Clinical and Experimental Obstetrics & Gynecology

Original Research

Uterine receptor activation in response to uterotonic agents in late-pregnancy rat myometrium following pretreatment with oxytocin: an *in vitro* study

Li-Mei Liao[1], Jian-Ying Hu[1], Ting-Ting Wang[1], Shao-Qiang Huang[1,*]

[1] *Department of Anaesthesia, Obstetrics & Gynecology Hospital, Fudan University, 200090 Shanghai, China*

*Correspondence: drhuangsq@163.com (Shao-Qiang Huang)

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Background: Long term exposure to oxytocin reduces the ability of myometrium to respond to oxytocin, leading to oxytocin receptor (OXTR) desensitization. In this study we analyzed the response to other uterotonics such as prostaglandin, as well as investigating prostaglandin E2 receptors (EP3) and prostaglandin F2α receptors (FP). We hypothesized that compensatory mechanisms would increase the expression and activation of FP and EP3 following OXTR desensitization. *Methods*: Myometrium from late-pregnancy rats was collected in order to assess mRNA expression levels for OXTR, FP, and EP3 using RT-PCR. This was done after 2 hours of pretreatment with 10^{-6} M oxytocin to induce OXTR desensitization, or equilibration in physiological salt solution (PSS). Myometrium was exposed to increasing concentrations of uterotonic agents (10^{-10} to 10^{-5} M) following 2 hours of pretreatment with 10^{-6} M oxytocin (experimental group) or with PSS (control group). Myometrium from the experimental group was washed with PSS and OXTR expression was assessed using Western blot and RT-PCR. *Results*: mRNA expression levels for EP3, FP and OXTR were not statistically different between the experimental (OXTR desensitization) and control groups. Compared to the control group, the (mean $\pm$ SD) contractile potency of carboprost (pEC50: 7.74 $\pm$ 0.56 vs 6.81 $\pm$ 0.25, P = 0.03) and maximal contractility of misoprostol (Emax(ratio): 4.44 $\pm$ 3.60 vs 1.32 $\pm$ 0.22, P = 0.02) were significantly increased in the OXTR desensitization group, while the contractility of oxytocin was significantly reduced (Emax(ratio): 1.62 $\pm$ 0.27 vs 2.82 $\pm$ 0.98, P = 0.015). No significant differences in myometrial OXTR expression were observed between the PSS, carboprost and misoprostol groups following OXTR desensitization. *Discussion*: Following OXTR desensitization of myometrium, FP and EP3 activation increased in a compensatory manner, but not FP and EP3 receptor expression.

Keywords

Myometrial contraction; Oxytocin receptor; PGE receptor; PGF2α receptor; Uterotonics

1. Introduction

Uterine contractions are usually strengthened by oxytocin, prostaglandin E2 (PGE2) and prostaglandin F2α (PGF2α) through their actions on oxytocin receptors (OXTR), PGE2 receptors (EP) and PGF2α receptors (FP), respectively [1–3]. At term, PGE2 mediates myometrial contraction mainly through the EP3 receptor subtype [4]. Generally recommended second-line agents for the treatment of uterine atony are misoprostol, a known EP3/EP2 agonist [2], and carboprost, which is a synthetic analog of PGF2α [5].

Postpartum hemorrhage (PPH) has an incidence of 4% and contributes to nearly one quarter of all maternal deaths worldwide [6], most of which occur due to uterine atony [7]. Oxytocin-induced or augmented labor contributes to uterine atony by causing desensitization of the myometrium to oxytocin [8, 9]. Currently, the treatment of uterine atony caused by oxytocin exposure is empirical [10, 11]. Therefore, the aim of the present study was to investigate changes in the receptors involved in myometrial contraction following OXTR desensitization, thus providing an experimental basis for the treatment of uterine atony.

Previous studies showed that OXTR expression was unable to increase at term in mice due to lack of the FP gene [12, 13]. Subsequent studies showed that treatment with PGF2α during pregnancy increased OXTR expression in human myometrial cells from the lower uterine segment and decreased it in the upper segment [14]. A recent study suggested that OXTR is an upstream regulator of cyclooxygenase-2, which facilitates the conversion of arachidonic acid to PGE2 and PGF2α [14]. OXTR antagonists inhibit PGF2α-induced contractions and inflammatory responses in the human myometrium [15]. These findings suggest that OXTR and FP expression and function may interact directly or indirectly in the perinatal uterus.

