Endometrial expression of vasopressin, oxytocin and their receptors in patients with primary dysmenorrhoea and healthy volunteers at ovulation

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Abstract

Objective: To investigate gene expressions for neurohypophyseal and ovarian hormones as well as their receptors in the endometrium of women with primary dysmenorrhoea and healthy subjects at ovulation.

Study design: A group of eight women with moderate to severe dysmenorrhoea and eight healthy subjects were compared in parallel between 18 and 35 years of age, regularly menstruating, non-overweight and nulliparous. The study was performed at The Department of Obstetrics and Gynecology, University Hospital of Lund, Sweden. Endometrial biopsies were taken around the time of ovulation, which was determined by repeated ultrasound examinations. Receptor and gene expressions for oxytocin and vasopressin in the tissue were measured.

Results: The gene expression for oxytocin receptor was significantly lower in dysmenorrhoic than in healthy women, in median 1.21 and 3.44 oxytocin-receptor/actin, respectively (p = 0.048). The expressions for oxytocin peptide, vasopressin V1a receptor, oestrogen receptor α, β and progesterone receptor did not differ between the two groups. Expression of vasopressin peptide was not detectable.

Conclusion: A lower oxytocin receptor gene expression at mid-cycle could be involved in the aetiology of primary dysmenorrhoea. However, the importance of a paracrine effect of oxytocin and its receptor at ovulation warrants further investigation.

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Keywords: Vasopressin; Oxytocin; Receptors

1. Introduction

Vasopressin may play a causative role in the increased myometrial activity and reduced uterine blood flow of primary dysmenorrhoea, since at early menstruation women with primary dysmenorrhoea have increased plasma concentration of the hormone [1–3]. Furthermore, administration of this peptide stimulates uterine activity and reduces blood flow in the uterus [4]. Involvement of vasopressin and oxytocin in primary dysmenorrhoea is also supported by the therapeutic effect of two substances that inhibit the uterine effect of these peptides, atosiban and SR 49059 [5,6]. A further observation supporting the involvement of the two peptides in primary dysmenorrhoea is that the concentration in plasma of oestradiol-17β premenstrually has been shown to be significantly higher in dysmenorrhoeic patients than in healthy women. Gonadotrophins, via ovarian steroids, influence the release of vasopressin and oxytocin. Oestradiol stimulates spontaneous and osmotically-induced output of the two neuropeptides, an effect counteracted by progestagens [7–10].

Vasopressin influences the uterus via its V1a receptors and, to some extent, oxytocin receptors [11]. Oxytocin is believed to have its contractile effect mainly in the pregnant uterus. It stimulates its own receptor, but cross-reacts also with the vasopressin V1a receptor [11]. The two peptides are not only synthesised in the central nervous system, but also seem to have other sources. We recently demonstrated
oxygen gene expression in the endometrium of non-pregnant women, with a peak at mid-cycle [12]. In comparison, the content of vasopressin mRNA in the uterus was sparse (unpublished). In the present study, we compared women with moderate to severe dysmenorrhea and healthy subjects with regard to their endometrial receptor and peptide gene expression for oxytocin and vasopressin as well as that for ovarian hormone receptors at mid-cycle.

2. Methods

A prospective parallel group study was performed in a total of 16 subjects. Eight of them suffered from moderate to severe dysmenorrhea, defined as a history of at least 6 month of painful menstruations necessitating drug therapy. The age in this group ranged from 22 to 34 years (mean 24.7 years). The women were regularly menstruating and non-overweight (BMI < 27). Eight healthy, regularly menstruating, non-overweight women aged 19–21 years (mean 20.4 years) constituted a “control” group. All were nulliparous and used contraceptives, although not an intrauterine device or hormonal contraception within three calendar months prior the start of the study. None had a previous history of inflammatory, circulatory or surgical disease of the abdomen or pelvis, and had normal ultrasound findings of the uterus and the adnexa. All women gave their written consent to participate. The study was conducted according to ICH guideline for good clinical practice and was approved by the local Ethics Committee.

2.1. Experimental procedure

Each subject underwent a screening visit seven days before an expected menstruation at which the medical history was obtained and a physical and gynaecological examination was performed. A urinary pregnancy test was done and blood samples were obtained for routine haematology, clinical chemistry and for screening of hepatitis A, B and HIV.

