Regulation of muscular contractions in the human Fallopian tube through prostaglandins and progestagens

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BACKGROUND: Transport of gametes and embryos is an important function of the Fallopian tube. Both muscular contractions and cilia activity are involved in the transportation. Prostaglandins (PGs) are known mediators of muscular contractility. PG receptors have previously been demonstrated in the human Fallopian tube. The aim was to study the effect of PGs and progestagens, antiprogestin, hCG and oxytocin on muscular contractions in the human Fallopian tube, and the hormonal regulation of PG receptors. METHODS: Twenty-two healthy women operated for benign causes were included in the study. The ampullary-isthmic junction of the Fallopian tubes was excised and used for *in vitro* contractility studies. The effect of PGE_1, PGE_2, $PGF_{2\alpha}$, progesterone, mifepristone, levonorgestrel, oxytocin and hCG on contractility was studied. Explants of Fallopian tubes were cultured for 24 h to study the effect of progestagens and hCG on the expression of PG receptors using immunohistochemistry and real-time PCR. RESULTS: Muscular contractions increased after treatment with $PGF_{2\alpha}$ and PGE_2 ($P < 0.05$). The contractions decreased after PGE_1, progesterone, levonorgestrel, mifepristone, oxytocin and hCG ($P < 0.05$). In tubal explant studies, relative mRNA expression of EP1, EP2, EP3 and FP increased after levonorgestrel treatment ($P < 0.05$). Mifepristone and levonorgestrel treatment increased immunostaining intensity of EP1 and EP2 protein, in lumen, muscle and vessels. Progesterone and mifepristone increased immunostaining of FP in vessels. CONCLUSIONS: These data suggest that the transport of gametes and embryos involves the action of PGs, progesterone, oxytocin and hCG on muscular contractility.

Keywords: hCG; mifepristone; progestagens; prostaglandins; tubal transport

Introduction

The transport of spermatozoa is believed to be aided by muscular contractions in the wall of the Fallopian tube (Mastroianni, 1999) and uterus (Kunz *et al.*, 1997). The regulation of muscular activity is influenced by adrenergic nerves, sex steroids (Sjoberg, 1967; Helm *et al.*, 1982), nitric oxide (Ekerhovd *et al.*, 1997; Ekerhovd *et al.*, 1999), oxytocin (OT) (Jankovic *et al.*, 2001) and prostaglandins (PGs) (Caschetto *et al.*, 1979; Lindblom *et al.*, 1983). The transport of spermatozoa from the vagina to the Fallopian tube can be very fast, and there is evidence that sperm can reach the Fallopian tube within minutes (Ahlgren, 1975; Ozgur *et al.*, 1997). Survival time of spermatozoa in the Fallopian tube has been demonstrated to be up to 85 h (Ahlgren, 1975). It has been suggested that the spermatozoa, after their initial transport, can be stored in the isthmic part of the Fallopian tube until ovulation (Hunter, 1987). Epithelial cells in the Fallopian tube enhance sperm motility (Murray and Smith, 1997) and promote the ability of the spermatozoa to bind to the zona pellucida (Ziskind *et al.*, 2000). Chemotaxis by progesterone from the cumulus cells

surrounding the oocyte is believed to be involved in the guidance of the spermatozoa during their movement toward to the oocyte (Teves *et al.*, 2006).

After ovulation, the ovum is picked up by the cilia in the fimbrial apparatus of the Fallopian tube, and thereafter transported into the ampulla, where it is fertilized (Pauerstein and Eddy, 1979; Lyons *et al.*, 2006). After fertilization the pre-embryo starts to cleave and develop during its journey through the Fallopian tube. Transport of the pre-embryo is believed to be aided mainly by the cilia in the tubal mucosa (Halbert *et al.*, 1989). The pre-embryo is retained in the Fallopian tube for ~3 days (Diaz *et al.*, 1980) before its transport into the uterine cavity where it can hatch and implant (Pauerstein and Eddy, 1979).

Spontaneous muscular activity of the Fallopian tube has been demonstrated *in vitro*. The amplitude of individual contractions was demonstrated to be similar during the different phases of the menstrual cycle, whereas the frequency of contractions increased during the periovulatory phase (Lindblom *et al.*, 1980). There are three layers of musculature in the Fallopian tube: outer longitudinal layer, a middle circular

layer and, in the intramural and proximal isthmic parts, an inner longitudinal layer. The frequency of contractions is higher in the circular than in the longitudinal musculature (Helm *et al.*, 1982).

PGs are important regulators of several reproductive processes, including ovulation, fertilization (Speroff and Ramwell, 1970) and implantation (Achache and Revel, 2006). The seminal fluid is rich in PGs, which are mainly produced in the seminal vesicles (Bendvold *et al.*, 1985). The seminal PGE is of importance for reproductive capacity and men with low levels of PGE in the seminal fluid have reduced fertility (Bygdeman *et al.*, 1970).

The human oviductal cells express cyclooxygenase (COX)-1, COX-2 and PG synthase (Arbab *et al.*, 2002), which make the Fallopian tubes capable of producing PGE_2, $PGF_{2\alpha}$ (Ogra *et al.*, 1974) and prostacyclin (PGI_2) (Huang *et al.*, 2002). The human embryo also produces PGE_2 in cell culture (Holmes *et al.*, 1990).

PGE_2, $PGF_{2\alpha}$ and PGI_2 exert their biological function through binding to their respective receptors, EP, FP and IP. There are four types of EP receptor (EP1–4) (Narumiya *et al.*, 1999) which all use different pathways for intracellular actions: EP1 is coupled to diacylglycerol/inositol triphosphate turnover and activation causes an increase in intracellular Ca^{2+}, EP2 and EP4 act by activation of adenylate cyclase, whereas EP3 inhibits adenylate cyclase. FP is coupled to stimulation of the phospholipase C—inositol (IP3) pathway and Ca^{2+} mobilization (Sugimoto *et al.*, 1992; Negishi *et al.*, 1993; Watabe *et al.*, 1993; Sando *et al.*, 1994; Ashby, 1998). We have previously shown that the receptors for PGE_2 and $PGF_{2\alpha}$ are present in the human Fallopian tube (Wanggren *et al.*, 2006).

