Oestrogen-dependent expression of LH/hCG receptors in pig Fallopian tube and their role in relaxation of the oviduct

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The current studies investigated the concentration and distribution of LH receptors in the oviduct of ovariectomized gilts at various times after administration of oestradiol benzoate (10 µg kg⁻¹ body weight) to determine whether LH participates in the regulation of oviductal contractions. Polyclonal antibodies to the LH receptor were used in immunocytochemical and western blot analyses of oviductal tissues. The mechanical activity of the isthmus and ampullar segments of oviduct, collected from 16 cyclic gilts, was recorded for 30 min after LH or hCG treatment. In the oviduct, there was little competition for receptor occupancy between hCG and pig FSH, bovine thyroid-stimulating hormone (TSH), pig growth hormone (GH) and pig prolactin (1.2, 0.1, 0.01 and < 0.001%, respectively) but pig LH could completely inhibit the binding of [¹²⁵I]hCG. Oestradiol benzoate increased ($P < 0.01$) the number of LH binding sites in oviduct 24, 48 and 72 h (0.60 ± 0.08 , 1.62 ± 0.15 , 2.48 ± 0.35 fmol mg⁻¹ protein; $n = 4$ per treatment, respectively) after injection compared with the control gilts treated with corn oil (0.20 ± 0.04 fmol mg⁻¹ protein; $n = 4$). The affinity of oviductal LH/hCG binding sites (K_d) varied from 4.0 to 8.5×10^{10} l mol⁻¹ and was similar to that of luteal cell binding sites (6.1×10^{10} l mol⁻¹). Oestradiol benzoate also resulted in more intense LH receptor immunostaining of the tubal mucosal epithelium, smooth muscle cells and blood vessels as compared with controls. Western blotting has revealed that the pig oviduct, similar to the corpus luteum, contains 75, 48 and 45 kDa immunoreactive LH receptor proteins. Treatment with LH *in vitro* (100 ng ml⁻¹) affected the contractility of oviduct. During the peri-ovulatory stage of the oestrous cycle, the amplitude, frequency and area under curve(s) of the isthmus decreased ($P < 0.05$), as did the frequency and area under curve ($P < 0.05$ and $P < 0.01$, respectively) of the ampulla ($n = 4$). The frequency and area under curve of the oviductal contractions were also significantly reduced during the early follicular phase of the oestrous cycle ($P < 0.05$). There was no effect of LH (or hCG) on the frequency and area under curve of the oviductal contractions during luteal stages of the oestrous cycle ($n = 8$). These data indicate that (1) the pig oviduct possesses immunoreactive and functional LH receptor, (2) oestradiol promotes the synthesis of LH receptor in the epithelium and smooth muscles, and (3) LH causes the relaxation of oviduct, especially during the peri-ovulatory stage of the oestrous cycle. In summary, the results of the present study indicate that LH can control oviductal contractions directly and may be partially responsible for the relaxation of isthmus during fertilization in pigs.

Introduction

LH and its agonist, hCG, play a crucial role in the regulation of gonadal physiology. However, the existence of specific, high-affinity, low capacity binding sites for LH/hCG has been reported in the pig (Ziecik *et al.*, 1986), rabbit (Jensen and Odell, 1988; Sawitzke and Odell, 1991), rat (Bonnamy *et al.*, 1990; Sawitzke and Odell, 1991), bovine (Friedman *et al.*,

1995) and human uterus (Reshef *et al.*, 1990; Bhattacharya *et al.*, 1993). Moreover, LH/hCG receptors are present in the myometrium (Reshef *et al.*, 1990; Környei *et al.*, 1993), endometrium (Reshef *et al.*, 1990; Bhattacharya *et al.*, 1993) as well as in the uterine broad ligament (Ziecik *et al.*, 1995). The myometrial LH/hCG receptors play a role in uterine hyperplasia and hypertrophy (Környei *et al.*, 1993), as well as in uterine motility (Flowers *et al.*, 1991; Ziecik *et al.*, 1993; Eta *et al.*, 1994), and hCG has a relaxing effect on pig (Flowers *et al.*, 1991; Ziecik *et al.*, 1993) and human (Eta *et al.*, 1994) myometrium.

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LH/hCG receptors are also present in the human Fallopian tube (Lei *et al.*, 1993). The tubal receptors are present mainly in mucosal cells, but also in the smooth muscle and blood vessels (Lei *et al.*, 1993). LH receptors have not yet been detected in the pig oviduct, but Derecka *et al.* (1995) showed the presence of LHR mRNA in the pig oviduct during the early luteal and follicular phase of the oestrous cycle. Given the extragonadal expression of the LH/hCG receptors, the purpose of the present study was to examine their regulation and function by assessing (a) the effect of oestradiol on the capacity and affinity of the LH/hCG binding sites in crude oviductal membrane fractions, and their distribution in the pig oviduct, and (b) to study the effect of LH on the contractility of different segments of the pig oviduct *in vitro*.

