Effects of human chorionic gonadotrophin on contractile activity of steroid-primed pig myometrium *in vitro*

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Summary. We examined the effects of (a) oestrogen and progesterone on concentrations of luteinizing hormone/human chorionic gonadotrophin (LH/hCG) receptors in uterine smooth muscle *in vivo* and (b) hCG on spontaneous myometrial contractions *in vitro*. Ovariectomized gilts received 2 ml corn oil (control; $n = 5$), 2 mg oestradiol benzoate ($n = 6$) or 20 mg progesterone ($n = 5$) for 5 days. Gilts were hysterectomized 8 h after the last injection and longitudinal sections of myometrium were incubated in modified Krebs' solution with 0 or 10 i.u. of hCG ($n = 10/\text{gilt}$) for 4 h at 37°C in 95% O₂:5% CO₂. After incubation, myometrial sections were placed in a tissue chamber perfused with Krebs' solution and mechanical activity was recorded for 30 min. Cell membrane fractions were prepared from myometrial tissue not used for in-vitro studies and analysed for LH/hCG receptors. Treatment with oestradiol benzoate increased ($P < 0.01$) the number of LH/hCG-binding sites compared with gilts receiving corn oil or progesterone. Incubation of myometrium with hCG reduced ($P < 0.01$) the frequency and amplitude of spontaneous uterine contractions in gilts treated with oestradiol benzoate. In contrast, hCG had no effect ($P > 0.05$) on the pattern of myometrial contractions in gilts given corn oil or progesterone. These results indicate that oestradiol promotes the synthesis of LH/hCG receptors in pig myometrium and incubation of oestrogen-primed tissue with hCG has a quiescent effect on myometrial contractility.

Keywords: LH; receptors; myometrium; uterine contractions; chorionic gonadotrophin; pig

Introduction

Uterine contractions are involved in a number of important reproductive processes (Finn & Porter, 1975; Huzar, 1983), including gamete transport (Blandau, 1969; Hawk, 1973; Hawk & Cooper, 1974), intrauterine migration of embryos (Pope *et al.*, 1982) and parturition (Taverne, 1982; Challis & Lye, 1986). Contractions of uterine smooth muscle during these physiological events are regulated by steroid hormones (Lye & Porter, 1978; Lye *et al.*, 1983b; Porter & Lye, 1983), prostaglandins (Rexroad & Barb, 1975; Gimeno *et al.*, 1979; Lye & Challis, 1982; Wainmann *et al.*, 1988), oxytocin (Liggins *et al.*, 1973; Cahill *et al.*, 1976), relaxin (Schwabe *et al.*, 1978; Szlachter *et al.*, 1980; Porter *et al.*, 1981) and biogenic amines (Rexroad & Barb, 1978).

Recently, specific high-affinity, low-capacity binding sites for luteinizing hormone/human chorionic gonadotrophin (LH/hCG) were identified in the myometrium of pigs (Ziecik *et al.*, 1986) and rabbits (Jensen & Odell, 1988). The presence of LH/hCG receptors in the myometrium raises the intriguing possibility that LH may have a direct effect on uterine function in addition to its indirect actions associated with the ovarian production of steroid hormones. However, neither the

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physiological significance nor hormonal regulation of myometrial LH/hCG receptors has been established. Therefore, in the present study, we examined the effects of (a) oestrogen and progesterone on the concentration of myometrial LH/hCG receptors *in vivo* and (b) hCG on the contractility of steroid-primed uterine smooth muscle *in vitro*.

Materials and Methods

Animals. Crossbred gilts (Yorkshire $\times$ Landrace $\times$ Duroc, 127–136 kg) were ovariectomized after sexual maturity and 3 months before the experiment began. They were housed individually in 1.2 $\times$ 1.3 m pens and received 2.8 kg of a 14% crude-protein, maize-soyabean meal diet per day during the study. Water was provided *ad libitum*.