Several important functions in female reproduction, such as ovulation and endometrial development, depend on progesterone (De Ziegler *et al.*, 1994). Progesterone is also believed to regulate tubal transport but this has not been proven in humans. The physiological effects of progesterone are mediated by its two progesterone receptor (PR) isoforms, PR-A and PR-B (Conneely *et al.*, 2003).

Mifepristone is a potent antiprogestin that blocks the action of progesterone at the receptor level (Klein-Hitpass *et al.*, 1991). Treatment with mifepristone has been shown to increase PR levels in the human endometrium and Fallopian tube (Christow *et al.*, 2002; Sun *et al.*, 2003). Mifepristone increases the sensitivity to PGE_2 in human myometrium (Bygdeman and Swahn, 1985), but the effect in the Fallopian tube is not yet known.

Other factors that may be involved in the regulation of muscular contractions in the Fallopian tube are hCG and OT. OT and hCG have been shown to have an inhibitory effect on muscular activity in the human as well as the porcine Fallopian tube (Gawronska *et al.*, 1999; Jankovic *et al.*, 2001). Expression of receptors for LH/hCG has been demonstrated in the human Fallopian tube (Lei *et al.*, 1993); however, their role in the physiology of the Fallopian tube is still unknown. We designed a study with the aim to reveal the hormonal regulation of muscular contractility in the human Fallopian tube.

Materials and Methods

Study subjects

Twenty-two healthy women with a mean age of 44 years (33–50 years), undergoing hysterectomy for benign causes or sterilization, were included in the study. Twenty of the women had not taken any hormonal treatment or used an IUD for at least 3 months prior to the study. Two women received pretreatment with an oral dose of mifepristone 50 mg every second day, for 3 months before operation. At surgery the Fallopian tubes were excised. One or two tubes were used for contractility studies. In three women, one of the Fallopian tubes was used for explant culture. In addition, endometrial biopsies were obtained from the women.

The Fallopian tubes used for the *in vitro* contractility experiments were immediately placed in ice-chilled Krebs–Ringer buffer solution (NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 1.0 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 24.8 mM, KH_2PO_4 1.2 mM and glucose 5.6 mM). Biopsies were obtained from the isthmic and the ampullary part of the Fallopian tubes. The biopsies were divided and one piece was fixed for immunohistochemistry and the other piece was snap-frozen for PCR. Fallopian tubes used for culture were immediately placed in sterile HBSS (Hanks' balanced salt solution, GIBCO-BRL, Invitrogen, Stockholm, Sweden).

In vitro contractility experiments

A total of 24 Fallopian tubes from 18 patients were used for *in vitro* contractility experiments. In four patients, biopsies were taken and the *in vitro* model was tested but no contractility registration was performed. The ampullary-isthmic part of the Fallopian tube was excised after being identified by inserting a 1 mm probe into the Fallopian tube (Lindblom *et al.*, 1978). Longitudinal tissue strips, $\sim 7 \times 1 \times 1$ mm consisting of the tubal muscular wall and mucosa, were prepared under a stereomicroscope. Each strip was placed in an organ chamber, filled with Krebs buffer solution at a constant temperature of 37°C and oxygenated with a gas mixture consisting of 95% O_2 and 5% CO_2. The strips were mounted under tension with a load equivalent to ~ 1 g. Contractions were recorded with a Grass FT03C force-displacement transducer and registered on paper with a Grass model 7 and 79D polygraph, and in a computer with the data acquisition program Windaq/XL (Dataq instruments, OH, USA). Experiments were carried out after ~ 30 min of equilibration. Progesterone, mifepristone, PGE_1, PGE_2, $PGF_{2\alpha}$, levonorgestrel, hCG and OT were administered separately, in two different concentrations and in different combinations, to the chambers at 20 min intervals after being dissolved in buffer solution. The final concentrations were 0.1 and 1 µM for progesterone, mifepristone, PGE_1, PGE_2, $PGF_{2\alpha}$ and hCG, 0.02 and 0.2 µM for levonorgestrel and 0.1 and 0.2 µM for OT.

Progesterone, PGE_1, PGE_2, $PGF_{2\alpha}$ and levonorgestrel were purchased from Sigma chemical company, St Louis, MO, USA and OT (Syntocinon) from Novartis, Sverige, AB, Täby, Sweden. Mifepristone (Mifegyne) was a gift from Exelgyn, Paris, France, and hCG (Ovitrelle) was kindly provided by Serono Nordic AB, Stockholm, Sweden.

Data processing

Contractility end-points were frequency of contractions (*f*) and area under the curve (AUC). Analyses of the contractions were made manually. The contractions were counted and measured for a period of 5 min, after an equilibration period of 3 min measured from when the drugs were added to the chambers. The contraction frequency (*f*) was calculated by dividing the number of contractions over the calculated time in minutes ($f = n_c/T$). The estimated AUC was calculated by adding all contractile force measurements ($fc_a - fc_x$), corrected for

the individual strip calibration (X_1), dividing them by time in minutes (T) and adding the change in baseline measurement (b) [$AUC_1 = ((fc_a + \cdots + fc_x)/(T \times 2 \times X_1) + (b/X_1))$]. In other words, the estimated AUC is the sum of all areas, each formed by a triangle between the time base and the height of each contraction, added to the basal level. The measurements and calculations for each drug were compared with the status of spontaneous contractions before the drugs were added and after pretreatment with active drugs (Crankshaw, 2001).