Materials and Methods

Hormone preparations

Purified hCG (CR-127; 14900 iu mg⁻¹) and bovine TSH (bTSH 11; NIH, Bethesda, MD) was provided by the National Hormone and Pituitary Program (NIDDKD, Rockville, MD). Pig prolactin (Biolactin; 19 iu mg⁻¹) was a gift of F. Ryszka (Silesian School of Medicine, Zabrze), and pig FSH (pFSH-B1), pig LH (pLH B-1; 1.7 iu mg⁻¹) and pig GH (pGH B-1; 1.54 iu mg⁻¹) was donated by the USDA Hormone Program, Bethesda, MD. Oestradiol benzoate was purchased from Polfa (Jelenia Góra).

Animals and experimental procedures

Sexually mature (8–10 months of age; 100–120 kg) crossbred gilts were ovariectomized under general anaesthesia 1 month before the first experiment. The animals were premedicated with Combelen (Biowet, Pulawy). As soon as the gilts became somnolent, anaesthesia was induced and maintained with Vetbital (Biowet; about 15–20 ml i.v.) through an ear vein. The ovariectomized animals received an i.m. injection of 2 ml oestradiol benzoate (1 mg 100 kg⁻¹ body weight) 24 h (*n* = 4), 48 h (*n* = 4) or 72 h (*n* = 4) before slaughter. Controls (*n* = 4) were given an i.m. injection of 2 ml corn oil 48 h before slaughter. The genital tracts were removed within 10 min after slaughter and transported on ice to the laboratory in less than 2 min. The tubes were carefully dissected from extraneous tissues. One Fallopian tube was used for LH/hCG receptor measurements and western blot analysis; the other was used for immunocytochemistry.

In the second experiment, oviducts were collected from 16 mature crossbred gilts. The animals were kept in separate pens and checked daily for heat in the presence of a boar. The first day of behavioural oestrus was established as day 0 of the oestrous cycle. The days of the oestrous cycle were additionally confirmed by the morphological appearance of the ovaries after slaughter (Akins and Morrisette, 1968; Leiser *et al.*, 1988). These parameters allowed the classification of the oviduct tissues into four major groups: (1) peri-ovulatory stage: days 18–2 (*n* = 4); (2) early luteal stage: days 5–8 (*n* = 4); (3) late luteal stage: days 13–15 (*n* = 4);

and (4) early follicular stage: days 16–17 (*n* = 4). In both experiments, gilts were slaughtered at the Institute's abattoir. The oviducts were collected 10 min after slaughter, placed immediately in cold Krebs' solution, transported to the laboratory and dissected. Subsequently, tissues were placed in tissue chambers within 15–20 min of death.

All experiments were performed in accordance with *Guiding Principles for the Care and Use of Research Animals* and the internal instructions of the Institute's Director.

LH/hCG receptor binding analyses

Tissue (oviducts from ovariectomized gilts in the first experiment and corpora lutea from mid-cycle gilts in the second experiment) were dissected, minced with scissors and weighed. The cell membrane fractions were obtained using the procedure described by Ziecik *et al.* (1986). Briefly, the tissues were homogenized in four bursts of 7 s at 4°C with an Ultra-Turrax homogenizer in six volumes (w/v) of 0.025 mol Tris-HCl l⁻¹ buffer pH 7.4, containing 0.25 mol of sucrose l⁻¹. The homogenate was then filtered through four layers of cheesecloth, and the filtrate centrifuged for 20 min at 800 g at 4°C. The resulting supernatant was centrifuged further for 1 h at 25 000 g at 4°C and the sediment resuspended in 3 ml of ice-cold 0.025 mol Tris-HCl l⁻¹ buffer, pH 7.2, containing 0.1% (w/v) BSA and 5 mmol MgCl₂ l⁻¹. The resulting suspension was stored at -70°C for further experimentation. A fraction of the membrane preparation without BSA addition was used for protein determination using the method of Lowry *et al.* (1951).

