Oxytocin Pretreatment Attenuates Oxytocin-induced Contractions in Human Myometrium In Vitro

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ABSTRACT

Background: Oxytocin receptor desensitization has been shown to occur in humans at biomolecular level and in isolated rat myometrium; however, its effect on human myometrial contractility has not been demonstrated. The objective of this in vitro study was to investigate the contractile response of human pregnant myometrium to oxytocin after pretreatment with different concentrations of oxytocin for variable durations.

Methods: Myometrial samples were obtained from 62 women undergoing elective cesarean deliveries under regional anesthesia. The strips were pretreated with oxytocin $10^{-10}$, $10^{-8}$, $10^{-5}$ M or physiological salt solution (control) for 2, 4, 6, or 12h, followed by a dose–response testing with oxytocin $10^{-10}$ to $10^{-5}$ M. Amplitude and frequency of contractions, motility index, and area under the curve during the dose–response period were recorded, analyzed with linear regression models, and compared among groups.

Results: Pretreatment with oxytocin $10^{-5}$ and $10^{-8}$ M significantly reduced motility index (estimate [standard error]: $-0.771$ [0.270] square root units, $P=0.005$ and $-0.697$ [0.293], $P=0.05$, respectively) and area under the curve ($-3.947$ [1.909], $P=0.04$ and $-4.241$ [2.189], $P=0.05$, respectively) compared with control group, whereas pretreatment with oxytocin $10^{-10}$ M did not significantly attenuate contractions. Increase in duration of oxytocin pretreatment from 2 to 12 h significantly decreased amplitude (type 3 generalized estimating equation analysis: chi-square = 14.0; $df=3$; $P=0.003$), motility index (chi-square = 9.3; $df=3$; $P=0.02$), but not the frequency of oxytocin-induced contractions.

Conclusion: Pretreatment with oxytocin decreases oxytocin-induced myometrial contractions in a concentration and time-dependent manner, likely as a function of the oxytocin receptor desensitization phenomenon.

OXYTOCIN is a neurohypophysial hormone that induces uterine contractility and is used for the induction and augmentation of labor as well as to decrease postpartum blood loss. It binds to myometrial oxytocin receptors to induce uterine contractility via activation of phospholipase C and release of inositol 1,4,5-triphosphate, 1,2-diacylglycerol, and intracellular calcium. Oxytocin receptors belong to the family of G-protein–coupled

What We Already Know about This Topic

- Prolonged exposure of rat and human myometrium to oxytocin in vitro leads to oxytocin receptor desensitization. Furthermore, it leads to decreased contractile response to oxytocin in rats, but whether this occurs in human myometrium is not known.

What This Article Tells Us That Is New

- In myometrial strips obtained at elective cesarean delivery from women not receiving oxytocin, in vitro pretreatment with oxytocin at concentrations higher than $10^{-10}$ M reduced the subsequent contractile response to oxytocin.
- These data suggest oxytocin-induced desensitization might explain the higher oxytocin dose required and more bleeding at cesarean delivery in women who have undergone oxytocin-augmented labor.

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receptors, and like other G-protein–coupled receptors undergo rapid molecular desensitization due to homologous stimulation.2 Continuous and prolonged exposure of cultured human myometrial cells to oxytocin in vitro down-regulates oxytocin receptors, and decreases oxytocin receptor messenger RNA (mRNA) expression and oxytocin binding sites, coincident with a loss of myometrial cellular responsiveness.3–6 This desensitization phenomenon has been shown to occur after both in vitro and in vivo exposure of myocytes to oxytocin.5,6

Desensitization of oxytocin receptors may have important clinical implications because oxytocin is the most commonly used uterotonic drug for prophylaxis and treatment of primary postpartum hemorrhage. The prolonged exposure of the uterus to supraphysiologic concentrations of oxytocin (for induction or augmentation of labor) may reduce the immediate postpartum effect of this first-line drug.7–9 In a recent study in pregnant rats, we demonstrated that pretreatment with oxytocin leads to a significant decrease in the in vitro oxytocin-induced myometrial contractions in a concentration-dependent manner, likely as a function of the desensitization phenomenon.10