Fallopian tube explant culture

To investigate if steroid hormones regulate PG receptors in the Fallopian tube, explants of Fallopian tube were cultured. Explants from three different patients were cultured. Preparations of the Fallopian tubes were performed in sterile conditions under laminar flow. The Fallopian tubes were opened longitudinally to expose the mucosa. The mucosa and the underlying muscular wall were cut into small pieces ($\sim 0.5 \times 1.5$ mm). The pieces were cultured on tissue culture inserts in separate wells (Costar Transwell, Milian SA, Geneva, Switzerland). At least four pieces, two for immunohistochemistry and two for real-time PCR, were used for each culture condition. The explants were cultured in Hams F12/Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum, charcoal treated (Invitrogen), penicillin–streptomycin 100 IU (Invitrogen) and L-glutamine 2 mM (Invitrogen). The culture was performed at 37°C, 5% CO_2 and 95% humidity. The following were added to different wells: progesterone to final concentrations of 100 nmol/l, mifepristone 100 nmol/l, levonorgestrel 10 nmol/l or hCG 10 μg/l. Explants were cultured for 24 h, and thereafter the explants were snap-frozen and stored in liquid nitrogen.

RNA and cDNA preparation

Total RNA was isolated using Quiagen kit (Quiagen AB, Solna, Sweden) according to the protocol from the manufacturer. One microgram of total RNA from each sample was reverse transcribed using deoxynucleotide triphosphates (10 mM each), random hexamer (250 ng/ml), ribonuclease inhibitor (40 U/μl) and superscript reverse transcriptase (200 U/μl), using the Superscript™ II RNase H⁻ Reverse Transcriptase Kit (Invitrogen).

Real-time PCR

Real-time PCR (Applied Biosystems, Foster City, CA, USA) was used to quantify the differential expression of EP1, EP2, EP3, EP4 and FP in the Fallopian tube explant cultures. Each sample was analyzed in duplicate. Experiments were performed on a 96 well array format, using the ABI 7500 instrument (Applied Biosystems). For EP1, a Taqman® probe was used. Each well was filled with Taqman® Universal PCR master mix (Applied Biosystems), $1\times$ Taqman® Gene expression assay mix for EP1 (Hs00168752_m1, Applied Biosystems) and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Hs02758991_g1, Applied Biosystems), 8 ng of cDNA and dH_2O added up to the final volume 15 μl. The target assay genes for EP2 (primer sequence: sense 5′-GAC CGC TTA CCT GCA GCT GTA C-3′; antisense 5′-TGA AGT TGC AGG CGA GCA-3′), EP3 (primer sequence: sense 5′-AAG GCC ACG GCA TCT CAG T-3′; antisense 5′-TGA TCC CCA TAA GCT GAA TGG-3′), EP4 (primer sequence: sense 5′-ACG CCG CCT ACT CCT ACA TG-3′; antisense 5′-AGA GGA CGG TGG CGA GAA T-3′) and FP (primer sequence: sense 5′-GCA GCT GCG CTT CTT TCA A-3′; antisense 5′-CAC TGT CAT GAA GAT TAC TGA AAA AAA TAC-3′) were incorporated along with GAPDH as internal control (primer sequence: sense 5′-GAA GGT GAA GGT CGG AGT CAA

C-3′; antisense 5′-CAG AGT TAA AAG CAG CCC TGG T-3′) (Sales et al., 2004; Perez-Novo et al., 2006), which was used to normalize the expression levels of target genes in each sample. The final reaction volume (10 μl) consisted of 5 ml SYBR green master mix (Applied Biosystems), forward and reverse primers for EP2, EP3, EP4 and FP (final concentration 0.3 μM) and cDNA at a final concentration of 0.4 ng/μl. A standard curve was performed for EP2, EP3, EP4 and FP using serial dilution of cDNA synthesized from the Fallopian tube. Denaturation was carried out for 10 min at 95°C, followed by 45 cycles of denaturation at 95°C for 15 s and combined primer annealing/extension at 60°C for 1 min. All reactions were performed in duplicate and the mean value of the threshold cycle (Ct, the start of exponential amplification) of each sample was normalized with their respective threshold cycle of GAPDH, obtaining the ΔCt value. Data were analyzed using SDS 1.3.1 software (Applied Biosystems). The data from the mucosa and the muscular layer were calculated together. Relative expression was calculated using the following formula: 100×2^{-DDCT} as is described in User Bulletin #2 from Applied Biosystems.

Immunohistochemistry

Paraffin embedded biopsies from cultured Fallopian tube explants were sectioned (4 μm) and mounted on glass slides. The samples were thereafter deparaffinated in Bioclear (CiAB, Stockholm, Sweden) and rehydrated in decreasing concentrations of ethanol, ending in distilled water. The samples were rinsed in phosphate-buffered saline (PBS), incubated in darkness for 10 min in H_2O_2 (3% in methanol) to block endogenous peroxidase activity and washed with PBS (with Albumin, bovine 0.05%). The slides were blocked with 1.5% goat serum in PBS for 30 min. The sections were then incubated with the primary antibody, diluted 1:100 for EP1, 1:350 for EP2, 1:50 for EP3, 1:700 for EP4 and 1:20 for FP overnight at 4°C. The antibodies for EP1, EP2, EP3 and EP4 receptors were rabbit polyclonal antibodies raised against synthetic peptides from the human EP1, EP2, EP3, EP4 and FP receptors, respectively (Catalog no. 101740, 10750, 10760, 10770 and 101802, Cayman Chemical, MI, USA). As negative control, the primary antibody was replaced with non-immune serum of equivalent concentration from the same species. The slides were washed in PBS and thereafter incubated with the secondary antibody diluted 1:300 (goat anti-rabbit) for 30 min at room temperature. The slides were then rinsed in PBS, prior to incubation with ABC complex (Vectastain Elite ABC immunoperoxidase detection system, Vector Laboratories Inc., Burlingame, CA, USA) according to the manufacturer's instructions. After washing with PBS, freshly prepared diaminobenzidine–hydrogen peroxide solution (DAB kit from Vector) was added to the slides, which were thereafter rinsed with distilled water. The slides were counterstained with Mayer's haematoxylin (VWR, Stockholm, Sweden), then washed in cold water, dehydrated in increasing concentrations of ethanol and mounted with Pertex (Histolab AB, Gothenburg, Sweden).