Human CG (CR-127) was labelled by the Iodogen method (Fraker and Speck, 1978). Na¹²⁵I iodide was purchased from Amersham International (Amersham, Bucks). The separation of protein-bound and free [¹²⁵I] was performed by chromatography on Sephadex G-50 (Pharmacia, Uppsala) columns (1 cm × 15 cm). The specific activity of the labelled hCG was determined by self displacement analysis on corpus luteum membrane preparations in the radioligand receptor assay (Ketelslegers *et al.*, 1975), and it varied from 50 × 10³ to 70 × 10³ c.p.m. ng⁻¹.

The cell membrane fraction was incubated with tracer in polystyrene tubes for 16 h at room temperature. The incubation mixture consisted of 0.1 ml 0.025 mmol Tris-HCl l⁻¹ (pH 7.2) containing 5 mmol MgCl₂ l⁻¹ and 0.1% (w/v) BSA (incubation buffer), 0.1 ml incubation buffer containing 25 000 c.p.m. [¹²⁵I]iodo-hCG and 0.2 ml of the receptor preparation. Preliminary experiments showed that binding of labelled hCG was proportional to the amount of receptor added from 0.6 to 2 mg protein of oviductal cell membrane preparation. Nonetheless, 1.0 mg receptor protein was used consistently to determine binding parameters in oviduct and 0.4 mg in corpora lutea and these values were normalized per milligram of protein for the presentation of data.

Two millilitres ice-cold incubation buffer was added at the end of the incubation period and the tubes were centrifuged at 1000 g for 30 min. The supernatants were removed by aspiration and the radioactivity of the pellets was determined in a γ-spectrometer with 75% counting efficiency. Non-specific binding was determined in the presence of 0.5

μg unlabelled hCG, and was found to be $< 5\%$ of the total ^{125}I -label added.

The receptor concentration and equilibrium association constant (K_d) of unoccupied binding sites were determined by Scatchard (1949) analysis. Six to eight subsaturating doses (0.03–5.00 ng) of unlabelled hCG were used for each receptor preparation in duplicate.

Immunoperoxidase staining of LH/hCG receptor

Three portions of each oviduct (isthmus, ampulla and infundibulum) were fixed for 6 h in 4% (w/v) paraformaldehyde in 0.1 mol PBS l^{-1} . Fixed tissues were stored in 18% (w/v) sucrose in PBS with 0.01% (w/v) sodium azide. Cryostat sections (8 μm) were air-dried, rinsed in 0.05 mol Tris-hydroxymethyl aminomethane l^{-1} -buffered saline l^{-1} (TBS), pH 7.6, and then placed in ethanol in ascending concentration series (50%, 70%, 96% of absolute alcohol). The sections were treated with 1% (v/v) H_2O_2 in methanol for 30 min to block endogenous peroxidases, and then in 0.75% (w/v) glycine in TBS for 30 min to block free aldehyde groups. After rinsing in TBS, the sections were incubated overnight with primary antibody (diluted 1:200; a rabbit polyclonal antiserum directed against the amino acids 15–38 of the N-terminal sequence of the rat LH receptor; a courtesy of P. C. Roche, Mayo Clinic, Rochester, MN). Antibody binding was detected with the ABC complex (avidin-biotin-horseradish peroxidase complex; Vectastain ABC kit from Vector Laboratories, Inc. Burlingame, CA). Peroxidase activity was revealed using 3,3'-diaminobenzidine as a substrate. Two types of controls were performed: (1) the primary antibody was omitted during the immunostaining procedure; (2) the primary antibody was substituted with non-specific immunoglobulin G (IgG) during the immunostaining procedure. The observations and photographs were made using a light microscope (Olympus IMT-2) equipped with Nomarsky optics.

Measurement of oviductal contractility in vitro

The tubes were dissected carefully from the mesosalpinx, utero-ovarian ligament and other surrounding tissues. Rings (3–4 mm long) from isthmus and ampullar parts of oviduct were dissected to explore the contractile activity of Fallopian tube. All these tubal manipulations were performed while the oviduct was immersed in chilled Krebs' solution. The preparations were then mounted in 5 ml tissue chambers containing Krebs' solution (120.3 mmol NaCl l^{-1} , 5.9 mmol KCl l^{-1} , 2.5 mmol CaCl_2 l^{-1} , 1.2 mmol MgCl_2 l^{-1} , 1.2 mmol NaH_2PO_4 l^{-1} , 15.5 mmol NaHCO_3 l^{-1} , 11.5 mmol glucose l^{-1} ; pH 7.4) that were exposed continuously to a mixture of 95% O_2 :5% CO_2 at 37°C. Two consecutive rings of ampulla or isthmus were used simultaneously in two separate tissue chambers. The rings were tied vertically at each edge with cotton threads; one edge was attached to an isometric smooth muscle transducer (HSE Isometric Force Transducer F30 Type 372, March-Freiburg) which was coupled to a physiological pen recorder (Graphtec-Watanabe Oscillo-