Although the phenomenon of desensitization has been extensively demonstrated in cultured human myometrial cells,5–6 it has not yet been shown in the isolated human myometrium, and there is no report in the literature using a reliable contractility model for in vitro study of the desensitized human myometrium. We hypothesized that, similar to the observations in human biomolecular and isolated rat myometrial contractility studies, the pretreatment of the human myometrium with oxytocin would cause further decrease in oxytocin-induced myometrial contractility in a concentration and/or time-dependent manner. The objective of this study was to investigate the in vitro contractile response of the human term pregnant myometrium to oxytocin, after pretreatment with different concentrations of oxytocin for variable durations.

Materials and Methods

After approval by the Research Ethics Board at Mount Sinai Hospital, Toronto, Ontario, Canada, the study was conducted in 62 nonlaboring term pregnant women undergoing elective cesarean deliveries (CD). We obtained written informed consent from all women enrolled in the study. Inclusion criteria were women with gestational age 37–41 weeks requiring an elective primary or first repeat CD under spinal anesthesia for any reason, although, most common indications were breech presentation or repeat CD. Exclusion criteria were: previous uterine surgery other than a single previous CD, a requirement for general anesthesia, and any condition predisposing to uterine atony and postpartum hemorrhage, such as abnormal placentation, multiple gestation, preeclampsia, macrosomia, polyhydramnios, uterine fibroids, bleeding diathesis, chorioamnionitis, or a previous history of postpartum bleeding. The study was registered with the ClinicalTrials.gov registry (NCT-00989027).

Reagents

Drug solutions were prepared by serial dilutions in sterile double-distilled water. Oxytocin (lyophilized powder), and all salts and reagents used in the preparation of the physiological salt solution (PSS), and 3-N-morpholino propanesulphonic acid solution, were obtained from Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada. The 3-N-morpholino propanesulfonic acid solution buffer (145 mM NaCl, 4.7 mM KCl, 1.5 mM CaCl2, 1.17 mM MgSO4·7H2O, 1.2 mM NaH2PO4·H2O, 3.0 mM 3-N-morpholino propanesulfonic acid solution, 5.0 mM glucose, and 2.0 mM pyruvate) and PSS (112 mM NaCl, 25 mM NaHCO3, 1 mM KH2PO4, 5 mM KCl, 1.2 mM MgSO4·7H2O, 11.5 mM glucose, and 2.5 mM CaCl2) were prepared in advance and stored at 4–8°C.10–12

Myometrial Strip Isolation and Preparation

The patients were administered spinal anesthesia by the attending anesthesiologist and underwent CD via a Pfannenstiel incision, as per the routine in this hospital. The obstetrician excised a small sliver of myometrium from the upper border of the incision on the lower uterine segment after the delivery of the neonate, just before oxytocin administration. The collected myometrial tissue was immediately immersed in the 3-N-morpholino propanesulfonic acid solution buffer, which was placed on ice for tissue dissection. Four longitudinal myometrial strips (2 × 2 × 10 mm3 each) were isolated and suspended vertically in individual, temperature-controlled organ bath chambers, containing 10 ml of PSS at pH 7.4. Each myometrial strip was clipped between the base of the organ bath chamber and an isometric force transducer (Radnoti four unit tissue-organ bath system, model 159920; Harvard Apparatus Canada, Saint-Laurent, Quebec, Canada). The organ bath solution was continuously aerated with 95% oxygen and 5% carbon dioxide at 37°C.

Contractility Analysis

As described in previous studies,10–12 during each experiment, each isolated myometrial strip was allowed to equilibrate in PSS at 1 g tension for at least 20 min of spontaneous regular contractions. If the spontaneous contractions did not start immediately, the PSS was changed by flushing every 10–15 min until spontaneous contractions were observed. After equilibration, each myometrial strip was exposed to an isomotic 120 mM of potassium chloride solution to induce a contraction that reflected the maximum contractile capacity of the tissue. The potassium chloride solution was then drained from the organ baths and any residual solution was removed by washing it three times with PSS.