Two persons evaluated the immunohistochemical staining independently, blinded to the identity of the samples. The staining was graded on a scale of 0 = no staining of cells, += faint staining, ++= moderate staining and +++= strong staining.

Statistics

Kruskal–Wallis one-way analysis of variance on ranks and Friedman's repeated measures analysis of variance on ranks were used to evaluate the contractility data. Kruskal–Wallis one-way analysis of variance on ranks was used to analyze the real-time PCR data. Pairwise multiple comparison procedures were performed according to Dunn's method (Sigmastat, Statistical Package for the Social Sciences INC,

Chicago, IL, USA). A *P*-value of <0.05 was considered statistically significant.

Ethics

All women gave their written informed consent prior to participating in the study. The study was approved by the Ethics Committee at the Karolinska University Hospital.

Results

Tubal contractility

Spontaneous contractions of the muscular strips occurred within 20 min in all the experiments. The basal frequency was higher during the periovulatory phase compared with the follicular and luteal phases. There was no difference in AUC between the menstrual phases (Table I).

Progesterone administration resulted in a marked dose-dependent reduction in frequency and AUC (Fig. 1A, Tables II

and III). Mifepristone administration had a similar but less pronounced effect on frequency and AUC compared with progesterone (Fig. 1B, Tables II and III). Mifepristone and progesterone in combination showed a more pronounced inhibitory effect on frequency and AUC compared with mifepristone alone (Fig. 1C, Tables II and III). Similarly, levonorgestrel strongly inhibited the frequency and reduced the AUC of the contractions (Fig. 1G, Tables II and III).

PGE_1 had an inhibitory effect on frequency only at the higher dosage, but reduced AUC at both concentrations when compared with the baseline (Fig. 1D, Tables II and III). In contrast, PGE_2 had a stimulatory effect on the contractions resulting in an increased AUC, but with unchanged frequency (Fig. 1E, Tables II and III). After administration of $PGF_{2\alpha}$, tubal contractions were more frequent and AUC increased (Fig. 1F, Tables II and III).

Pretreatment *in vitro* with progesterone, mifepristone or co-administration of both for 20–40 min prior to PGE_1 or PGE_2

Table I. Baseline median frequency (*f*) and median AUC, measured in longitudinal strips of the ampullary-isthmic junction of the human Fallopian tube during different menstrual phases.

Menstrual cycle phase	Number of measurements	Baseline frequency contractions/min	Number of measurements	Baseline AUC (mN/min)
Follicular phase	42	3.7 (0.7–8.3)*	72	4.8 (0.1–26.8)[ns]
Periovulatory phase	30	4.5 (2.0–7.2)*	33	3.8 (1.0–10.8)[ns]
Luteal phase	38	3.2 (0.6–7.2)*	44	3.8 (0.2–17)[ns]

There was a significant difference for *f* ($P = 0.003$*) but not for AUC ([ns]) between the different menstrual phases. Statistics according to Kruskal–Wallis analysis of variance on ranks. Data are given as median and range. mN, millinewton.
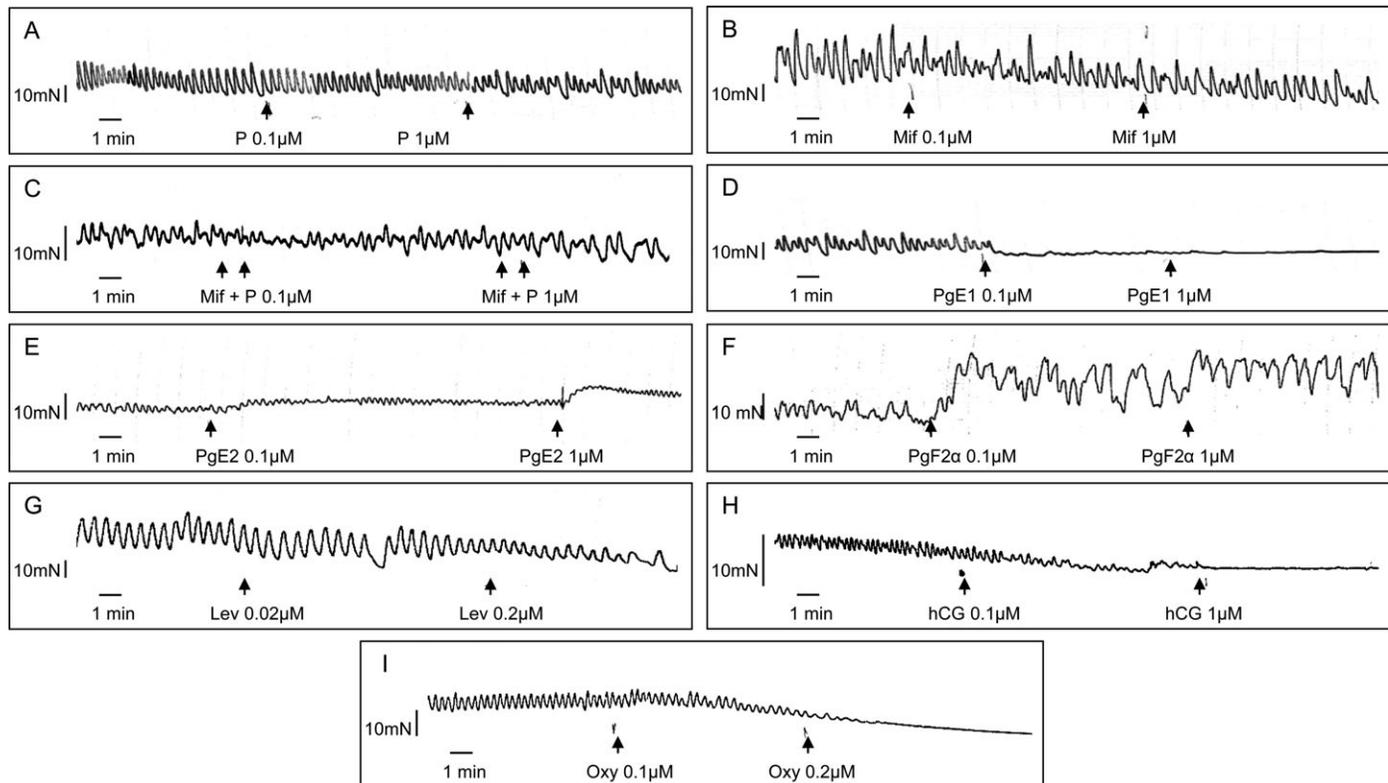