Experimental design. Gilts received daily i.m. injections of either 2 ml corn oil (control; $n = 5$), 2 mg oestradiol benzoate ($n = 6$) or 20 mg progesterone ($n = 5$) for 5 consecutive days. Oestradiol benzoate and progesterone were purchased from Sigma Chemical Company, St Louis, MO. Corn oil (2 ml) was the vehicle for all steroid hormones.

Blood samples were collected from a jugular vein via venepuncture 4 h before the first injection and 8 h after the last injection of each treatment. Blood samples were allowed to clot at 4°C and serum was collected by decanting the supernatant after refrigerated centrifugation at 1200 g for 20 min. Serum was stored at -20°C .

Gilts were hysterectomized 8 h after receiving their last injection on Day 5. Anaesthesia was induced with 1 g of sodium thiopental (i.v.) and maintained with a closed-circuit system of halothane, nitrous oxide and oxygen. After hysterectomy, uteri were transported to the laboratory on ice. Myometrium was separated from endometrium by gentle scraping. Longitudinal sections of myometrium (2 mm wide $\times$ 20 mm long; 55 ± 0.02 mg) were removed from 5 locations equidistant between the utero-tubal junction and the cervix on each uterine horn ($n = 10/\text{gilt}$). Each of these myometrial strips was further divided into 2 sections (2 mm wide $\times$ 10 mm long; 25 ± 0.03 mg) and incubated in modified Krebs' solution with either 0 or 10 i.u. hCG ($n = 10/\text{gilt}/\text{in-vivo treatment}$) for 4 h at 37°C in 95% O_2 :5% CO_2 . Incubation conditions were selected to promote maximum binding of hCG to myometrial receptors (Ziecik *et al.*, 1986). The ionic composition of the modified Krebs' solution was 25 mmol Ca^{2+}/l , 134 mmol Cl^{-}/l , 15.5 mmol $\text{HCO}_3^{-}/\text{l}$, 1.2 mmol $\text{H}_2\text{PO}_4^{-}/\text{l}$, 5.9 mmol K^{+}/l , 1.2 mmol Mg^{2+}/l , 137.4 mmol Na^{+}/l and 11.5 mmol glucose/l. The solution was equilibrated with a mixture of 95% O_2 and 5% CO_2 .

Immediately after incubation, myometrial strips were suspended in 3.8-ml tissue chambers perfused with modified Krebs' solution at 37°C. The Krebs' solution in the chamber was exposed continuously to a mixture of 95% O_2 :5% CO_2 . Isometric contractions were recorded by suspending uterine strips from an isometric transducer (Narco Bio-systems, Inc., Houston, TX), which was coupled to a physiological pen recorder (Myograph F-60; Narco Biosystems, Inc., Houston, TX); initial resting tension was adjusted to 500 mg. Mechanical activity was recorded for 30 min after the muscle began to contract spontaneously and showed a stable pattern of rhythmic contractions (~ 15 min). At the end of each recording period, myometrial strips were exposed to 0.09M-KCl. Data from strips not producing a contractile response to KCl were not included in the statistical analyses. Before the addition of KCl, 24 of the myometrial strips from gilts treated with oestradiol benzoate *in vivo* and hCG *in vitro* were selected at random and exposed to 0.9 μM prostaglandin (PG) F-2 α (Lutalyse, The UpJohn Co., Kalamazoo, MI). Mechanical activity was recorded for an additional 30 min.

LH/hCG receptor binding analyses. Myometrium not used for in-vitro studies was analysed for LH/hCG receptors. Cell membrane fractions were prepared according to the procedures of Ziecik *et al.* (1986). Protein and DNA contents of the receptor preparation were determined according to the methods of Lowry *et al.* (1951) and Burton (1956), respectively. The assay used for measurement of membrane-bound, myometrial LH/hCG receptors has been described and validated previously (Ziecik *et al.*, 1986; Pomp *et al.*, 1988). Specific activities of [^{125}I] iodo-hCG (CR-121, 13450 i.u./mg) were determined by self displacement in a radioligand receptor assay (Ketelslegers *et al.*, 1975) and averaged 45 $\mu\text{Ci}/\mu\text{g}$. The mean maximum binding of iodinated hCG preparations in the presence of excess receptor was 61.3%. Non-specific binding was measured by the addition of 1 μg unlabelled hCG and was <2% of the total [^{125}I] added. Concentrations of unoccupied binding sites and affinity constants (K_a) were determined by Scatchard analysis (Scatchard, 1949). Seven subsaturating quantities of unlabelled hCG (0.01–5 ng) were used for each receptor preparation. Sensitivity of the receptor assay was 0.15 fmol/mg protein.