graphic Recorder WR 3320 Mark VII, Type MK7-2DL) under a passive tension of 10 mN, and the other was tied to a hook at the bottom of the bath. The mechanical activity was recorded 30 min after the muscle began to contract spontaneously and it showed a stable pattern of rhythmic contractions. LH or hCG was added to the tissue chambers and the mechanical activity was recorded for the next 30 min. After changing Krebs' solution, tissues recovered to resting contraction within about 20–30 min and only segments maintaining the ability to contract were used in the final analysis. The chart speed was set at 25 mm min^{-1} to allow for analysis of areas under the curves (AUC).

Frequency, amplitude and AUC measurements were carried out for three different 10 min periods: I, 10 min before the addition of hormone to the tissue chamber; II, 10 min after addition of the hormone; and III, between 20 and 30 min after the addition of the hormone. The measurement alone during period I served as control for measurements II and III, which were expressed as percentages of measurement I. A similar method was used in a study by Ekerhovd *et al.* (1997).

Western blotting

Membrane fractions of oviducts and corpora lutea were prepared as described by Zieck *et al.* (1986) with the exception that 0.2 mol Tris-HCl l^{-1} , pH 7.2 buffer, containing 0.1 mol phenylomethylsulphonyl fluoride l^{-1} to inhibit the protein activity, was used. Aliquots (70 μg) of protein were dissolved in double-strength loading buffer consisting of 0.5 mol Tris-HCl l^{-1} (pH 6.8), 4% (w/v) SDS, 20% (v/v) glycerol and 2% (v/v) 2-mercaptoethanol and separated on 10% (w/v) SDS-PAGE (polyacrylamide gel electrophoresis in the presence of SDS). The separated proteins were electroblotted onto 0.1 μm nitrocellulose membranes for 2 h at 250 mA in a 0.025 mol Tris-HCl l^{-1} buffer (pH 8.2), 0.192 mol glycine l^{-1} , containing 0.1% (w/v) SDS. The non-specific binding sites were blocked with 5% (w/v) non-fat dry milk in 0.1 mol Tris-HCl l^{-1} (pH 7.4), 0.15 mol NaCl l^{-1} , 0.1% (v/v) Tween-20 (TBST buffer) overnight at 4°C. The membranes were then incubated with a 1:1000 dilution of the LH receptor antibody (PR) for 1.5 h at 22°C and washed three times for 10 min each with TBST. The washed blots were reincubated for 1 h at 22°C with a 1:3000 dilution of biotinylated antirabbit IgG, washed with TBST and incubated for 1 h at 22°C with 1:3000 dilution of ABC complex (Vectastain ABC kit, Vector Laboratories, Inc. Burlingame, CA). TBS containing 0.01% (v/v) H_2O_2 and 0.04% (w/v) 3, 3'-diaminobenzidine was used to develop colour.

Statistical analysis

The data were analysed by ANOVA followed by Duncan's new multiple range post-hoc test. The results are expressed as means $\pm$ SEM. The number of tubal preparations n was equal to the number of animals per group since one segment of isthmus or ampulla from each gilt was taken for measurement of oviductal contractility *in vitro*.

Fig. 1. Saturation curve and Scatchard plot of hCG binding to pig oviductal membrane preparation. $K_a = 4.9 \times 10^{10} \text{ l mol}^{-1}$.

Fig. 2. Hormonal specificity of ^{125}I -labelled hCG to pig oviduct. Crude membrane preparation (1 mg protein) of oestradiol-treated gilts was incubated with 25000 c.p.m. of ^{125}I -labelled hCG in the presence of increasing amounts of hormones. Means of duplicates from a representative of two experiments (with a mixed receptor sample from control and oestradiol-treated gilts) are shown. GH, growth hormone; PRL, prolactin; TSH, thyroid-stimulating hormone.