Individual strips were then pretreated with oxytocin at 10−10, 10−8, or 10−5 M concentration (experimental group)
or bathed in PSS without oxytocin (control group) for a 2-, 4-, 6-, or 12-h period. After pretreatment, the strips in the control group were given fresh PSS, and all oxytocin-pretreated strips in the experimental groups were washed twice with PSS. All strips were then subjected to a dose–response testing with oxytocin $10^{-10}$ M to $10^{-5}$ M (fig. 1), increased cumulatively in a pattern of one log molar concentration every 10 min. At the end of the dose–response period, the myometrial strips were washed with PSS and exposed to a final isosmotic 120 mM potassium chloride solution to assess the muscle viability. The strips were then removed from the apparatus, manually dried and weighed.

The amplitude and frequency of myometrial contractions were continuously recorded by force displacement transducers connected to a data acquisition system (MP 100 with Acknowledge 3.9.0 software; Biopac System Inc., Goleta, CA). The data sets from samples that failed to contract or those with technical errors were discarded.

**Statistical Analysis**

In each group, the amplitude (g) and frequency (number of contractions in 10 min) of contractions were recorded, and the motility index (amplitude × frequency/10 min; Montevideo units) and the area under the curve (AUC; integral force; gram × minute) were derived to reflect the uterine activity and the strength of contractions, respectively. These parameters measured during the dose–response period were compared among various study groups. We used linear regression models (maximum likelihood estimates for parameter determination) adjusted for repeated measures through a compound symmetry covariance structure. Generalized estimating equations (GEE) were generated, including duration of oxytocin preexposure, oxytocin concentration during preexposure, and oxytocin concentrations during the dose–response period, as independent variables. Outcomes (amplitude, frequency, motility index, and AUC) were square root (SR) transformed (selected on the basis of Akaike information criteria) to adjust for their skewed distribution of both motility index and AUC. The model was adjusted for baseline tone, weight of sample, maximum amplitude after potassium (administered before the pretreatment period), and measures of amplitude, frequency, motility index, and AUC of baseline contractions, during the equilibration period. All these values were added as variables in the multivariable regression model. The statistical analyses were performed using SAS statistical software v9.2 (The SAS Institute, Cary, NC). All hypotheses were tested using two-tailed testing, and a value of $P$ less than 0.05 was considered statistically significant.

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**Fig. 1.** Experimental design. Myometrial samples were exposed to oxytocin $10^{-10}$, $10^{-8}$, or $10^{-5}$ M concentration (experimental groups) or physiological salt solution (PSS; control groups) for either 2, 4, 6, or 12 h and then subjected to increasing concentrations of oxytocin $10^{-10}$ to $10^{-5}$ M. Potassium (K) stimulation was done to assess the muscle viability.
**Results**

Patients were recruited for this study between August 2008 and December 2010. Of 129 women approached for participation in the study, 65 consented, and myometrial samples were obtained from 62 subjects. Each sample was divided into four pieces for four separate experiments, and a total of 163 experiments were conducted successfully (fig. 2). Among the excluded experiments, there was a failure to obtain oxytocin-induced contractions during the dose–response in 0, 6, 4, and 15% of samples in the 2-, 4-, 6-, and 12-h groups, respectively. The clinical characteristics of the patients were similar among the study groups (table 1).