We hypothesized that desensitization of OXTR would result in compensatory increases in the expression and activation of FP and EP3. We also hypothesized that the effects of prostaglandins and oxytocin on OXTR expression might be different in myometrium with OXTR desensitization. The primary goal of this *in vitro* study was therefore to explore changes in EP3, FP and OXTR expression in late-pregnancy rat myometrium following pretreatment with oxytocin, as well as their activation effects. The second goal was to investigate the effect of prostaglandins on OXTR expression.

2. Methods

2.1 Experimental animals

All animal experiments were approved by the Animal Ethics Committee of the Shanghai Medical College, Fudan University [Approval number: 201907007Z]. Pregnant Sprague-Dawley rats (age 12 weeks, weight 280 to 350 g, 16 days of gestation as determined by the presence of a copulatory plug at gestational day 0 or 1) were purchased from Shanghai Jiesijie Experimental Animal Co. Ltd. (No. 1068, Zhaotai Road, Minhang District, Shanghai, China). The rats were housed for four days in the animal facility and maintained on *ad libitum* standard rat chow and tap water in a 12:12 hour light-dark cycle. The temperature of the breeding room was kept at 20~25 °C and the relative humidity at 50~65%.

2.2 Myometrial strip isolation and preparation

Rats were euthanized by injecting excess pentobarbital (140 mg/kg) through the right groin and into the abdominal cavity. After carefully removing the contents of the uterine cavity, the remaining uterine smooth muscle was placed in physiological salt solution (PSS: 120 mM NaCl, 5.9 mM KCl, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 11.5 mM dextrose, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$) precooled to 4 °C. The adipose and vascular tissues surrounding the uterine tissue were carefully separated and removed under the microscope. For the isometric contraction experiment, the uterine tissue was cut into 7-mm-long and 3-mm-wide strips along the longitudinal axis of the muscle [15]. Three or four myometrial strips were obtained from each rat. Longitudinal myometrial strips were vertically suspended in a thermostatic water bath at 37 °C with 35-mL liquid at pH 7.4. Gas (95% oxygen and 5% carbon dioxide) was pumped into the liquid continuously [16]. Myometrial contractions were continuously recorded using an isometric force transducer connected to a 4-channel physiological signal acquisition and processing system (Jide Experimental Instrument Factory, Shanghai, China). The myometrial contraction record was analyzed using the RM6240 series multi-channel physiological signal acquisition and processing system. Each myometrial strip was equilibrated in PSS at 1-g tension for 40 min to achieve regular contraction and to adapt to the environment. When the myometrium reached a regular contraction pattern, each myometrial strip was stimulated with 96-mM KCl to induce a contraction that reflected the maximum contractile capacity of the tissue [16]. The KCl solution was then drained from the organ bath and residual solution was removed by washing three times with PSS [16]. The prepared myometrial strips were used for subsequent experiments. The flow diagram for the experiments is shown in Fig. 1.

3. Calculation of contractile activity

The contractile activity of the myometrium was calculated as the average tension (g) × frequency (contractions/15 min). The ratio of contractility was used for statistical analysis and was calculated as the contractile activity at each drug concentration divided by the spontaneous activity. The spontaneous activity of each myometrium strip was calculated during 15 min of stable contractions prior to the addition of 10^{-6} M oxytocin/PSS.

4. *Experiment 1*: mRNA expression for EP3, FP and OXTR

4.1 Inducing the desensitization of OXTR

Experiment 1: three myometrial strips were obtained from each of four rats and randomly distributed to the baseline, control and experimental groups. The baseline group was used to extract total RNA just after muscle strip preparation. In the experimental group, myometrial strips were pretreated with 10^{-7} M oxytocin for 15 minutes and then with 10^{-6} M oxytocin for 2 hours to induce OXTR desensitization. Myometrium strips were then rested in fresh PSS for 10 min to adapt to the subsequent lower concentration of 10^{-7} M oxytocin (Fig. 2A) [17]. The myometrium contractile activity in 10^{-7} M oxytocin was assessed before and after the induction of OXTR desensitization and the values were compared. The control group was exposed to PSS rather than 10^{-6} M oxytocin for 2 hours, and the contractile responses compared to those observed in the experimental group. The myometrial strips were collected at the end of the contraction experiment. Reverse transcription-polymerase chain reaction (RT-PCR) assays were used to evaluate the mRNA expression of EP3, FP and OXTR in the baseline, experimental and control groups.