Figure 1: Examples of registrations of contractions from the longitudinal muscular layer of human Fallopian tubes following the addition of (**A**) progesterone (P), (**B**) mifepristone (Mif), (**C**) co-administration of mifepristone and progesterone, (**D**) prostaglandin (PG)E_1, (**E**) PGE_2, (**F**) PGF_2α, (**G**) levonorgestrel (lev), (**H**) hCG and (**I**) Oxytocin (oxy) (all given as final concentration). *Y*-axis: contraction force in millinewton (mN) and *X*-axis: time in minutes (min).

Table II. Effect on median f measured in longitudinal strips of the ampullary-isthmic junction of the Fallopian tube after treatment with different drugs.

Drug	Number of measurements	Baseline frequency contractions/min	Final concentration (μM)	Frequency contractions/min	Final concentration (μM)	Frequency contractions/min
Progesterone	35	4.2 (1.7–8.3)	0.1	3.7 (1.2–7.3)[a]	1	3.4 (0–7.2)[a,b]
Mifepristone	29	3.6 (0.7–7.0)	0.1	3.7 (1.6–7.3)[ns]	1	3.4 (0.8–4.7)[a,b]
Progesterone + Mifepristone	29	3.6 (1.6–7.7)	0.1	3.1 (1.2–8.0)[ns]	1	2.6 (0.4–7.2)[a,b]
PGE$_1$	26	3.9 (0.8–7)	0.1	3.1 (1.0–6.8)[ns]	1	2.6 (0.6–6.6)[a]
PGE$_2$	39	3.0 (0.4–5.6)	0.1	3.0 (0.4–7.0)[ns]	1	3.0 (0.2–6.6)[ns]
PGF$_{2\alpha}$	46	3.0 (1.0–7.2)	0.1	3.8 (0.8–9.8)[a]	1	3.9 (1,4–8.4)[a]
Levonorgestrel	12	4.2 (0.6–6.0)	0.02	3.8 (0.4–5.0)[a]	0.2	1.0 (0.4–4.2)[a]
hCG	3	4.6 (4.0–5.4)	0.1	3.8 (1.8–4.0)[a]	1	1.0 (1.0–2.8)[a]
Oxytocin	5	6.0 (3.2–7.4)	0.1	3.2 (1.6–3.8)[ns]	0.2	2.6 (2.2–3.0)[ns]

Statistics were performed according to the Friedman repeated measures analysis of variance on ranks, multiple comparison procedures according to Dunn's method; [a]significantly different from control frequency; [b]significantly different from the frequency after treatment at the lower concentration, $P < 0.05$ and [ns]not significantly different. Data are given as median and range. PG, prostaglandin.

Table III. Effect on median AUC measured in longitudinal strips of the ampullary-isthmic junction of the Fallopian tube after treatment with different drugs.

Drug	Number of measurements	Baseline AUC (mN/min)	Final concentration (μM)	AUC (mN/min)	Final concentration (μM)	AUC (mN/min)
Progesterone	34	6.7 (0.4–26.4)	0.1	4.0 (−0.5–17.2)[a]	1	1.9 (−1.6–8.6)[a,b]
Mifepristone	31	4.3 (0.7–26.8)	0.1	3.0 (0.0–19.3)[a]	1	2.3 (−0.8–22.9)[a,b]
Progesterone+Mifepristone	27	3.5 (1.5–9.6)	0.1	2.6 (0.1–8.3)[a]	1	1.2 (0.2–8.1)[a,b]
PGE$_1$	24	2.7 (−0.4–11.2)	0.1	1.4 (−3.1–8.1)[a]	1	0.9 (−3.3–6.6)[a]
PGE$_2$	36	0.9 (−1.6–7.4)	0.1	1.7 (−0.7–14.3)[ns]	1	2.2 (−1.6–24.5)[a]
PGF$_{2\alpha}$	32	2.0 (1.0–9.7)	0.1	7.0 (1.0–16.6)[a]	1	7.7 (0.7–40.7)[a]
Levonorgestrel	12	2.3 (0.2–15.2)	0.02	−0.1 (−9.8–4.6)[a]	0.2	−1.4 (−13.1–2.7)[a]
hCG	3	2.3 (1.4–3.8)	0.1	0.7 (0.5–1.0)[a]	1	−0.4 (−0.8–0.4)[ns]
Oxytocin	3	2.4 (0.1–8.3)	0.1	−0.2 (−0.9–3.3)[ns]	0.2	−7.0 (−8.7—3.9)[a]

Statistics were performed according to the Friedman repeated measures analysis of variance on ranks, multiple comparison procedures according to Dunn's method; [a]significantly different from control AUC; [b]significantly different from AUC after treatment with the lower concentration, $P < 0.05$. Data are given as median and range.

administration did not significantly influence the response (data not shown). Co-administration of mifepristone and progesterone 40 min before administration of PGF$_{2\alpha}$ significantly reduced the AUC at the higher dose of PGF$_{2\alpha}$ treatment. The frequency was not affected by pretreatment with progesterone, mifepristone or a combination of both.

Administration of hCG resulted in a decreased contractility, with reduced frequency at the higher dose and reduced AUC at the lower dose (Fig. 1H, Tables II and III). Administration of OT showed a tendency toward initial stimulation followed by inhibition of the contractions and AUC was reduced at the higher dose (Fig. 1I, Tables II and III).

