Contractile reactivity of human myometrium in isolated non-pregnant uteri

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BACKGROUND: Perfusion of the isolated uterus has been shown to be a feasible experimental system for studies of the human endometrium and myometrium. Utilizing our established experimental perfusion model we perfused 20 uteri for 27 h and investigated the contractile reactivity of the myometrium in response to 17β-estradiol (E2) and oxytocin (OT). METHODS: Uteri of group A (n = 4) were stimulated with OT; group B (n = 4) was treated continuously with E2; group C (n = 4) received both E2 and OT for 27 h; group D (n = 4) was perfused for 27 h with E2 with the addition of OT for the last 3 h of the experiment; group E (n = 4) as control group remained without any treatment. The pressure and duration of uterine contractions were recorded during the entire perfusion period using intramural and endoluminal pressure catheters. RESULTS: Compared to the other treatment groups and controls, the most effective myometrial activity was achieved in group D during the OT stimulation period. No relevant myometrial activity was detected in the control group. CONCLUSIONS: Continuous E2 treatment, with the addition of OT for the last 3 h of the 27 h perfusion period, led to the most pronounced uterotonic effects in the presented experimental condition.

Key words: human non-pregnant uterus/myometrial contractility/oxytocin/perfusion of the isolated uterus

Introduction

Myometrial contractions of the human non-pregnant uterus are assumed to be receptor-mediated via the oxytocin receptor (OTR) which can be modulated by steroid hormones and oxytocin (OT) respectively (Bulletti et al., 1986, 1993; Maggi et al., 1992; Kimura, 1998; Tahara et al., 2000; Richter et al., 2003, 2004). Based on these observations, some authors postulate that idiopathic pelvic complaints—one of the most frequent clinical symptoms of the non-pregnant, menstruating woman—are causally related to hypercontractility of the non-pregnant myometrium (Ekström et al., 1992; Åkerlund et al., 1995; Noe et al., 1999; Åkerlund, 2002). Furthermore, uterine hyperactivity may contribute to the control of other important clinical pathologies such as embryo implantation failure, abortion and preterm labour (Fuchs et al., 1998; Goldenberg, 2002; Gimpl and Fahrenholz, 2001; Goldenberg et al., 2000). For further understanding of these clinical disorders, several investigators have pointed out the importance of in vivo studies to examine the related physiological and pathophysiological conditions in the non-pregnant and pregnant human uterus.

Perfusion of the isolated non-pregnant human uterus simulating physiological conditions, first described by Bulletti et al. (1986), has been shown to be a promising experimental system to investigate myometrial activities stimulated by ovarian hormones (Bulletti et al., 1993; Richter et al., 2000). In particular, previous results showed that the OTR expression of myometrial cells can be affected by stimulation with 17β-estradiol (E2) and OT not only in the pregnant uterus, but also in the non-pregnant uterus (Richter et al., 2003, 2004). In the present study we addressed the question as to what degree uterine contractions can be induced by treatment with different combinations of E2 and OT.

Materials and methods

Surgical specimens and perfusion procedure

Twenty uteri in the proliferative phase of the menstrual cycle were obtained by standard abdominal or vaginal hysterectomy for benign conditions. Oral contraceptives or hormone therapy were discontinued 6–8 weeks prior to the operation, and hormone serum levels for each patient were evaluated on the day of operation for the assessment of E2, progesterone, LH and FSH. Ethical committee approval for the study protocol and written informed consent of the patients was obtained prior to the investigation.

The pre-operative selection of the patients, cannulation of both uterine arteries, bilateral flush perfusion followed by the original perfusion procedure were performed identically as previously described in detail (Bulletti et al., 1986, 1993; Richter et al., 2000, 2003, 2004). Appropriate oxygenation of the perfusion medium (modified heparinized Krebs–Henseleit bicarbonate buffer) was monitored by taking arterial and venous samples of perfusate every 60 min with syringes for measurements of pH, partial pressure of oxygen (pO2),
partial pressure of carbon dioxide (pCO₂), lactate, lactate dehydrogenase (LDH) and creatine kinase (CK). These samples were analysed as previously described (Richter et al., 2000, 2003). Organ weights were always measured immediately before and after perfusion.