4.2 Real-time RT-PCR

Total RNA was extracted using TRIzol reagent (Life Technologies, 21-22F, L'Avenue, 99 Xianxia Road, Changning District, Shanghai, China) and cDNA was synthesized using the ReverTra Ace qPCR RT Kit (FSQ-101; TOYOBO, Osaka, Japan) according to the manufacturer's instructions. Quantitative RT-PCR was carried out using SYBR® Green Real-time PCR master mix (TOYOBO) with a CFX96^{TM} Real-Time System instrument (BIO-RAD, Hercules, CA, USA). Each reaction was run in triplicate to minimize variation. Gene expression was normalized to the mean expression of the housekeeping gene GAPDH. The experiment was repeated four times. The PCR primers were as follows: GAPDH forward primer 5′-TGCACCACCAACTGCTTAGC-3′anreverse primer 5′-GGCATGGACTGTGGTCATGAG-3′; OXTR forward primer 5′-TAGGTGATGGCGTATGTTTGTG-3′and reverse primer 5′-GTTGTCTGATGGCTGAGTCCC-3′; EP3 forward primer 5′-ACTGTCCGTCTGCTGGTC-3′and reverse primer 5′-CCTTCTCCTTTCCCATCTG-3′; FP forward primer 5′-GAGATTTAGACGGAAGTCGAAGG-3′ and reverse primer 5′-GTGATCACCAGGCCACTAGC-3′.