The effect of progestagens on the regulation of PG receptors in explants

Levonorgestrel treatment caused an increase in the expression of EP1, EP2, EP3 and FP mRNA ($P < 0.05$, Fig. 2). Staining of EP1 protein was more intense in all cell types versus control after mifepristone and levonorgestrel treatment (Table IV and Fig. 3). Staining intensity of EP2 was more pronounced in lumen and muscle after mifepristone and in muscle after levonorgestrel treatment (Table IV and Fig. 3). Immunostaining of EP3 was strong both in treated and control samples, whereas immunostaining of EP4 was weak both in treated and control

samples (Table IV and Fig. 3). The immunostaining of FP was more intense in vessels after treatment with progesterone and mifepristone (Table IV and Fig. 3).

The effect of hCG on the regulation of PG receptors in explants

The mRNA expression was not significantly different after hCG treatment compared with untreated controls. Treatment with hCG showed slight increase of immunostaining in lumen for EP2 and in vessels for FP, whereas a slight decrease was observed for in lumen for EP3. (Table IV, Figs 2 and 3).

Discussion

Transport of the gametes and the pre-embryo is an important task of the Fallopian tube. The transport is believed to be facilitated through ciliary activity and muscular contractions. In the present study, we found that both PGs and hormones can regulate the tubal contractility.

We found that the basal frequency of muscular contractions was elevated during the periovulatory period compared with the follicular and luteal phases. This is in agreement with earlier studies of contractile patterns in muscular strips of the Fallopian tube (Lindblom et al., 1980). The intensified
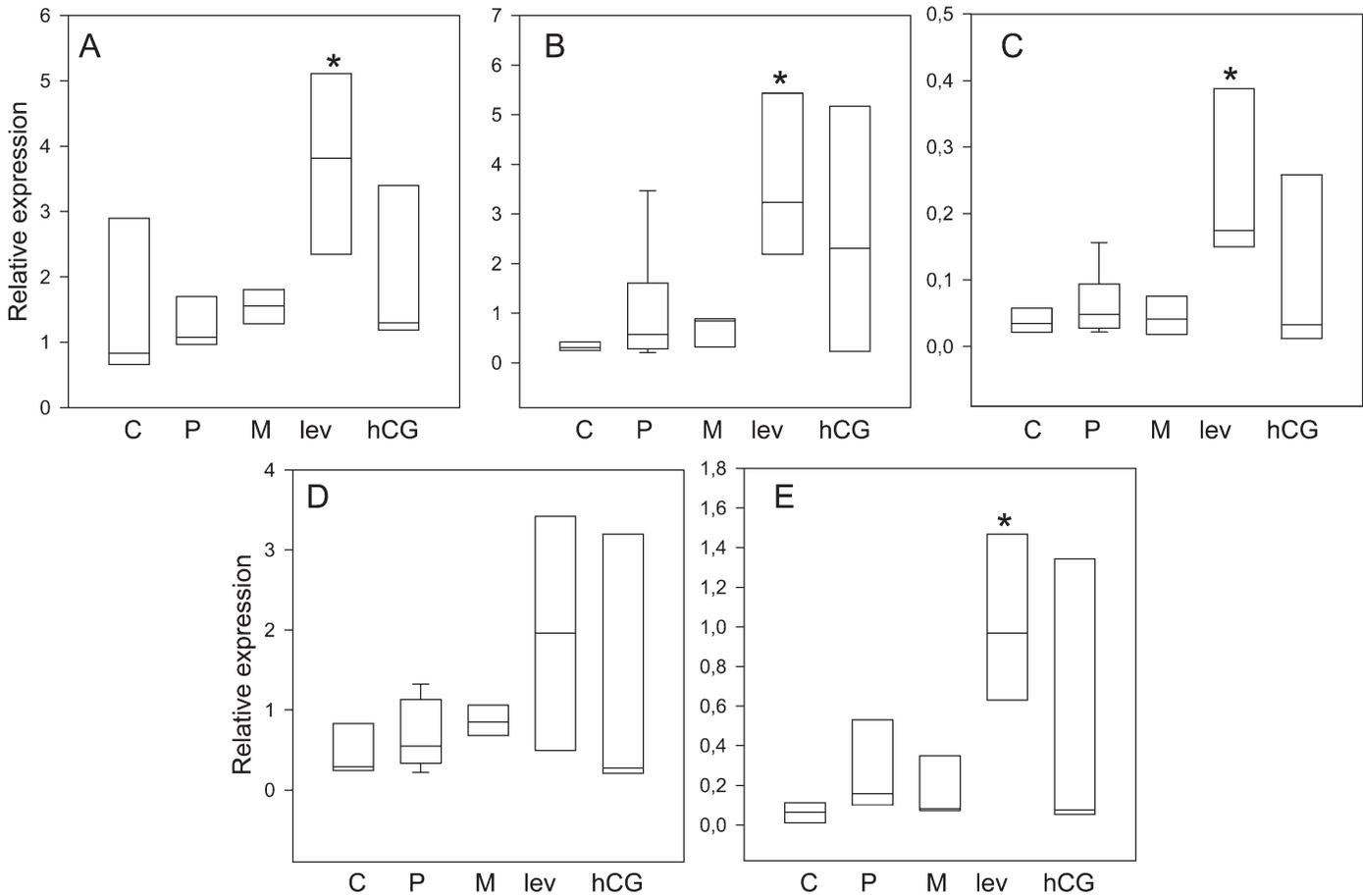
Figure 2: Relative expression of PG receptor (EP1−4 and FP) mRNA in cultured explants of Fallopian tube for the control (C) and after treatment for 24 h with progesterone (P, final concentration 100 nmol/l), mifepristone (M, 100 nmol/l), lev (10 nmol/l) and hCG (10 μg/l). (**A**) EP1, (**B**) EP2, (**C**) EP3, (**D**) EP4 and (**E**) FP. Statistics according to Kruskal–Wallis one-way analysis of variance on ranks; asterisk is significantly different from control, $P < 0.05$.

Table IV. Immunohistochemistry staining intensity for the PG receptors EP1–4 and FP in different cell types of Fallopian tube tissue after 24 h of culture.