To investigate the myometrial reactivity, i.e. amplitudes and duration of uterine contractions, to OT (group A) or E₂ (group B) alone and to a combined treatment with E₂ and OT (group C, group D), we carried out the following study. While the entire experiment was running, all specimens were perfused for 27 h with the modified heparinized Krebs–Henseleit bicarbonate buffer (Richter et al., 2000); uteri of group A received only OT stimulation during the whole experimental period (n = 4) (Figure 1a); uteri of group B were treated only with E₂ (1.0 ng/min) throughout the perfusion period (n = 4) (Figure 1b); uteri of group C were perfused with E₂ (1.0 ng/min) and with OT throughout the perfusion period (n = 4) (Figure 1c); uteri of group D were stimulated by E₂ perfusion (1.0 ng/min) throughout the experimental period and with OT perfusion for the last 3 h of the experiment (n = 4) (Figure 1d); the control group (group E) remained without any treatment (n = 4) (Figure 1e).

Based on data of oxytocin doses for induction and augmentation of labour (Müller et al., 1992) and the previous observations of the dynamics of the oxytocin receptor (OTR) under E₂ and OT stimulation (Richter et al., 2003, 2004) OT was added beginning with 8 mIU/min and increased by doubling the previous concentration every hour. OT stimulation was stopped when relevant uterine contractions occurred or the recommended maximum dose of 30 mIU/min was reached. All perfusion and monitoring proceedings were performed as previously described (Richter et al., 1998, 2000, 2003, 2004).

**Signal acquisition of uterine activity**

For the examination of the uterine pressure profile, two catheter systems and a digital data acquisition system were used. First, usual 20G cannulas (Braun, Melsungen, Germany), prepared with three side-opening perforations of 1.5 mm in diameter and then covered with a 0.5 mm silicon rubber slice and filled up bubble-free with distilled water, were inserted in the median line of the uterus 1.5 cm into the fundus, corpus and the isthmus uteri of the uterine wall. The second catheter system consisted of a microtip catheter with two measuring sensors as usually used for urodynamic examinations (Raumedic catheter, Rehau, Germany), which was also filled up bubble-free with distilled water and then inserted into the uterine cavity.

All catheters were connected via silicone rubber lines (diameter: 1.5 mm) to the digital data acquisition system DASYLab 5.0 (Data-log, Mönchengladbach, Germany) which consisted of an analogue–digital transducer, a high speed 16-Bit analogue–digital multifunction PCI-card (PCI-9118HR) and the data acquisition software.

The intramural and the intraluminal pressure as well as the perfusion flow and pressure rates monitored by the roller perfusion pumps (Storz, Tuttingen, Germany) were all measured in mmHg and continuously recorded during the entire experimental period.

**Statistical analysis**

All biomathematical calculations were performed using the Stat View 5.0 statistical software (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA) and the Sigma Plot 2000 graphic software (SPSS, Chicago, IL, USA). The recorded data were statistically analysed considering each 5, 15 and 30 min time-interval separately and demonstrated as mean values ± SEM. Biochemical perfusion parameters were statistically evaluated using the Kruskal–Wallis one-way analysis of variance for non-parametric data as previously described (Richter et al., 2000).

The degree and duration of intramural and intraluminal pressure were analysed by analysis of variance (ANOVA) followed by Fisher’s protected least significance difference (PLSD) test and paired t-tests. P < 0.05 was considered significant.

**Results**

**Myometrial activity**

The myometrial activity was measured continuously using the described intramural and endoluminal catheters. For all groups, analysis of the intramural and endoluminal pressure showed similar values for the amplitude and duration of the uterine contractions. The values of the duration of contractions showed significant differences between fundus, corpus and isthmus uteri. Data for this parameter are demonstrated as pooled data with mean values ± SEM.