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Duration of Oxytocin Exposure</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>12 h</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>31.6 (4.6)</td>
<td>33.3 (4.2)</td>
<td>33.0 (2.9)</td>
<td>35.4 (5.8)</td>
<td>0.22</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.2 (7.6)</td>
<td>29.1 (4.0)</td>
<td>29.9 (5.1)</td>
<td>28.9 (6.3)</td>
<td>0.27</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>38.7 (0.8)</td>
<td>38.9 (0.9)</td>
<td>38.9 (0.7)</td>
<td>39.1 (0.8)</td>
<td>0.58</td>
</tr>
<tr>
<td>Gravidity</td>
<td>2 (1–3)</td>
<td>2 (2–4)</td>
<td>2 (1–3)</td>
<td>2.5 (1–5)</td>
<td>0.52</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (0–2)</td>
<td>1 (1–2)</td>
<td>1 (0–2)</td>
<td>1 (0–1)</td>
<td>0.13</td>
</tr>
<tr>
<td>Indication for cesarean delivery</td>
<td>Primary (N = 11)</td>
<td>3 (27.3%)</td>
<td>0 (0%)</td>
<td>3 (18.8%)</td>
<td>5 (50%)</td>
</tr>
<tr>
<td></td>
<td>Repeat (N = 37)</td>
<td>8 (72.7%)</td>
<td>11 (100%)</td>
<td>13 (81.2%)</td>
<td>5 (50%)</td>
</tr>
<tr>
<td></td>
<td>Breech presentation (N = 8)</td>
<td>3 (27.3%)</td>
<td>1 (9.1%)</td>
<td>2 (12.5%)</td>
<td>3 (30%)</td>
</tr>
</tbody>
</table>

Values are shown as mean (SD), median (range), or N (%).

BMI = body mass index; N = number of patients (data obtained from these patients was used for final analysis [patients with excluded samples or experiments are not included in the table]).

**Effect of the Duration of Oxytocin Pretreatment on the Dose–Response Curves**

After adjustment for all covariates and baseline contractility parameters, the duration of oxytocin preexposure had significant effect on all the contractility parameters, except frequency. The amplitude, motility index and AUC during the dose–response period decreased with the increased duration of oxytocin preexposure from 2 to 12 h; however, the effect was mainly driven by the 6- and 12-h preexposure groups. Longer preexposure was associated with significantly lower amplitude (type 3 GEE analysis: chi-square = 14.0; df = 3; P = 0.003), motility index (type 3 GEE analysis: chi-square =

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**Fig. 3.** The dose–response curves for the motility index (square-root) of the myometrial strips stimulated with oxytocin after pretreatment with oxytocin $10^{-10}$, $10^{-8}$, or $10^{-5}$ M concentration at each time point (2, 4, 6, and 12 h). The graphs are based on modelized data.
Effect of the Concentration of Oxytocin Pretreatment on the Amplitude and Frequency of Contractions

After adjustment for all covariates and baseline contractility parameters, we found that the amplitude of contractions was significantly lower during the dose–response period with oxytocin pretreatment $10^{-5}$ M ($P = 0.02$; type 3 GEE for all four concentrations: $P = 0.06$), however, there was no statistically significant difference between $10^{-8}$ M ($P = 0.07$) or $10^{-10}$ M ($P = 0.93$) pretreatment groups and the control groups (table 2). The frequency of contractions was significantly lower with oxytocin pretreatment $10^{-5}$ and $10^{-8}$ M ($P = 0.03$ and $P = 0.002$, respectively; type 3 GEE for all four concentrations: $P = 0.02$) as compared with the control groups, however, there was no difference between oxytocin $10^{-10}$ M pretreatment and the control group (table 2). The mean (standard error) values for the amplitude and frequency of contractions in various oxytocin pretreatment groups are shown in table 3.

**Table 2.** Comparison of Contractility Parameters of Various Oxytocin Pretreatment Concentration Groups with the Control Group

<table>
<thead>
<tr>
<th>Pretreatment Concentration</th>
<th>Amplitude (EST (SE))</th>
<th>$P$ Value</th>
<th>Frequency (EST (SE))</th>
<th>$P$ Value</th>
<th>AUC (EST (SE))</th>
<th>$P$ Value</th>
<th>Motility Index (EST (SE))</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-5}$ M</td>
<td>$-0.172$ (0.074)</td>
<td>0.02</td>
<td>$-0.219$ (0.099)</td>
<td>0.03</td>
<td>$-3.947$ (1.909)</td>
<td>0.04</td>
<td>$-0.771$ (0.270)</td>
<td>0.005</td>
</tr>
<tr>
<td>$10^{-8}$ M</td>
<td>$-0.125$ (0.070)</td>
<td>0.07</td>
<td>$-0.319$ (0.103)</td>
<td>0.002</td>
<td>$-4.241$ (2.189)</td>
<td>0.05</td>
<td>$-0.697$ (0.293)</td>
<td>0.02</td>
</tr>
<tr>
<td>$10^{-10}$ M</td>
<td>$-0.005$ (0.066)</td>
<td>0.93</td>
<td>$-0.122$ (0.114)</td>
<td>0.28</td>
<td>$-0.859$ (1.869)</td>
<td>0.65</td>
<td>$-0.263$ (0.282)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Values are expressed in square root units. $P < 0.05$ was considered statistically significant.