Treatment	EP1			EP2			EP3			EP4			FP		
	L	M	V	L	M	V	L	M	V	L	M	V	L	M	V
Control	++	+	+	++	+	++	+++	+++	++	++	+	+	++	++	+
Progesterone	+	+	+	++	++	+	+++	++	++	+	+	++	++	++	+++
Mifepristone	++	++	++	+++	++	++	+++	++	++	+	+	+	++	++	+++
Levonorgesterel	+++	++	++	++	++	++	+++	++	++	++	+	+	++	+	+
hCG	++	+	+	+	++	++	++	++	++	++	+	+	++	++	++

L, lumen, M, muscle and V, vessels.

contractions are probably due to the rise in endogenous estradiol during the periovulatory period. It is plausible that increased contractions in the longitudinal layer of the Fallopian tube play a physiological role in aiding the transport of spermatozoa before, and at the time of, ovulation.

Progesterone has been suggested to have an inhibitory action on tubal activity, as high progesterone levels in the luteal phase coincides with reduced frequency of contractions (Lindblom *et al*., 1980). This was confirmed in our *in vitro* studies where a dose-dependent inhibitory effect of progesterone on the Fallopian tube muscle activity was seen. This inhibitory effect of progesterone might be of importance in relaxation

of the physiological sphincter in the isthmus, allowing cilia to transport the pre-embryo into the uterine cavity (Mastroianni, 1999). A similar regulatory effect on transport of the pre-embryo has previously been shown in rabbits (Singh *et al*., 1990).

Mifepristone is a potent antiprogestin (Teutsch *et al*., 1988), which acts by competitive binding to the PR and thereby inhibiting transcription induced by progesterone binding (Klein-Hitpass *et al*., 1990). Mifepristone can also display some agonist effects on its target organ depending on hormonal status (Spitz *et al*., 1996). Interestingly, mifepristone seems to have an agonist effect in the Fallopian tube, in contrast to
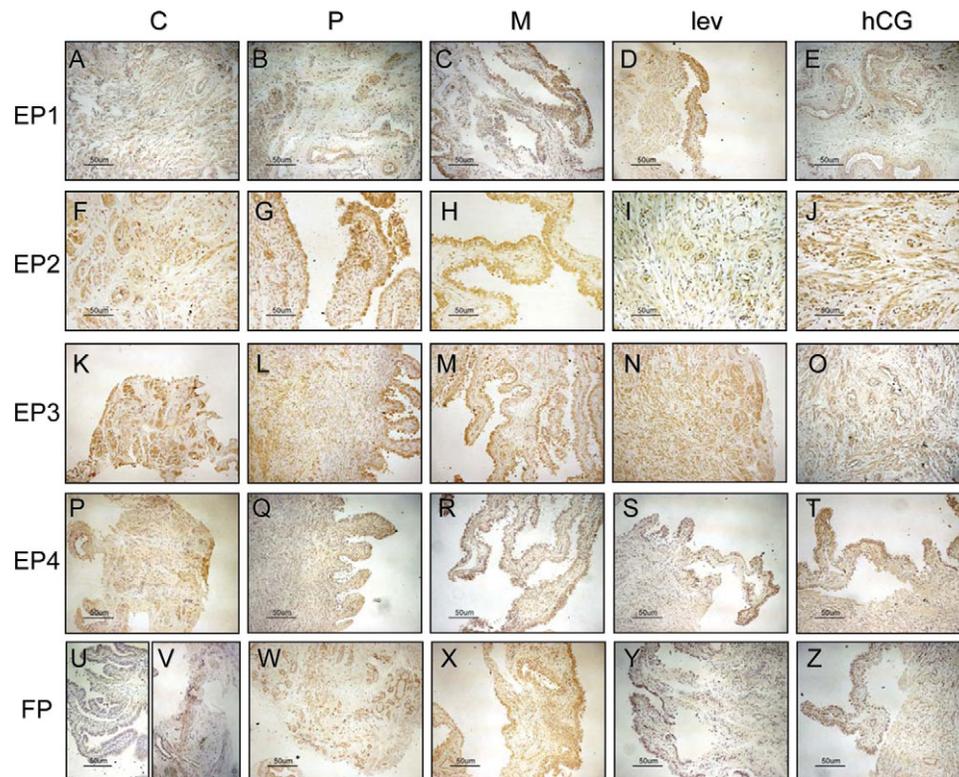
Figure 3: Examples of immunostaining for EP1–4 and FP in the Fallopian tube after different *in vitro* culture conditions: (**A**) EP1 control without treatment (**C**). (**B**) EP1 after P treatment (**P**). (**C**) EP1 after mifepristone treatment (**M**). (**D**) EP1 after levonorgestrel treatment (lev). (**E**) EP1 after hCG treatment (hCG). (**F**) EP2, (**C**). (**G**) EP2, after (**P**). (**H**) EP2 after (**M**). (**I**) EP2, after lev. (**J**) EP2, after hCG. (**K**) EP3, (**C**). (**L**) EP3, after (**P**). (**M**) EP3, after (**M**). (N) EP3, after lev. (**O**) EP3, after hCG. (**P**) EP4, (**C**). (**Q**) EP4, after (**P**). (**R**) EP4, after (**M**). (**S**) Ep4, after lev. (**T**) EP4, after hCG. (**U**) Negative control. (**V**) FP, (**C**). (**W**) FP, after (**P**). (**X**) FP, after (**M**). (**Y**) FP, after lev. (**Z**) FP, after hCG.

the antagonistic effect shown in the uterine myometrium (Bygdeman and Swahn, 1985).

In the present study, mifepristone seemed to act as an agonist, resulting in an inhibitory effect on contractility in the longitudinal muscular layer to a slightly lower degree than progesterone.