**Group A**

In group A, uteri (n = 4) were treated with OT during the entire experimental period (Figure 1a). The increase of the OT dose caused a significant increase of the amplitude of contractions for all localizations compared to the control uteri (n = 4) in the first 5 h of perfusion (P < 0.0001) (Figure 2a). Considering all data of group A, the maximum pressure values were detected in this period with significant differences between fundus, corpus and isthmus uteri (P < 0.0001) (Figure 2a). After a significant decrease of contractions in the period from 5 to 9 h of perfusion (P < 0.0001 for fundus, corpus and isthmus uteri) no significant effects were seen. The calculated mean values ± SEM are demonstrated in Figure 2a. The mean of the pressure was calculated using the Kruskal–Wallis one-way analysis of variance for non-parametric data as previously described (Richter et al., 2000).
relevant changes could be measured in the further course of the experiment (Figure 2a). Comparing contractions of fundus, corpus and isthmus uteri with each other, the difference in the first 9 h of perfusion was significant ($P < 0.0001$). Further observation during the continuing experiment showed significance of fundus versus corpus ($P < 0.01$), fundus versus isthmus uteri ($P < 0.0001$), corpus versus isthmus uteri ($P < 0.01$), corpus versus controls ($P < 0.0001$) and isthmus uteri versus controls ($P < 0.0001$) (Figure 2a).

Regarding the data for the duration of uterine contractions, significant differences were reached for fundus versus isthmus uteri ($P < 0.0001$), fundus versus controls ($P < 0.0001$), corpus versus isthmus uteri ($P < 0.01$), corpus versus controls ($P < 0.0001$) and isthmus uteri versus controls ($P < 0.0001$) (Figure 2a).

Figure 2. (a) The stimulation with OT only led to significant changes in the first hours of the experiment followed by a relative uterine quiescence in the further course of perfusion. (b) The significant increase of uterine contractions caused by $E_2$ treatment occurred in the first 3 h and is followed by a pronounced undulation of fundal and corporal activity throughout the continuing experiment. (c) The exposure to $E_2$ and OT achieved maximum myometrial contractility in the first 5 h of perfusion with undulant uterine activity throughout the further course of perfusion. (d) The most pronounced and significant changes in group D were detected during the OT stimulation in the last 3 h of perfusion. (e) Uterine contractions measured after the onset of oxytocin administration at 24 h of perfusion time. Both endoluminal and intramural sensors detect a significant increase of myometrial activity already in the first 30 min of oxytocin stimulation. (f) Uterine contractions measured in the last hour of the experiment. Compared to the onset of oxytocin stimulation the unaltered dose of 8 mIU/min has led to a further distinct increase in uterine activity detected by both endoluminal and intramural catheters. (g) Fundal activities of the respective groups compared to each other.
uteri ($P < 0.05$), fundus versus controls ($P < 0.01$) and corpus versus controls ($P < 0.05$) (Figure 3).

**Group B**
Comparing all localizations to each other, including the control group, the stimulation of uteri of group B ($n = 4$) with $E_2$ (1.0 ng/min) throughout the perfusion period (Figure 1b) caused significant changes of the myometrial activity ($P < 0.0001$) (Figure 2b). The most relevant increase was detected in the first 3 h of perfusion followed by a pronounced undulation of the contraction activity during the further treatment with $E_2$ (Figure 2b). For the duration of contractions, significant differences were measured as follows: fundus versus corpus ($P < 0.05$), fundus versus isthmus uteri ($P < 0.01$), fundus versus controls ($P < 0.001$), corpus versus isthmus uteri ($P < 0.05$), corpus versus controls ($P < 0.01$) and isthmus uteri versus controls ($P < 0.05$) (Figure 3).