AUC = area under the curve; EST = estimate; SE = standard error.
in Table 4. Overall, there was a statistically significant effect of the pretreatment concentration on the motility index during the dose–response period (P = 0.02). The pretreatment concentrations of 10⁻⁵ M (P = 0.005) and 10⁻⁴ M (P = 0.02) were associated with lower motility index than the control group, whereas the 10⁻¹⁰ M concentration did not differ (P = 0.35) from control (Table 2; Fig. 3). For the pretreatment concentration of 10⁻⁵ M, the effect of decreased myometrial contractility versus control was preserved at 2 h (estimate [EST; standard error] = −5.883 [5.081] SR unit; P = 0.002) and 4 h (EST = −10.164 [2.269] SR unit; P < 0.001), but not at 6 h (EST = −2.530 [2.667] SR unit; P = 0.34) or 12 h of preexposure (EST = −5.883 [5.081] SR unit; P = 0.25). The pretreatment with 10⁻⁸ and 10⁻¹⁰ M oxytocin did not have any statistically significant effect on the AUC for any pretreatment duration (Fig. 4).

Effect of Oxytocin Pretreatment on the Slopes of the Dose–Response Curves

Increasing the concentration of oxytocin by 10⁻¹ M increments from 10⁻¹⁰ to 10⁻⁵ M during the dose–response period led to a statistically significant change in the motility index (P < 0.001) as well as the AUC (P < 0.001) in a consistent manner, with an initial increase in the slope until 10⁻⁶ M concentration, which was followed by a plateau. The response was similar for all durations of preexposure (i.e., at 2, 4, 6, and 12 h). There was no difference in the slopes of the dose–response curves for motility index and AUC between oxytocin-pretreated and control groups, or across the various durations of exposure (type 3...
GEE analysis for preexposure time interaction with concentration: $P = 0.09$ for motility index and $P = 0.20$ for AUC). The lack of interaction between the effect of preexposure concentration and duration suggests that these variables are independent of each other (i.e., additive), but are not multiplicative of each other. The representative recordings of myometrial contractions after pretreatment with various concentrations of oxytocin for 2 h are shown in figure 5.

**Discussion**

Our study demonstrates that oxytocin-induced contractile response of the isolated term human myometrium is attenuated by previous oxytocin exposure, and suggests that this response is dependent on both concentration and duration of oxytocin preexposure. Our results reproduce our previous findings in rat myometrium and are, therefore, of practical importance because this effect may be seen with clinically used doses of oxytocin.\(^{10}\) The similarity of the response in both rat and human myometrium is an important validation step in utilizing the rat myometrium model for in vitro myometrial contractility studies.

The most likely explanation for our findings, in keeping with the existing evidence in the literature, is the oxytocin receptor desensitization phenomenon.\(^3\)–\(^6\) Desensitization of receptors is a complex physiologic process, one that has evolved to prevent endogenous hyperstimulation by impairing signal transduction during prolonged receptor activation. The possible mechanism of oxytocin receptor desensitization involves phosphorylation, uncoupling from the G protein and internalization, leading to recycling of the receptor to the cell membrane or degradation by lysosomes.\(^15\) This phenomenon has been shown in a variety of ways at the biomolecular level.