Hormone analyses. Serum concentrations of LH were determined by radioimmunoassay (Stevenson *et al.*, 1981; Armstrong & Britt, 1987). Purified LH (LER-786-3) was used for preparation of the radioiodinated antigen and standard. Assay sensitivity was 0.2 ng/ml and intra-assay coefficient of variation for a single assay was 7.5%.

A radioimmunoassay procedure described by Guthrie & Bolt (1983) and modified by Esbenshade & Britt (1985) was used to determine concentrations of follicle-stimulating hormone (FSH). Anti-pFSH (USDA-10-1010), [^{125}I] labelled pFSH (USDA-FSH-PPI) and pFSH (USDA-FSH-B-1) were used as the first antibody, radiolabelled ligand and standard, respectively. Intra-assay coefficient of variation for a single assay was 9.7%. Assay sensitivity was 6 ng/ml.

Serum concentrations of oestradiol-17 β and progesterone were determined by radioimmunoassay described by Cox & Britt (1982) and Stevenson *et al.* (1981), respectively. For oestradiol-17 β , assay sensitivity was 2 pg/ml and the intra-assay coefficient of variation was 8%. Cross-reactivity of the oestradiol-17 β antiserum used in the radioimmunoassay with oestradiol benzoate was 97.3%. For progesterone, assay sensitivity was 0.2 ng/ml and the intra-

assay coefficient of variation was 7.7%. All samples were quantified in a single radioimmunoassay for each steroid hormone.

Statistical analysis. Treatment effects on myometrial contractions were evaluated by three criteria: amplitude (mg), frequency (number/min) and duration (s) (Rexroad & Barb, 1975; Kawarabayashi *et al.*, 1989). Data on contractions were analysed by analysis of variance procedures for a split-split-slot design using the General Linear Models procedure of the Statistical Analysis System (Snedecor & Cochran, 1980). The main plot was in-vivo treatment (corn oil, oestradiol benzoate or progesterone); the subplot was in-vitro treatment (0 or 10 i.u. hCG) and the sub-subplot was uterine location (1–10). There were no effects ($P > 0.05$) of uterine location or its interactions and, therefore, data were reanalysed using a statistical model for a split-plot design (Snedecor & Cochran, 1980). A significant in-vivo $\times$ in-vitro treatment interaction was present ($P < 0.05$). This model was modified to compare differences between myometrial strips receiving 0 or 10 i.u. of hCG within each of the in-vivo treatments (Steel & Torrie, 1980).

Receptor concentrations and hormone data were analysed by least-squares analysis of variance procedures. Treatment mean comparisons for all data were made using Tukey's standardized range test (Steel & Torrie, 1980).

Results

LH/hCG receptors

Gilts receiving oestradiol benzoate for 5 consecutive days had higher ($P < 0.01$) concentrations of myometrial LH/hCG receptors than females given corn oil or progesterone. Affinity constants (K_a) for receptors were not different ($P > 0.05$) among treatments and averaged $7.8 \times 10^{10}/\text{mol}$, $8.5 \times 10^{10}/\text{mol}$ and $7.7 \times 10^{10}/\text{mol}$ for control gilts and those treated with oestradiol benzoate and progesterone, respectively.