**Group C**
Uteri of group C ($n = 4$) received OT and $E_2$ (1.0 ng/min) for 27 h (Figure 1c). For OT, it was necessary to apply the maximum dose after 4 h of perfusion. Under these conditions

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*Figure 2. Continued*
maximum increase of contractions was detected for all localizations in the first 5 h of stimulation ($P < 0.0001$) (Figure 2c). In the following 3 h of perfusion fundal activity decreased significantly ($P < 0.0001$) and remained stable throughout the continuing experiment. The contractile activity of the corpus was characterized by a distinct undulation of the measured contractions ($P < 0.0001$) (Figure 2c). The activity of the isthmus uteri after 5 h of perfusion showed significant changes ($P < 0.01$). The measurement of the duration of contractions showed the following results: fundus versus corpus ($P < 0.01$), fundus versus isthmus uteri ($P < 0.001$), fundus versus controls ($P < 0.0001$), corpus versus isthmus uteri ($P < 0.01$), corpus versus controls ($P < 0.0001$), isthmus uteri versus controls ($P < 0.001$) (Figure 3).

**Group D**

The uterine activity of group D ($n = 4$) was modulated by stimulation with E$_2$ (1.0 ng/min) for 27 h and OT in the last 3 h of the experiment (Figure 1d). In this case the initial dose of 8 mIU/min was sufficient to achieve a steadily increasing uterine contraction within all localizations throughout the OT treatment period ($P < 0.0001$) (Figure 2d–f). In the first 24 h of perfusion the sole E$_2$ stimulation caused changes in the myometrial activity similar to the analogous treatment of group B ($P < 0.0001$) concerning fundus, corpus and isthmus uteri compared to each other (Figure 2d). Concerning the duration of contractions the following were significant: fundus versus corpus ($P < 0.001$), fundus versus isthmus uteri ($P < 0.0001$), fundus versus controls ($P < 0.0001$), corpus versus isthmus...
uteri \((P < 0.001)\), corpus versus controls \((P < 0.0001)\), isthmus uteri versus controls \((P < 0.001)\).

Comparing all groups the maximum degree of contractions can be achieved in the fundus uteri independent of the course of stimulation (Figure 2a–e). Yet, the most pronounced changes occur in those groups with a combined stimulation of E\(_2\) and OT (Figure 2e).

### Perfusions and biochemical evaluations

All uteri were removed from pre-menopausal women in the proliferative phase of the cycle. The median age of the patients was 38 years (range: 34–43 years). In seven cases a contraceptive or hormone treatment with various estrogen–gestagen combinations was discontinued 6–8 weeks prior to the operation. No other patients had any hormonal therapy. Hormone serum levels were given as median (range) levels as follows: estrogen: 112.8 (85.4–151.2) pg/ml; progesterone: 1.04 (0.73–1.38) ng/ml; LH: 7.2 (5.4–8.1) IU/l; FSH: 4.9 (3.7–5.8) IU/l. Similar to our previous results (Richter et al., 1998, 2000, 2003, 2004) all hypoxia and cytolysis parameters (pH, pO\(_2\), pCO\(_2\), lactate, LDH, CK) remained stable throughout the entire perfusion period as a sign of physiological oxygen consumption of the perfused organ. All perfusions were maintained in physiological ranges with constant flow rates of 15–35 ml/min through each artery and pressure rates from 60 to 140 mmHg (data not shown). Organ weights did not show significant differences within the respective group at \(t = 0\) h versus \(t = 27\) h (cumulative mean
organ weights measured in grams ± SD); group A: 92.3 ± 9.3 versus 96.3 ± 7.4; group B: 90.5 ± 15.9 versus 94.0 ± 12.1; group C: 103.3 ± 21.7 versus 106.5 ± 26.6; group D: 84.8 ± 8.7 versus 88.5 ± 10.9; group E (control): 84.3 ± 7.8 versus 86.0 ± 5.9.

Discussion
This study presents our results for the uterine reactivity to uterotonic drugs obtained by utilizing our established experimental system of the perfusion of the isolated human uterus (Richter et al., 1998, 2000).