Endometrial biopsies were taken around the time of ovulation, which was determined by repeated ultrasound examinations starting on cycle days 13–14. The sampling was adjusted on the basis of vaginal ultrasound findings of a follicle diameter of 18 mm or bigger. Tissue aliquot measuring approximately 3 mm × 3 mm × 3 mm were sampled using a uterine curette (Uterine explora curette, Milex, Chicago, USA) and were then directly snap frozen in liquid nitrogen and stored at −80 °C.

2.2. Real time PCR-RNA extractions

Total RNA was extracted from frozen tissue using a TissueLyser® and RNeasy® Mini Kit (QIAGEN GmbH, Germany) according to the manufacturer’s instructions. The quality of RNA was determined by electrophoresis on a 1.0% agarose/6.7% formalin denaturing gel with 1xMOPS buffer. RNA loading mix (GenHunter, Nashville, TN, USA) was used to verify the 18S and 28S RNA bands under UV light. Only samples with visible 18S/28S bands were included for further analyses.

2.3. cDNA synthesis

RNA was reverse-transcribed according to protocols from Applied Biosystems in a 50 μl reaction containing: 0.5 μg toxytocinal RNA, and final concentrations of 1 × TaqMan RT buffer, 5.5 mmol/L MgCl2, 500 μmol/L dNTP, 2.5 μmol/L random hexamers, 0.4 IU/μl RNase inhibitor, and 1.25 IU/μl multiscribe reverse transcriptase. The reaction mixture was incubated at 25 °C for 10 min, at 48 °C for 30 min and then 5 min of inactivation at 95 °C. The samples were stored at −20 °C until further use.

2.4. Real time PCR amplification

Gene transcripts were quantified using real time PCR on ABI PRISM® 7000 sequence detection system (Applied Biosystems). Primers and probes were ordered from assays on-Demand™ (Applied Biosystems). Each primer pair was located on different axons of the investigated gene in order to avoid genomic DNA contamination. Oligonucleotide probes were labelled with fluorogenic dye, 6-carboxyfluorescein (Fam). PCR reactions were carried out in a 25 μl final volume containing final concentrations: 1× Universal PCR Master Mix (Applied Biosystems), 1× Assaymix (Applied Biosystems), 0.25 μmol/L probe, 0.9 μmol/L of forward and reverse primers, respectively, and 1 μl of 10 ng/μl of a DNA aliquot. The thermal cycling conditions were initiated by UNG activation at 50 °C for 2 min and an initial denaturation at 95 °C for 10 min, then 40 cycles at 95 °C for 15 s, annealing at 60 °C for 1 min. Two negative controls, without template, were included in every amplification. RNA samples were tested for genomic DNA contamination prior to further investigation. For each reaction, duplicate assays were carried out. Transcript of β-actin mRNA, as a housekeeping gene, was quantified to normalize each sample and results are expressed as relative values.

2.5. Statistical analysis

The sample size was determined and based on our previous experience of exploratory studies of uterine gene expressions, rather than formal power calculations. The real time PCR oxytocin- and vasopressin V1a-receptor data are presented as a scatter plot, the Mann–Whitney rank sum test was used to compare the groups, p < 0.05 was considered significant.

3. Results

One of the women in the dysmenorrhoea group and three in the control group did not appear to ovulate and were
therefore excluded from analysis. This left seven participants in the former and five in the latter group. The mean length of menstrual cycles, endometrial thickness and main follicular size at the time of biopsy in the two groups of remaining women were similar (Table 1).

Real time PCR results for oxytocin- and vasopressin V1a-receptor gene expression in the endometrial samples obtained at the time of ovulation are shown in Fig. 1. Expression of the oxytocin-receptor mRNA was significantly lower in the dysmenorrhoea group than in the control group with medians of 1.21 and 3.44 oxytoin-receptor/actin, respectively ($p = 0.0484$). mRNA for vasopressin V1a receptor was expressed in all samples with medians of 0.30 and 0.33 oxytocin-receptor/actin, respectively ($p = 0.5367$). For the peptides, mRNA expression for oxytocin was 0.86 and 1.13 oxytocin/actin in dysmenorrheic and healthy subjects, respectively ($p = 0.4254$). Expression for vasopressin peptide was below the limit of detection in all biopsies.