Results

Concentration of LH/hCG receptor in oviduct

The number of LH/hCG receptors and their apparent association constant (K_a) were measured at equilibrium (Fig. 1). The Scatchard plots prepared from oviductal tissues were linear (Fig. 1). The specificity of ^{125}I hCG binding to oviductal cell membrane preparations is shown (Fig. 2). There was very little competition between hCG and pFSH, bTSH, pGH and pig prolactin (1.2, 0.1, 0.01 and $<0.001\%$, respectively) but pLH could completely inhibit the binding

Fig. 3. Concentration of LH/hCG receptors (means $\pm$ SEM) in the oviduct of control (ovariectomized) gilts, and those ovariectomized and treated with oestradiol benzoate 24, 48 and 72 h before tissue collection. Corpora lutea (CL) from mid-cycle mature gilts represent a positive control tissue. Asterisks indicate values significantly different from controls, * $P < 0.05$; ** $P < 0.01$.

of ^{125}I hCG. The gilts treated with oestradiol benzoate had a higher ($P < 0.05$ – 0.01) concentration of oviductal LH/hCG receptors than those given corn oil (Fig. 3). The effect was time-dependent, without affecting the receptor affinity. This parameter (K_a) averaged between 4.0×10^{10} and $8.5 \times 10^{10} \text{ l mol}^{-1}$ for control gilts and those treated for various times with oestradiol benzoate. The affinity of uterine LH/hCG binding sites of oviduct was similar to that measured for corpus luteum ($K_a = 6.1 \times 10^{10} \text{ l mol}^{-1}$). However, the number of binding sites in the oviductal tissue of gilts treated with oestradiol benzoate (0.60 – $2.48 \text{ fmol mg}^{-1} \text{ protein}$) was 4–30-fold less ($P < 0.01$) than the receptor binding of the pig corpora lutea ($16.8 \pm 2.4 \text{ fmol mg}^{-1} \text{ protein}$). The specificity of ^{125}I hCG binding to other tissues was determined by incubating crude membrane preparations of skeletal muscle and kidney. Only non-specific binding of ^{125}I hCG was found in these tissues.

Immunoperoxidase staining of LH/hCG receptor

The immunostaining of gilt oviducts for the LH receptor are shown (Figs 4 and 5). This immunostaining was absent when the receptor antibody was omitted or substituted with non-specific immunoglobulin G (IgG) during the immunostaining procedure (Fig. 4l). Three parts of the pig oviduct, the isthmus (Fig. 4a,d,g,j), ampulla (Fig. 4b,e,h,k) and infundibulum (Fig. 4c,f,i), were immunopositive for the LH receptor. Immunoreactivity for LH receptor in the Fallopian tube sections was seen in the epithelium of the tubal mucosa, smooth muscle cells and the blood vessel endothelium (Fig. 4a–k). Tubal sections from control pigs revealed only minor LH receptor immunostaining (Fig. 4a–c). In all pigs treated with oestradiol, the most noticeable immunostaining was seen in the mucosal epithelium (Fig. 4e–k), where the apical regions of epithelial cells had more intense staining than the underlying cytoplasm. The muscle

Fig. 4. Immunocytochemistry for LH/hCG receptors in the gilt oviduct. (a,b,c) Ovariectomized controls; (d,e,f) ovariectomized gilts treated with oestradiol benzoate 24 h before tissue collection; (g,h,i) ovariectomized gilts treated with oestradiol benzoate 48 h before tissue collection; (j,k,l) ovariectomized gilts treated with oestradiol benzoate 72 h before tissue collection; (l) Control in which non-specific IgG was substituted for primary antibody. (a,d,g,j,l) Isthmus; (b,e,h,k) ampulla; (c,f,i) infundibulum. Arrowheads represent a positive reaction in endothelial cells of blood vessels; arrows represent a positive reaction in the smooth muscle cells. Scale bars represent (a-c, f-l) 250 µm and (d,e) 500 µm.

Fig. 5. A typical control (without LH addition) polygraph tracing of the isthmus ring segment of a pig oviduct (a), and a representative myograph tracing showing the relaxing effect of LH on the pig oviduct isthmus ring segment (b). Both oviducts were collected during the peri-ovulatory stage of the oestrous cycle.

cells of myosalpinx also showed the presence of LH receptors (Fig. 4d,g,j), but the staining was less intense than it was in the mucosal layer. Tubal blood vessels contained LH receptors, but these were limited to the endothelium (Fig. 4d,k).

Contractility of oviductal segments

In the preliminary experiment, the effects of different doses of LH and hCG on oviductal contractility *in vitro* were tested. The lowest dose that consistently decreased contractility in all segments tested (100 ng LH ml⁻¹ and 134 ng ml⁻¹ hCG) was used in all further experiments. FSH (pFSH-B1) and prolactin (Biolactin) tested at concentrations between 20 and 100 ng ml⁻¹ showed no effect on oviductal contractility.

