in agreement with the visual appraisal of the CL performed in the present study. Beads containing E_2 also modify the composition of the uterine flushings and the morphology of the endometrium in a way similar to pregnancy (Keys, 1987). Therefore, it was expected that PGE binding sites of gilts treated with beads containing E_2 would respond similarly to those of pregnant gilts. The results obtained showed otherwise, suggesting that insertion of Silastic beads impregnated with E_2 into the uterine lumen of cycling gilts affects some characteristics of the endometrium in a still unknown way. It is possible that the introduction of Silastic beads within the uterine lumen had an effect on PGE binding sites that was not mediated by either E_2 or PGE_2 from the beads. For instance, the enhancement of $PGF_{2\alpha}$ concentration within the uterine lu-

men in bead-treated gilts might have directly regulated PGE binding sites or modulated factors that influence PGE binding sites. Alternately, the peak release of E_2 on the day following bead insertion (data not shown) might have overly increased the estrogen content of the uterine lumen compared to the slight increase normally occurring in pregnant gilts on days 11–12 of pregnancy (Geisert *et al*, 1982), inducing important changes in PGE binding sites.

In conclusion, the results showed no difference between day 15 cycling or pregnant endometrium for high- and low-affinity PGE binding site concentrations and apparent dissociation constants in porcine endometrium, even with endometrial samples taken adjacent or far from sites of first conceptus attachment. However, Silastic beads impregnated with E_2 and PGE_2 and inserted into the uterine lumen on day 10 of the cycle increased the high-affinity binding site concentrations and reduced the low-affinity apparent dissociation constants to pregnant and cycling gilts for reasons that still remain unknown. PGE_2 could be involved in the morphological changes suggesting an increase in vascular permeability at the time of attachment in the pig (Keys and King, 1988) or in the increase in blood flow through the uterus (Magness *et al*, 1983), as well as other uterine modifications in early pregnancy, but the involvement of its binding site in the process remains to be determined.

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