Robinson et al. worked with an in vitro human myocyte culture model and showed that cells preexposed to oxytocin $10^{-8}$ M undergo desensitization in the form of

### Table 4. Motility Index and Area under the Curve of Myometrial Contractions during the Dose–Response Period at Various Concentrations and Durations of Oxytocin Pretreatment

<table>
<thead>
<tr>
<th>Pretreatment Concentration</th>
<th>Oxytocin Dose–Response</th>
<th>Motility Index (MVU)</th>
<th>Area under the Curve (gm*min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^{-10}$ M</td>
<td>$10^{-9}$ M</td>
<td>$10^{-8}$ M</td>
</tr>
<tr>
<td>Control</td>
<td>3.01 (0.66)</td>
<td>6.48 (1.13)</td>
<td>15.02 (2.05)</td>
</tr>
<tr>
<td>Oxytocin $10^{-10}$ M</td>
<td>1.94 (0.41)</td>
<td>4.09 (0.61)</td>
<td>9.60 (1.51)</td>
</tr>
<tr>
<td>Oxytocin $10^{-8}$ M</td>
<td>1.67 (0.43)</td>
<td>3.00 (0.63)</td>
<td>10.10 (2.21)</td>
</tr>
<tr>
<td>Oxytocin $10^{-5}$ M</td>
<td>5.06 (1.19)</td>
<td>4.62 (1.13)</td>
<td>6.24 (1.05)</td>
</tr>
<tr>
<td>Pretreatment time</td>
<td>2 h</td>
<td>5.75 (1.64)</td>
<td>9.11 (2.38)</td>
</tr>
<tr>
<td></td>
<td>4 h</td>
<td>3.26 (0.60)</td>
<td>5.72 (0.75)</td>
</tr>
<tr>
<td></td>
<td>6 h</td>
<td>2.12 (0.33)</td>
<td>3.74 (0.49)</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>1.52 (0.30)</td>
<td>2.46 (0.48)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1,050.82</td>
<td>1,458.31</td>
<td>2,089.80</td>
</tr>
<tr>
<td>Oxytocin $10^{-10}$ M</td>
<td>856.68</td>
<td>1,265.11</td>
<td>1,834.92</td>
</tr>
<tr>
<td>Oxytocin $10^{-8}$ M</td>
<td>799.13</td>
<td>1,091.23</td>
<td>1,994.95</td>
</tr>
<tr>
<td>Oxytocin $10^{-5}$ M</td>
<td>1,307.53</td>
<td>1,217.94</td>
<td>1,409.85</td>
</tr>
<tr>
<td>Pretreatment time</td>
<td>2 h</td>
<td>1,588.04</td>
<td>1,942.65</td>
</tr>
<tr>
<td></td>
<td>4 h</td>
<td>1,163.41</td>
<td>1,592.98</td>
</tr>
<tr>
<td></td>
<td>6 h</td>
<td>881.92</td>
<td>1,118.53</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>525.26</td>
<td>620.05</td>
</tr>
</tbody>
</table>

Values are expressed as mean (standard error). Motility index = amplitude × frequency per 10 min, expressed as MVU. MVU = Montevideo units.
inhibition of oxytocin-induced calcium efflux. This effect is seen within 3 h of oxytocin stimulation, resulting in 50% desensitization at 4.2 h and complete desensitization within 6 h.³

Phaneuf et al.⁵ demonstrated the same phenomenon by reporting a 50-fold down-regulation of oxytocin receptor mRNA in myometrium obtained at emergency CD, when labor had been stimulated with intravenous oxytocin for more than 12 h. The same investigators subsequently demonstrated that in women with spontaneous and induced labors, resulting in CD after more than 10 h of labor, oxytocin receptor mRNA levels decreased by 60 and 300 times, respectively, when compared with nonlaboring women.⁶ They also found a decrease in oxytocin binding related to the increasing duration and dose of oxytocin infusion administered for labor induction.⁶

Previous in vitro studies in rat myometrium demonstrated inhibition of oxytocin-induced myometrial contractions after exposure to oxytocin 10⁻⁸ M for 1 h,¹⁰¹¹ without any obvious dependence on the duration of preexposure. In the human myometrial preparation used in our current study, however, pretreatment with either oxytocin 10⁻⁵ M for at least 2 h, or 10⁻⁸ M for at least 4 h was required for producing such effect. This suggests that with a low concentration of oxytocin preexposure, a longer duration of exposure might produce similar inhibitory effects on myometrial contractility, whereas, with a higher concentration, this effect may be seen earlier. We observed that with increasing concentration of oxytocin pretreatment, both amplitude and frequency of contractions during the dose–response are attenuated, thereby, decreasing the motility index as well as the AUC. However, the decrease in the motility index and