Hormone concentrations

Concentrations of LH, FSH and progesterone were similar ($P > 0.05$) in all gilts before the onset of the experiment (time, -4 h; data not shown). Mean values were 2.0 ng/ml, 25.1 ng/ml and 1.2 ng/ml for LH, FSH and progesterone, respectively. Concentrations of oestradiol were below the sensitivity of the assay (non-detectable). In gilts that had received corn oil, oestradiol benzoate or progesterone for 5 days, LH and FSH were not significantly different among treatments (Table 1), but administration of progesterone and oestradiol benzoate resulted in substantial increases ($P < 0.01$) in serum concentrations of progesterone and oestradiol-17 β , respectively. Serum concentrations of progesterone increased from 2.0 to 9.4 ng/ml in gilts receiving progesterone. Similarly, in females treated with oestradiol benzoate, oestradiol-17 β concentrations increased from non-detectable concentrations (< 2 pg/ml) before injections to 131.4 pg/ml on the final day of steroid administration.

Table 1. Mean concentrations of luteinizing hormone/human chorionic gonadotrophin (LH/hCG) receptors in porcine myometrium and serum concentrations of progesterone, oestradiol-17 β , LH and follicle-stimulating hormone (FSH) in ovariectomized gilts treated with corn oil (control), oestradiol benzoate or progesterone

	Control	Oestradiol benzoate	Progesterone	Pooled s.e.
LH/hCG receptors				
fmol/mg protein	0.19	0.71*	0.22	0.04
fmol/mg DNA equivalent	0.38	1.49*	0.54	0.14
Progesterone (ng/ml)	1.4	1.2	9.4*	1.4
Oestradiol-17 β (pg/ml)	7.2	131.4*	7.8	15.7
LH (ng/ml)	1.8	0.8	1.1	0.4
FSH (ng/ml)	27.2	15.1	21.3	4.8

*Values significantly different from control, $P < 0.01$.

Table 2. Mean ($\pm$ s.e.) frequency, amplitude and duration of spontaneous contractions in porcine myometrium incubated with 0 or 10 i.u. human chorionic gonadotrophin (hCG) from gilts given corn oil (control), oestradiol benzoate or progesterone for 5 consecutive days. (n = number of myometrial sections)

Treatment	Frequency (no. min)	Amplitude (mg)	Duration (s)	n
Control				
0	2.4 ± 0.3	142.2 ± 10.1	25.1 ± 3.0	47
10 i.u. hCG	2.1 ± 0.2	139.2 ± 9.8	28.6 ± 4.1	48
Oestradiol benzoate				
0	1.8 ± 0.2	371.7 ± 10.3	34.8 ± 4.1	59
10 i.u. hCG	$0.3 \pm 0.1^*$	$235.3 \pm 14.1^*$	42.7 ± 5.1	58
10 i.u. hCG + prostaglandin F-2 α	1.4 ± 0.4	354.3 ± 21.5	47.1 ± 5.7	24
Progesterone				
0	1.2 ± 0.2	51.9 ± 6.1	47.3 ± 4.2	46
10 i.u. hCG	0.8 ± 0.3	47.4 ± 8.4	58.3 ± 5.6	49

*Values significantly different from strips not incubated with hCG (0) in control gilts and those treated with oestradiol benzoate or progesterone, $P < 0.01$.

Fig. 1. Patterns of spontaneous contractions of porcine myometrium from gilts receiving corn oil *in vivo* and (a) 0 or (b) 10 i.u. human chorionic gonadotrophin *in vitro*.

Myometrial contraction

Incubation with hCG *in vitro* had no effect ($P > 0.05$) on the amplitude, frequency or duration of myometrial contractions in gilts given corn oil or progesterone, but hCG reduced ($P < 0.01$) the frequency and amplitude of spontaneous contractions in gilts treated with oestradiol benzoate (Table 2 and Figs 1, 2 and 3). Mechanical activity of uterine smooth muscle incubated with hCG in gilts receiving oestradiol benzoate increased ($P < 0.01$) after administration of PGF-2 α and was similar ($P > 0.05$) to the pattern observed from myometrium not exposed to hCG.