The human Fallopian tube expresses the receptors for PGE_1, PGE_2 (EP1–4) and $PGF_{2\alpha}$ (FP), which was previously shown using immunohistochemistry (Wanggren *et al.*, 2006). PGE_2 can either induce contraction via EP1 and EP3 or relaxation via EP2 and EP4 (Senior *et al.*, 1993; Negishi *et al.*, 1995). The EP3 receptor generally stimulates smooth muscle contraction (Narumiya *et al.*, 1999). In the porcine uterus, EP3 was shown to mediate a contractile effect on longitudinal muscle, although there was almost no effect on circular muscles (Cao *et al.*, 2002). Human *in vitro* studies have also shown that PGE_2 has different effects on the circular and longitudinal musculature of the Fallopian tube, inhibiting the circular and stimulating the longitudinal musculature (Lindblom *et al.*, 1979). This is in accordance with our findings, which show a dose-dependent increase in AUC, but not in frequency of contractions, induced by PGE_2. Studies in mares have shown that an increased proportion of spermatozoa pass into the Fallopian tube in the presence of PGE (Troedsson *et al.*, 2005). Human *in vivo* studies have shown that PGE_2 can induce relaxation of the Fallopian tubes and at the same time stimulate uterine activity, suggesting that relaxation of the tubal isthmus induced by

seminal PGE_2 can facilitate sperm penetration into the Fallopian tube, in the same way as for PGE_1 (Coutinho and Maia, 1971).

In the Fallopian tube, PGE_1 is known to inhibit muscular contractions (Lindblom *et al.*, 1978). This was confirmed in the present study, where we found a dose-dependent inhibitory effect on frequency and AUC. The seminal fluid is rich in PGE_1 and one of its actions might be to relax the tubal isthmus, allowing sperm to be transported up into the Fallopian tube (Lindblom *et al.*, 1983). Pretreatment with progesterone, mifepristone or both, 20–40 min before PGE_1 treatment, did not significantly change the response to PGE_1. This may be explained by the relatively short-time elapsing between pretreatment and treatment. Earlier studies on human myometrium have revealed an increased sensitivity to PG at 24 h with a maximum effect on uterine contractions after 36–48 h (Bygdeman and Swahn, 1985; Bygdeman *et al.*, 1991).

$PGF_{2\alpha}$ facilitates muscular contractility through binding to its specific receptor FP (Narumiya *et al.*, 1999). FP is involved in the activation of phospholipase C and mobilization of the IP3 pathway. FP induces Ca^{2+} via a G-coupled receptor (Jabbour and Sales, 2004).

Treatment with $PGF_{2\alpha}$ resulted in an increase in muscular contractility, which is in accordance with earlier *in vitro* studies (Lindblom *et al.*, 1978). Mifepristone increases PR expression in the Fallopian tube (Sun *et al.*, 2003) in contrast

to PGF$_{2\alpha}$ and PGE$_2$ receptors, which diminish after mifepristone treatment (Wanggren *et al.*, 2006).

Treatment with progesterone or mifepristone prior to PGF$_{2\alpha}$ did not significantly influence the frequency of contractions, but mifepristone and progesterone in combination significantly reduced the AUC. It can be suggested that one function for PGF$_{2\alpha}$ in the Fallopian tube is to regulate the isthmic sphincter, and thereby retain the pre-embryo for ∼3 days. After relaxation of the sphincter by progesterone action, the pre-embryo is transported into the uterine cavity (Spilman and Harper, 1975; Croxatto *et al.*, 1978).

Combined oral contraceptives have been shown to decrease muscular activity in the Fallopian tube (Lindblom *et al.*, 1980). The present *in vitro* study showed that treatment with levonorgestrel markedly inhibited muscular contractions. This could be an additional contraceptive function of levonorgestrel as it may affect the transport of the pre-embryo through the Fallopian tube. The tubal explants study also showed that levonorgestrel caused a radical rise in receptor expression, especially for EP1, EP2 and EP3, but also for EP4 and FP. This effect might be of importance for the contraceptive function of levonorgestrel and might also explain the elevated risk of tubal pregnancy following levonorgestrel treatment (Sheffer-Mimouni *et al.*, 2003).

In the present study, hCG resulted in an inhibition of muscular activity. Measurable amounts of hCG produced by the pre-embryo can be detected in culture medium from the blastocyst stage (Fishel *et al.*, 1984; Woodward *et al.*, 1993, 1994). Secreted hCG may act through the hCG receptors in the Fallopian tube (Lei *et al.*, 1993) resulting in decreased contraction of the circular musculature. This is probably not a primary function but may serve as an additional backup system. hCG might also act through up-regulation of COX-2 expression and subsequent increase in PGE$_2$ (Han *et al.*, 1996), which can lead to relaxation of the circular muscle layer of the ampullary-isthmic part of the Fallopian tube (Lindblom *et al.*, 1978), which will allow the pre-embryo to be transported by cilia down through the Fallopian tube into the uterine cavity (Halbert *et al.*, 1989). Injection of equine CG up-regulates PRs in mouse oviducts. Endogenous progesterone might have a physiological function by regulating relaxation of the isthmic sphincter.

OT administration resulted in a short contractile response followed by a pronounced inhibition of muscular activity. OT has previously been shown to relax the muscles in the human Fallopian tube through a specific effect on OT receptors (Jankovic *et al.*, 2001). This is in agreement with the observations in our study. OT could, through simultaneous relaxation of the isthmic part of the Fallopian tube and contractions in the uterus (Wildt *et al.*, 1998), be of importance for rapid transport of sperm from the vagina to the Fallopian tube (Kunz *et al.*, 2007).

Fallopian tube explant studies showed a trend for an increase in the mRNA expression of PG receptors after all the tested treatments in comparison to the control. This was most pronounced after levonorgestrel treatment which was also, to some extent, seen on the protein level. This suggests that progestagens not only have an immediate effect on Fallopian tube

activity, but also have an indirect effect through regulation of PG receptor quantity (Shao *et al.*, 2006). Mifepristone had the same effect as progesterone on the relative expression of PG receptors.

PGs, progesterone, hCG and OT seem to have a role to play in the transport of gametes and pre-embryos in the Fallopian tube. Knowledge of the mechanisms of action for PGs, progestagens, hCG and OT is of importance in understanding the effects of different drugs, such as non-steroidal anti-inflammatory drugs, progestins, antiprogestins and gonadotrophins on fertility. This can be valuable in future improvements in IVF treatment or in designing new contraceptives.

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