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**Fig. 5.** Representative data showing the contraction tracings. The effects of increasing concentrations of oxytocin (10⁻¹⁰ to 10⁻⁵ M) on the contractions of isolated myometrial strips pretreated with either PSS or 10⁻¹⁰, 10⁻⁸, or 10⁻⁵ M concentration of oxytocin for 2 h are shown. PSS = physiological salt solution.
AUC seen with increasing the duration of preexposure to oxytocin are likely to be predominantly due to the change in amplitude, rather than in frequency of contractions. Our results also suggest that the rat myometrium may be prone to the desensitization effect at lower oxytocin concentrations than in human myometrium, but further studies are warranted to confirm these findings.

The findings of our study may have important clinical implications. Previous studies have demonstrated that women with oxytocin-augmented labors require nine-fold greater doses of oxytocin \( (ED_{90} \ [95\% \ CI] = 2.99 \ [2.32–3.67] \ IU) \), to produce effective uterine contractions during CD as compared with nonlaboring women \( (ED_{90} \ [95\% \ CI] = 0.35 \ [0.18–0.52] \ IU) \). Despite the use of this higher dose of oxytocin, these women bled twice as much as the nonlaboring women \((1,178 \ [716] \ vs. 693 \ [487] \ ml)\). A meta-analysis of 11 randomized clinical trials on the use of oxytocin for labor induction demonstrated that more aggressive or high-dose oxytocin protocols resulted in more episodes of uterine hyperstimulation, lower rate of spontaneous vaginal delivery, and higher incidence of postpartum maternal infection and hemorrhage, compared with low-dose regimens, in which doses were not increased more than every 30 min. Similarly, a recent study demonstrated a higher incidence of severe postpartum hemorrhage secondary to uterine atony when oxytocin was used for labor augmentation in larger doses and for longer durations. These clinical findings are likely to be due to oxytocin receptor desensitization and signal attenuation that have been demonstrated by molecular mechanism and are now confirmed to affect myometrial contractility in vitro.

The concentrations of oxytocin in our study are likely to be on the upper limit of physiological levels during labor \((10^{-10} \ M)\) and/or circulating levels observed with high doses of oxytocin induction protocols \((10^{-8} \ M)\) in humans. Although there is a huge variability of these data in the literature, the plasma levels of oxytocin in laboring women, and those with oxytocin augmentation, have been shown to vary from \(10^{-9}\) to \(10^{-12} \ M\) and the concentrations reached after delivery are likely to be much higher due to the practice of administration of high doses of oxytocin within a short timeframe. In fact, plasma levels of oxytocin have been shown to correlate with the rate of oxytocin infusion rather than with the uterine activity during labor. The rationale for choosing different preexposure durations was based on the time-dependent oxytocin receptor desensitization seen in human myometrium.

The most important limitation of our study is that it was an in vitro preparation. The effect of the same magnitude may be difficult to achieve in in vivo settings. Our experimental model produced consistent results up to 6-h duration, showing increased myometrial inhibitory effect with increasing concentration of oxytocin pretreatment. However, at 12 h, such effect was no longer detectable, which could be related to the limitations of the model itself. We observed a higher failure rate and an overall reduction in myometrial contractility during the dose–response period in the 12-h group, perhaps due to tissue exhaustion and/or deprivation of metabolites in the in vitro settings. Hence we suggest that this experimental model should not be used for pretreatment duration longer than 6 h.

Our findings suggest the need for a reappraisal of current regimens used for prevention and treatment of primary postpartum hemorrhage in women who have received oxytocin augmentation of labor. Further, our study attests to the validity of an in vitro model, demonstrating reduced response to oxytocin after oxytocin pretreatment of the human myometrium. Our model can be used for future in vitro studies on the oxytocin-pretreated human myometrium, to define new strategies to optimize uterine contractility, and reduce complications associated with postpartum hemorrhage.

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