Discussion

Results from the present study demonstrate that oestradiol is involved in the regulation of LH/hCG-binding sites in pig myometrium. This is evident in that concentrations of myometrial LH/hCG

Fig. 2. Patterns of spontaneous contractions of porcine myometrium from gilts receiving oestradiol benzoate *in vivo* and (a) 0 or (b) 10 i.u. human chorionic gonadotrophin (hCG) *in vitro*; (c) stimulation by prostaglandin F-2 α on oestrogen-primed uterine smooth muscle incubated with 10 i.u. hCG.

Fig. 3. Patterns of spontaneous contractions of porcine myometrium from gilts receiving progesterone *in vivo* and (a) 0 or (b) 10 i.u. human chorionic gonadotrophin *in vitro*.

receptors were 3–4-fold greater in ovariectomized gilts given oestradiol benzoate than in their control counterparts. Similar stimulatory effects of oestrogens on LH receptor concentrations have been observed in corpora lutea (Garverick *et al.*, 1982) and granulosa cells (Richards *et al.*, 1976; Hsueh *et al.*, 1984). Thus, even though a number of substances are probably involved in the regulation of gonadotrophin receptors, enhancement of LH/hCG receptor concentrations may be a fairly ubiquitous response of female reproductive tissues to oestrogenic compounds.

From our results, one would expect changes in concentrations of myometrial LH/hCG-binding sites during the oestrous cycle to reflect the secretory pattern of oestradiol. However, Ziecik *et al.* (1986) reported that myometrium from the late follicular phase (Days 18–20) contained fewer LH/hCG receptors than myometrium from the luteal phase. Pigs in our study were given oestradiol benzoate or progesterone for 5 days prior to the quantification of LH/hCG receptors. Because of hormone concentrations during the oestrous cycle (Van de Wiel *et al.*, 1981), females in the study of Ziecik *et al.* (1986) were probably exposed to lower concentrations of oestradiol for a shorter period and higher concentrations of progesterone over a longer period than gilts used in our study. Thus, differences in the magnitude and duration of steroid hormone exposure between the two studies could explain the observed variation in concentrations of myometrial receptors.

In terms of functional significance, our results demonstrate that incubation of myometrium from oestrogen-primed tissue with hCG produced a quiescent effect on the spontaneous mechanical activity of uterine smooth muscle. Modifications in myometrial contraction usually are the result of changes in frequency and amplitude of individual contractions (Taverne, 1982; Challis & Lye, 1986). While reductions in both these variables were observed in the present study, the quiescent effect of hCG primarily was due to a decrease in frequency. This is shown by the frequency of spontaneous contractions being reduced by 83%, whereas amplitude decreased only 36% in response to incubation with hCG. Whether this quiescence was due to direct action of hCG on oestrogen-primed muscle cells or was mediated via the production of some other substance is not known.

PGF-2 α stimulated spontaneous contractions and essentially overcame the quiescent effects of hCG on oestrogen-primed myometrium *in vitro*. Stimulation of myometrial contractions by prostaglandins in the presence of compounds that induce relaxation of uterine smooth muscle is a common phenomenon *in vivo* (Lye & Challis, 1982; Challis & Lye, 1986). Consequently, it has been suggested that many substances which influence myometrial activity exert their effects via prostaglandin synthesis and release (Hawk, 1975; Lye *et al.*, 1983a). Accordingly, Lye *et al.* (1983a) provided evidence that the biphasic contractile response of ovine myometrium to oestrogens was associated with the production of prostaglandins. Potential relationships between oestrogens, LH/hCG receptors and prostaglandins in the hCG-induced quiescence of myometrial contractions observed in our study remain to be determined. However, it appears that the mechanical activity of the porcine uterus is controlled by complex mechanisms involving a number of different factors.

In summary, oestradiol benzoate increased the concentration of myometrial LH/hCG receptors *in vivo*. Incubation of oestrogen-primed tissue with hCG had a quiescent effect on uterine smooth muscle contractions *in vitro*. Thus, it seems plausible that LH may be involved with the regulation of myometrial activity independent of its effect on the ovarian production of steroid hormones. The physiological significance of myometrial LH/hCG receptors during specific reproductive events, such as the oestrous cycle and pregnancy, remains to be determined.

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