5. *Experiment 2*: effect of uterotonic agents on OXTR expression

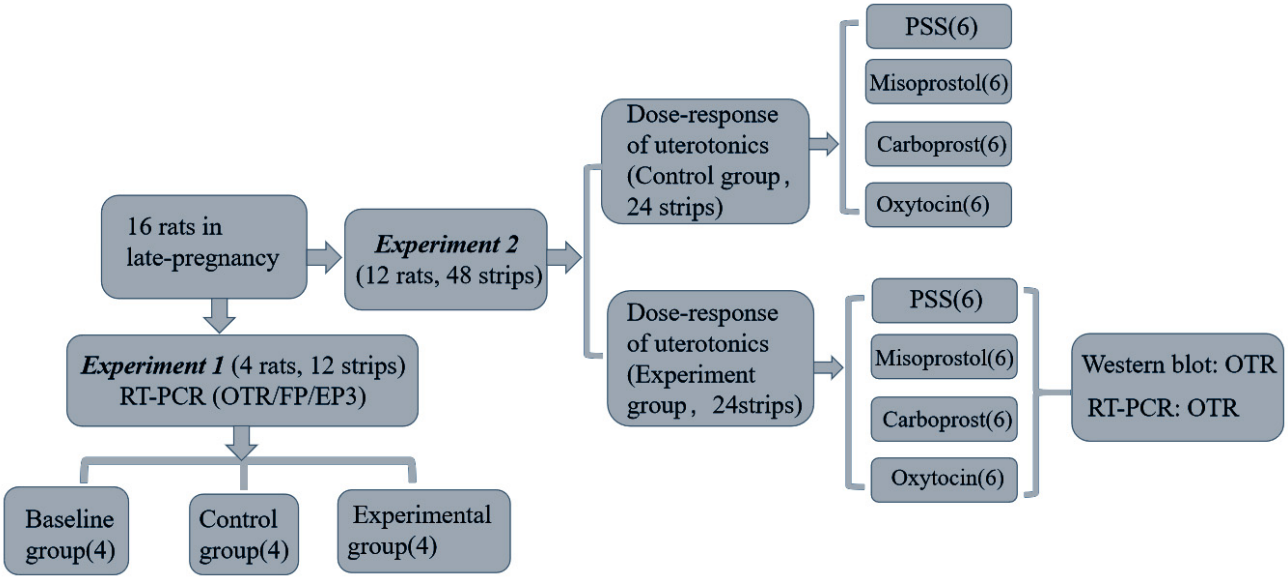

Fig. 1. Flow diagram of the study. A total of 16 late-pregnancy rats were used. In ***Experiment 1***, 3 myometrial strips were isolated from each of 4 rats and randomly distributed to baseline, control (equilibration in PSS for 2 hrs) and experimental groups (treatment with 10^{-6} M oxytocin for 2 hrs). In ***Experiment 2***, 4 myometrial strips were isolated from each of 12 rats in order to test the dose-response of oxytocin (10^{-10} to 10^{-6} M), misoprostol (10^{-10} to 10^{-5} M), carboprost (10^{-10} to 10^{-5} M) and PSS. Before the dose-response testing, two myometrial strips were equilibrated in PSS for 2 hrs (control group) and the other two were treated with 10^{-6} M oxytocin for 2 hrs (experimental group). The control group and the experimental group for the same drug were evaluated simultaneously, and the uterine smooth muscle isolated from the same rat. The number in each box reflects the sample size.

5.1 Contractility analysis

Experiment 2: following treatment with PSS (control group) or 10^{-6} M oxytocin (experimental group) for 2 hours, the myometrial strips were subjected to dose-response testing with oxytocin (10^{-10} to 10^{-6} M), misoprostol (10^{-10} to 10^{-5} M), carboprost (10^{-10} to 10^{-5} M) or PSS (Fig. 2B) [18–20]. For each drug the control and experimental group tests were conducted simultaneously and the myometrium samples were obtained from the same rat. According to a previous study, a concentration of 10^{-7} M oxytocin produces the maximum contraction in isolated rat myometrium [16]. In our preliminary experiments, 10^{-5} M oxytocin induced tetanic contraction of the myometrium with high mean tension. Therefore, the maximum concentration of oxytocin used in the present study was 10^{-6} M. After finishing the uterotonic-stimulated contractions, each myometrial strip was stimulated with 96-mM KCl to evaluate its activity. Following completion of the contraction experiment, myometrial strips from the experimental groups were divided into two halves to evaluate the expression of OXTR. One of the halves was used for Western blot analysis and the other for RT-PCR.

5.2 Expression of OXTR mRNA

OXTR mRNA expression was evaluated using the same method described for real-time RT-PCR.

5.3 Expression of OXTR proteins

Western blot analysis: Tissue proteins were extracted using a radioimmune precipitation assay RIPA lysis buffer (P0013B, Beyotime, Jiangsu, China) and separated by 10% SDS-PAGE. The detached protein was transferred onto a polyvinylidene difluoride (PVDF) membrane. The membrane was then blocked with 5% skim milk dissolved in PBST (PBST: KCl, 2.68 mM; KH_2PO_4, 1.47 mM; NaCl, 136.89 mM; $Na_2HPO_4.12H_2O$, 8.06 mM; 0.1% TWEEN-20.) for 2 hours at room temperature to reduce nonspecific background. PVDF membranes were incubated at 4 °C overnight with primary antibodies to oxytocin receptor (1 : 5000; ab181077 supplied by abcam) and GAPDH (1 : 10,000; ab181602 supplied by abcam). Each membrane was then incubated with a secondary antibody (1 : 5000; 7074P2; CST) for 2 hours at room temperature. Finally, the membranes were treated with enhanced chemiluminescence (Bio-Rad) and observed using a Western blot visualizer (Tanon 5500; Tanon, Shanghai, China). The experiment was repeated four times and the intensity of bands was quantified by Image J.

6. Statistical analysis

All data are expressed as mean ± standard deviation (SD). The concentration-response curve was obtained by taking drug concentrations as the abscissa and ratio of contractility as the ordinate variable. The curve was fitted using Prism 8 (GraphPad Prism Software, San Diego, CA, USA) . The Student's *t*-test was used to compare differences between the control and experimental groups for each uterotonic agent. The potency pEC50 (negative logarithm of the molar concentration required to elicit 50% maximum contraction response) and efficacy (maximum response [Emax(ratio)]) of