Expression of mRNA for estrogen receptors $\alpha$ and $\beta$, as well as for progesterone receptor, are shown in Table 2. All results fell within normal range of our laboratory.

### Table 1

Mean values (±S.E.) of cycle length (range in brackets), as well as endometrial thickness and main follicle size at the time of biopsy in dysmenorrhoeic women and healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>Dysmenorrhoic patients ($n = 7$)</th>
<th>Healthy volunteers ($n = 5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle (days)</td>
<td>28.9 (24–36)</td>
<td>29.4 (27–31)</td>
</tr>
<tr>
<td>Endometrium (mm)</td>
<td>12.2 ± 0.9</td>
<td>11.2 ± 1.7</td>
</tr>
<tr>
<td>Follicle size (mm)</td>
<td>22.4 ± 1.5</td>
<td>20.6 ± 1.3</td>
</tr>
</tbody>
</table>

### Table 2

Expression of ovarian hormone receptors in endometrial biopsies obtained at the time of biopsy in dysmenorrhoeic women and healthy volunteers

<table>
<thead>
<tr>
<th>Receptor type</th>
<th>Dysmenorrhoic patients ($n = 7$)</th>
<th>Healthy volunteers ($n = 5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen $\alpha$</td>
<td>59.5 ± 7.0</td>
<td>70.8 ± 19.9</td>
</tr>
<tr>
<td>Estrogen $\beta$</td>
<td>4.0 ± 0.3</td>
<td>5.6 ± 1.8</td>
</tr>
<tr>
<td>Progesterone</td>
<td>19.4 ± 1.2</td>
<td>16.6 ± 4.25</td>
</tr>
</tbody>
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Medians of respective hormone receptor/actin ± S.E. are given and compared.

### 4. Discussion

During this study we looked at gene expression for oxytocin and vasopressin in the endometrium at the time of ovulation. Other researchers have shown an increase in plasma levels of oxytocin and vasopressin during the follicular phase, reaching maxima at this time of the menstrual cycle and decreasing during the luteal phase [10,13], a finding supported in our studies where we observed a higher content of mRNA for oxytocin in glandular cells of the endometrium with maximum level at mid-cycle [12]. These findings are in agreement with previous observations by other groups in which oxytocin and vasopressin V1a receptor genes have been shown to be present in uterine tissue from non-pregnant women [14–19].

A novel finding of the present study was the significantly lower endometrial concentration of oxytocin receptor mRNA in patients with dysmenorrhoea than in healthy women at midcycle. No difference was seen in vasopressin V1a receptor mRNA between the groups. These results are difficult to interpret, since the interaction of oxytocin and vasopressin with their receptors at different parts of the uterus is complicated and not fully understood [19]. However, dysmenorrheic women could have an elevated estradiol level in plasma at the time of ovulation as was found in late luteal phase by our group [3]. The higher concentration of estradiol could then cause an increased secretion to plasma of oxytocin. In fact, we recently found (unpublished) an increased concentration of circulating oxytocin at ovulation in dysmenorrheic women. This increased level of circulating oxytocin could then cause a down-regulation of oxytocin, by decreasing its synthesis. Whether or not the lower oxytocin receptor expression could also cause a higher sensitivity to vasopressin around the onset of menstruation remains to be investigated.

We demonstrated expression of mRNA for estrogen receptors $\alpha$ and $\beta$ as well as for progesterone in the endometrium. These findings are in agreement with previous observations [20]. The concentrations were in ranges as previously described for normal human endometrial tissue with a more pronounced expression of the estrogen receptor $\alpha$ compared to that of $\beta$ type [20]. The concentrations did not differ between dysmenorrheic and healthy women, indicat-
ing that ovarian steroid receptors are of minor importance in the aetiology of primary dysmenorrhoea.

In conclusion, our results point out a more important involvement of oxytocin in the non-pregnant uterus than earlier assumed, particularly in patients with dysmenorrhoea. It is possible that oxytocin is important in sexual function, as suggested on the basis of menstrual cycle-related changes in plasma oxytocin [13]. Other areas where oxytocin could be involved are sperm and egg transport in the uterus.

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References