There are numerous experimental perfusion models reporting on uterine contractions under various conditions for various purposes. On one hand there are in vitro models such as animal models of different species (Engstrom et al., 1999; Ahn et al., 2004; Ocal et al., 2004; Raynal and Houdeau, 2004; Tsatsaris et al., 2004; Mota-Rojas et al., 2005). On the other hand there are models utilizing perfusion systems of
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separated myometrial stripes obtained from the uterine incision during Caesarean section at different stages of pregnancy (Kawarabayashi et al., 1990; Pierzynski et al., 2004; Young and Zhang, 2004; Hurd et al., 2005). There exist several in vivo studies in humans for questions on the field of uterine hyperperistalsis in connection with dysmenorrhoea and endometriosis (Åkerlund et al., 1995; Åkerlund, 2002; Bossmar et al., 1995; Ijland et al., 1998; Kunz et al., 1998; Leyendecker et al., 1996, 2004; Kunz and Leyendecker, 2002; de Gestel et al., 2003). However, experimental models simulating physiological conditions for understanding the physiology and pathophysiology of uterine contractions and contractile disorders are important.

Previous investigations emphasized the feasibility of the perfusion of the isolated human uterus for various purposes avoiding the risks of in vivo testing for the patient (Bulletti et al., 1986, 1987, 1988a,b, 1993; Richter et al., 2000, 2003, 2004). The original model of the perfusion of the isolated uterus was first described by Bulletti et al. (1986). Our further developed perfusion system—as performed in the present study—has been validated biologically by extensive biochemical, light- and electron-microscopic examinations (Richter et al., 2000). Both uterine perfusion models have been confirmed to be a new scientific approach, especially for mid-term and long-term perfusion experiments (Bulletti et al., 1987, 1988a,b; Richter et al., 1998, 2000, 2003, 2004). However, the scientific information resulting from a perfused uterus represents the electrical and mechanical activities of all tissues and cells of the organ as they occur in vivo, indicating proper nutrition and oxygenation of the perfused uterus (Bulletti et al., 1993; Richter et al., 2000, 2003, 2004).

The analysis of uterine contractility and its regulation may contribute to the investigation of important clinical pathologies such as dysmenorrhoea, failure of sperm and/or oocyte transportation, embryo implantation failure, spontaneous abortion and preterm labour (Ekström et al., 1992; Starbuck et al., 1998; Goldenberg et al., 2000; Åkerlund, 2002). Previous investigations have suspected that such hypercontractility of the non-pregnant uterine myometrium is mediated through myometrial receptors (Ekström et al., 1992; Åkerlund, 1994, 2002; Åkerlund et al., 1995). In particular, these results in both pregnant and non-pregnant myometrium have shown that there are good correlations between myometrial OTR density and contractile effects. It was assumed that in the non-pregnant myometrium, oxytocin acts specifically on its own receptor (Ekström et al., 1992; Åkerlund et al., 1995; Bossmar et al., 1995). These authors found that oxytocin seemed to be less important for the aetiology of uterine hyperactivity of dysmenorrhoea than vasopressin. In this context various data concerning receptor-specific antagonists for hypercontractility of the non-pregnant and pregnant uterus have been available hitherto (Åkerlund, 2004). Contractions of both the non-pregnant and pregnant uterus can undoubtedly also be a result of stimulation by endogenous prostaglandins.

Its inhibitors play a central role in the therapeutic principles of dysmenorrhoea, and, occasionally also, because of its side-effects for the unborn child, in preterm labour. Previous results showed that oxytocin treatment of estrogen-primed non-pregnant rats down-regulated uterine contractile responsiveness to prostaglandin F2α, leaving mRNA values of the prostaglandin F2α receptor unchanged (Engstrom et al., 2000). Furthermore, the contractile in vivo response remained unaltered by receptor-specific antagonism with atosiban (Engstrom et al., 2000). Investigations on cyclic and early pregnant swines showed that the prostaglandin F2α release by oxytocin stimulation occurs mainly in the late luteal phase whereas responsiveness to oxytocin was suppressed to maintain corpus luteum function during early pregnancy (Carnahan et al., 1996). We did not address this particular question in the present study but the role of endogenous prostaglandins in our experimental condition can be further investigated.

Our team have demonstrated previously that the dynamics of the oxytocin receptor (OTR) expression can be modulated by stimulation with E2 and OT, not only in the pregnant but also in the non-pregnant human uterus (Richter et al., 2003, 2004). Based on these observations the data of the present study confirm that the receptor-mediated reactivity of the non-pregnant myometrium can be modulated by E2 or OT treatment or by different combinations of both.