The pattern of spontaneous oviductal contractions differed among the various stages of the oestrous cycle. The highest frequencies of isthmus contractions (contractions per 10 min) were found during the early luteal stage (167.50 ± 17.50) and early follicular stage (98.80 ± 6.90) and compared with lower frequencies in the peri-ovulatory (60.40 ± 9.60) and late luteal stages (64.00 ± 8.50 ; $P < 0.05$). The typical contractions of the isthmus during the peri-ovulatory stage of the oestrous cycle are shown without hormone addition (Fig. 5a) and during LH treatment (Fig. 5b). In addition, a higher frequency of contractions was recorded in the ampulla during the early luteal phase (99.50 ± 8.50) than during the peri-ovulatory, late luteal and follicular stages of the oestrous cycle (52.20 ± 7.20 , 61.70 ± 5.00 and 55.00 ± 4.60 , respectively). The early luteal stage was characterized by the lowest amplitude (tissue tension; mN) of isthmus contractions (0.48 ± 0.05) and compared with the peri-ovulatory, late luteal and follicular stages (3.73 ± 0.47 ; $3.18 \pm$

1.80 and 1.28 ± 0.20 , respectively; $P < 0.05$). There was no difference between the early (2.52 ± 1.10) and late (2.73 ± 0.58) luteal stages in the amplitude of contractions in the ampulla, but the amplitude of this contraction increased slightly in peri-ovulatory (4.27 ± 0.62) and early follicular (3.50 ± 0.70) stages of the oestrous cycle. The areas under curve (arbitrary units) during the peri-ovulatory, early and late luteal, and follicular stages did not differ significantly in isthmus (5.98 ± 0.28 ; 5.38 ± 0.67 ; 5.28 ± 0.87 and 6.58 ± 0.40 , respectively) and ampulla (6.45 ± 0.46 ; 4.80 ± 0.09 ; 5.67 ± 0.31 and 4.70 ± 0.54 , respectively) of the oviduct.

The contractile parameters of the isthmus in response to LH are shown (Table 1). The frequency of contraction decreased by 23% 20–30 min after addition of LH ($P = 0.06$) only during the peri-ovulatory stage of oestrous cycle; no effect was observed in the other stage of the cycle. LH significantly reduced the amplitude of spontaneous contractions of the isthmus in the peri-ovulatory ($P < 0.05$), early luteal ($P < 0.05$) and late luteal ($P < 0.05$) stages of the oestrous cycle. The reduction of isthmus AUC was statistically significant only in the peri-ovulatory stage of the oestrous cycle ($P < 0.05$). In the ampulla, the frequency of contraction decreased after LH addition in all stages of the oestrous cycle (Table 2). However, the reduction of the frequency of contractions 20–30 min after hormone administration reached statistical significance ($P < 0.05$) only during the peri-ovulatory and early follicular stages. The highest differences were observed in the reduction of ampullar AUC. The effect of LH was most pronounced during the peri-ovulatory ($P < 0.01$) and early follicular stages ($P < 0.05$). LH reduced the ampullar AUC up to 65.5 and 79.5% of controls in the peri-ovulatory and early follicular stages, respectively ($P < 0.05$ – 0.01). The results indicate that LH had the most pronounced effect on the inhibition of spontaneous contractions *in vitro* in both the

Table 1. Mean ($\pm$ SEM) frequency, amplitude and area under curves of spontaneous contraction of the pig isthmus collected during different stages of the oestrous cycle and incubated with 100 ng LH ml⁻¹

Stage of oestrous cycle	Frequency		Amplitude		Area under curves	
	II	III	II	III	II	III
Days 18–2 (peri-ovulatory)	101.1 $\pm$ 9.3	77.4 $\pm$ 7.8	83.4 $\pm$ 8.6	68.4 $\pm$ 7.3*	87.2 $\pm$ 5.1	70.9 $\pm$ 8.7*
Days 5–8 (early luteal)	94.5 $\pm$ 5.9	98.7 $\pm$ 0.7	105.8 $\pm$ 4.0	79.2 $\pm$ 3.8*	97.9 $\pm$ 8.1	91.2 $\pm$ 12.8
Days 13–15 (late luteal)	104.3 $\pm$ 6.9	82.3 $\pm$ 11.8	93.1 $\pm$ 6.9	59.4 $\pm$ 15.1*	104.3 $\pm$ 4.3	87.1 $\pm$ 9.7
Days 16–17 (early follicular)	94.9 $\pm$ 3.1	99.5 $\pm$ 4.21	112.7 $\pm$ 11.9	112.8 $\pm$ 15.7	100.2 $\pm$ 2.9	94.5 $\pm$ 9.4

The responses are expressed as percentage of initial values (period I: from 10 to 0 min before hormone treatment; period II: from 0 to 10 min; and period III: from 20 to 30 min after LH addition).

* $P < 0.05$ versus period I in rows.