Regarding the results of group A (only OT treatment), the increase in myometrial activity during the first hours could only be achieved by increasing the OT dose given. Our results for the density of myometrial OTR of the non-pregnant myometrium (Richter et al., 2003, 2004) may explain on the one hand this initial uterine reactivity to OT and on the other hand the significantly lower extent and duration of the contractions compared to group C (E2 + OT for 27 h) and group D (E2 for 27 h + OT in the last 3 h of perfusion). Once the maximum OT dose was reached, no further increase but rather a decrease of contractile activity occurred. These changes might be explained by previous observations which have shown that long-term OT stimulation of human non-pregnant myometrial cells for up to 48 h led to OTR mRNA down-regulation including transcriptional suppression and destabilization of mRNA by RNA-binding proteins while the total amount of receptor protein seemed to be unaffected (Phaneuf et al., 1997, 2000). Results of group B (E2 for 27 h) confirm previous data that stimulation with high-dose E2 increases myometrial activity (Bulletti et al., 1993). Similar to group A (OT for 27 h), contractions rose significantly in the first hours of perfusion (Figure 2b). But in contrast to group A, no relevant decrease, rather a slight increase without significance, was measured in the further course of perfusion (Figure 2b and e). Furthermore, myometrial activity caused by E2 seems to be more effective, as degree and duration of contractions reach significant differences compared to group A (Figures 2e and 3).

The effect of stimulation with E2 and OT for 27 h (group C) is demonstrated in Figure 2c and e. Similar to group A (OT) the significant increase of contractions in all three localizations in the first 5 h of the treatment is followed by a relevant decrease in the following 3 h of perfusion concerning fundus and corpus (Figure 2c). While further stimulation did not affect the activity of fundus and isthmus uteri, significant differences of the amplitude of the contractions occurred in the corpus (Figure 2c). The course of the changes of group C (E2 and
OT for 27 h), which on a significantly higher level are very similar to both the changes in group A (OT for 27 h) and group B (E2 for 27 h) (Figure 2c and e), may be explained by our previous observations that the myometrial OTR expression as well as the OTR sensitivity to OT are significantly influenced by E2 and OT stimulation respectively (Richter et al., 2003, 2004).

Regarding the results of group D (E2 for 27 h and OT for the last 3 h of perfusion, Figure 1d), the uterine activity to a large degree reflects our data for the expression of the myometrial OTR under analogous experimental conditions (Figure 2d) (Richter et al., 2003, 2004). In the first 24 h of perfusion the E2 stimulation caused changes in contractions analogous to group B (E2 for 27 h) (Figure 2b and d). Additional treatment with OT starting from 24 h of the experiment led to a pronounced increase of both the amplitude and duration of uterine contractions throughout the further course of perfusion with significant differences from the other groups (Figure 2d and 3).

Furthermore, considering all data and comparing all groups with each other, the most effective contractions resulted from the fundal activity (Figure 2a–e). On closer observation the treatment with E2 and OT (group C and D) caused the most distinct uterine contractions compared to stimulation with E2 or only OT (group A and B) (Figure 2e). These results could be explained by our previous observations which demonstrate a significant rise in the myometrial OTR by E2 and OT stimulation and showed a significantly increasing density of myometrial OTR from the isthmus uteri to the fundus uteri (Richter et al., 2003, 2004).

In conclusion, the present data reveal that the uterine activity of an isolated, perfused, non-pregnant human uterus can be affected by stimulation with E2 and/or OT. Treatment courses using different combinations of E2 and OT in most of cases caused significant myometrial contractions,signifying the vitality of the myometrial tissue throughout the experimental period.

With respect to our data on the influence of E2 and OT stimulation on the myometrial OTR expression utilizing the equivalent experimental system (Richter et al., 2003, 2004) and the results of the present study, the perfusion of the isolated non-pregnant human uterus has been confirmed to be a suitable experimental system for investigations of the physiology of myometrial contractions and might be an ideal model to study the mechanism of action of uterotonics and tocolytic drugs.

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