Table 2. Mean ($\pm$ SEM) frequency, amplitude and area under curves of spontaneous contraction of the pig ampulla collected during different stages of the oestrous cycle and incubated with 100 ng LH ml⁻¹

Stage of oestrous cycle	Frequency		Amplitude		Area under curves	
	II	III	II	III	II	III
Days 18–2 (peri-ovulatory)	87.7 $\pm$ 3.8	71.1 $\pm$ 11.5*	100.6 $\pm$ 9.0	69.8 $\pm$ 17.2	89.8 $\pm$ 3.6	65.5 $\pm$ 11.2**
Days 5–8 (early luteal)	89.4 $\pm$ 0.4	70.4 $\pm$ 13.5	84.0 $\pm$ 19.0	86.7 $\pm$ 13.3	108.6 $\pm$ 9.3	90.3 $\pm$ 5.7
Days 13–15 (late luteal)	96.8 $\pm$ 2.5	87.2 $\pm$ 9.1	88.7 $\pm$ 11.8	75.8 $\pm$ 14.2	95.3 $\pm$ 4.2	91.1 $\pm$ 5.5
Days 16–17 (early follicular)	98.9 $\pm$ 2.5	93.5 $\pm$ 1.6*	92.0 $\pm$ 5.1	88.7 $\pm$ 6.7	95.6 $\pm$ 1.8	79.5 $\pm$ 7.5*

The responses are expressed as percentage of initial values (period I: from 10 to 0 min before hormone treatment; period II: from 0 to 10 min; and period III: from 20 to 30 min after LH addition).

* $P < 0.05$; ** $P < 0.01$ versus period I in rows.

isthmus and ampulla during the peri-ovulatory stage of oestrous cycle (Tables 1 and 2). The inhibition of the amplitude and frequency of the contractions, as well as the reduction of the AUCs, took place gradually in about 10 min after the LH addition (Fig. 5).

LH/hCG receptor protein

Western blotting with polyclonal LH/hCG receptor antibody showed that oviducts contained 75, 48 and 45 kDa proteins in all pigs treated with oestradiol (Fig. 6, lanes 1 and 2). Corpora lutea contained the same 75, 48, and 45 kDa receptor proteins (Fig. 6, lane 3). The skeletal muscle did not show receptor protein (data not shown).

Discussion

These experiments provide evidence that the pig oviduct is a target organ for LH. The immunocytochemical studies

revealed that LH receptors are present mainly in the mucosal epithelial cells of the oviduct, which is in agreement with a previous study of Lei *et al.* (1993) of the human oviduct. The presence of LH/hCG receptor mRNA transcripts in human (Lei *et al.*, 1993) and pig (Derecka *et al.*, 1995) oviducts, as well as receptor protein in cultured mucosal cells of the human oviduct (Han *et al.*, 1996), has been reported. Comparison of the immunostaining intensities revealed that the cells of myosalpinx expressed lower amounts of LH/hCG receptors than the epithelial mucosal cells of pig oviduct. A similar distribution of LH receptors was found in women (Lei *et al.*, 1993). LH/hCG receptors in smooth muscle cells were also found in the myometrial cells of pigs (Ziecik *et al.*, 1986) and women (Reshef *et al.*, 1990; Környei *et al.*, 1993), and in the superficial smooth muscle cell layer of the broad ligament in pigs (Ziecik *et al.*, 1995).

Western blotting studies showed that pig corpora lutea and oviducts contain mainly 75 kDa receptor species, which corresponds to a calculated molecular mass for LH receptor in pigs (Loosfelt *et al.*, 1989). However, in the present study, the 75 kDa band is rather the glycosated 68 kDa form

Fig. 6. Western immunoblotting for LH/hCG receptors. Lane 1, oviduct from ovariectomized gilt treated with oestradiol benzoate 48 h before tissue collection; lane 2, oviduct from ovariectomized gilt treated with oestradiol benzoate 24 h before tissue collection; lane 3, pig corpora lutea. Forty milligrams of protein of each tissue was used.

described by Vu Hai-Luu Thi *et al.* (1992) in pig testis. The 48 and 45 kDa receptor variants detected in pig corpora lutea and oviducts are apparently forms lacking transmembrane or intracellular domains. Comparison of the results of the present study with previous results revealed that the receptors in pig oviduct do not differ qualitatively and quantitatively from those in the other extragonadal positive tissues (myometrium, endometrium and umbilical cord). The negative control tissues, liver, kidney and skeletal muscle, do not contain LH receptors.

The oviduct is a natural extension of the uterine horn and both structures share a common origin from Müllerian ducts (Hunter, 1988); therefore, it is not surprising that LH receptors are present in the oviduct. Oviductal LH binding was dependent on oestradiol stimulation as is LH binding in the myometrium (Flowers *et al.*, 1991). The results of the present study showed that LH inhibited the amplitude of isthmic contractions in gilts from peri-ovulatory, early luteal and late luteal stages of the oestrous cycle, as well as reducing the AUC of the isthmus during the peri-ovulatory stage. In the ampulla, LH reduced the AUC and the frequency of contractility in gilts from the peri-ovulatory and early follicular stages of the oestrous cycle. Hence, LH can modulate the spontaneous contractions of the pig oviduct directly. In all cases, gradual inhibition of the spontaneous contractions were observed within 10 min after the addition of LH (or hCG; data not shown). LH inhibited the frequency and amplitude and reduced the AUC particularly in the isthmus and ampulla of the peri-ovulatory stage. The site of the effect is in agreement with the radioligand and immunocytochemical studies showing that oestradiol can be responsible for the expression of LH receptors in the pig oviduct.

The relaxing effect of gonadotrophin on smooth muscle contraction in the reproductive tract has been reported. Flowers *et al.* (1991) showed that hCG has a quiescent effect on myometrial spontaneous contractility, reducing the frequency and amplitude of spontaneous contractions only in gilts treated with oestradiol benzoate. Similar results were obtained during the studies *in vivo* of Ziecik *et al.* (1993), in which it was demonstrated that hCG treatment decreased the

myometrial electrical activity in pigs primed with oestrogen. In the human myometrium, Eta *et al.* (1994) found that hCG can inhibit the amplitude of oxytocin-stimulated contractions directly during the proliferative, but not the secretory, phase of the cycle. Although the mechanism of the LH effect on oviduct is not clear, it is possible that it acts through receptors situated both in epithelial and smooth muscle cells. Han *et al.* (1996) reported that cultured cells of human oviductal mucosa respond to hCG stimulation with increased cyclooxygenase 2 mRNA expression and protein concentrations, and with increased PGE₂ production (PGE₂ is an important factor known to cause oviductal relaxation). However, LH can affect oviductal smooth muscle cell contractions directly through an increase in cAMP accumulation, as was found in pig myocytes (Kisielewska *et al.*, 1996).

The major function of the Fallopian tube is the transport of gametes to the ampullary-isthmic junction, the usual site of fertilization and transport of embryos to the uterus. Two basic mechanisms are involved in this transport: the beat of cilia and the waves of smooth muscle activity in the myosalpinx (Hunter, 1988). Many studies show that ovarian steroid hormones (Hunter, 1988; Ayad *et al.*, 1994), the autonomic nervous system (Rodríguez-Martínez, 1984; Kaleczyc, 1994), prostaglandins (Spilman, 1974; Borda *et al.*, 1980; Rodríguez-Martínez and Einarsson, 1985) and endothelin (McGovern *et al.*, 1992; Rosselli *et al.*, 1994) are involved in control of oviductal function. Hunter (1984) suggested that ovulation can somehow co-ordinate the transport of gametes in the pig oviduct to the ampullar-isthmic junction. However, the transport of spermatozoa from the isthmus to the site of fertilization was synchronized by ovulation in the New World monkey, *Cebus apella* (Ortiz *et al.*, 1995), although the exact nature of the mechanism controlling the passage of gametes and synchronizing the process of fertilization remains unknown (Hunter, 1988; Ito *et al.*, 1991). Stimulation with oestradiol for 24–72 h caused a significant increase in LH/hCG receptors in the oviduct of ovariectomized gilts. The duration of the natural 'surge' of oestradiol that triggers the initiation of the pre-ovulatory LH surge in intact, cyclic gilts is about 48–72 h (Brinkley, 1981; Ziecik *et al.*, 1987). Under normal physiological conditions, it is possible to increase LH/hCG receptors in the pig oviduct and to prepare it for LH action. In the present *in vitro* studies of oviduct contractility, the greatest effect of LH on relaxation of oviduct occurred during the peri-ovulatory stage of the oestrous cycle. The results of this investigation indicate that LH should be added to the list of factors affecting oviduct function. We hypothesize that the pre-ovulatory surge of LH, in addition to causing the rupture of follicles and luteinization of granulosa and theca cells, also plays an important role in controlling oviductal contractions, and is a major factor responsible for opening the ampulla-isthmic junction for spermatozoa and for synchronization of fertilization of ova in the ampulla. The relaxing action of LH may also facilitate the passage of embryos through the isthmus towards the uterus.

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