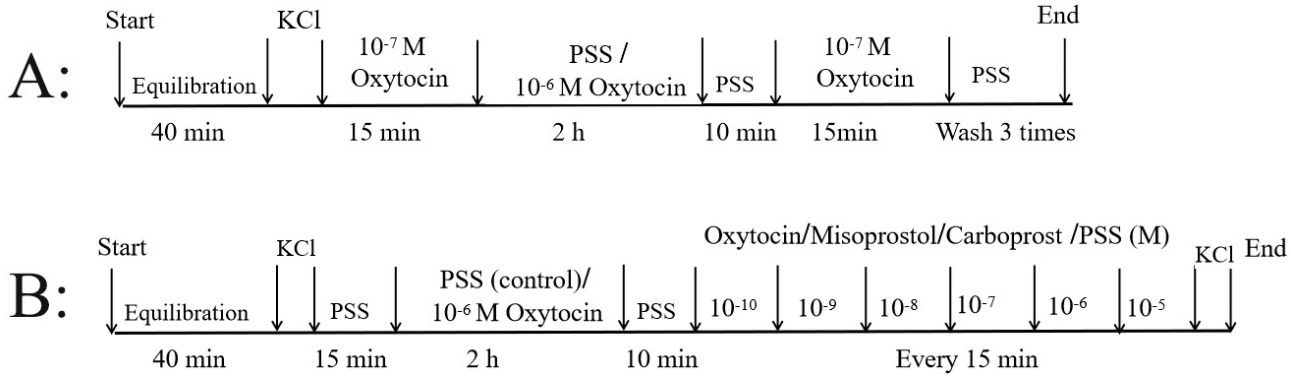

Fig. 2. Experimental evaluation of myometrial contraction. Experiment 1: following treatment with PSS or 10^{-6} M oxytocin (to induce oxytocin receptor desensitization) for 2 hrs, myometrial strips were washed 3 times with PSS and assessed for mRNA expression of OXTR, EP3 and FP receptors (A). Experiment 2: following treatment with PSS (control group) or 10^{-6} M oxytocin (experimental group) for 2 hrs, myometrial strips were exposed to different uterotonic agents. Only myometrium samples from the experimental group were collected for evaluation of oxytocin receptor expression (B). KCl, potassium chloride; PSS, physiological salt solution.

each drug were compared between groups with one-way analysis of variance (ANOVA) and Dunnett's post hoc (2-sided) test using SPSS Statistics 22 software (IBM, Armonk, NY, USA). In all cases, differences were considered significant at a P value of < 0.05.

7. Results

Myometrial contractile amplitude and frequency showed no significant changes during continuous exposure to PSS for 2 hours (Fig. 3A1). The response to 10^{-7} M oxytocin before and after this process also showed no difference. However, the baseline level, amplitude, and frequency of myometrial contraction gradually decreased during 2 hours of 10^{-6} M oxytocin treatment (Fig. 3A2). The contraction response of myometrium to 10^{-7} M oxytocin was significantly weaker than prior to the 10^{-6} M oxytocin pretreatment ($P = 0.005$) (Fig. 3B). The mRNA expression levels of EP3, FP and OXTR did not change significantly with and without oxytocin pretreatment (Fig. 3C1–C3).

Without oxytocin pretreatment, the concentration-response curve for misoprostol was flat. However, with oxytocin pretreatment the myometrial contractility increased rapidly between misoprostol concentrations of 10^{-10} to 10^{-7} M (Fig. 4). Misoprostol had a more obvious contractile effect on the myometrium after oxytocin pretreatment compared to the control (mean (SD) Emax(ratio): 4.44 $\pm$ 1.47 vs 1.32 $\pm$ 0.22, $P = 0.02$). There was no significant difference in the maximum myometrial contraction effect produced by carboprost with or without oxytocin pretreatment. However, carboprost significantly enhanced the contractile potency of myometrium pretreated with oxytocin compared to the control (pEC50: 7.74 $\pm$ 0.56 vs 6.81 $\pm$ 0.25, $P = 0.03$). At any oxytocin concentration, myometrial contractility induced by oxytocin in the experimental group was significantly lower than that observed in the control group (Emax(ratio): 1.62 $\pm$ 0.27 vs 2.82 $\pm$ 0.98, $P = 0.015$). Myometrial contractile activity in the control group was greater than that of the oxytocin pretreatment group at any time in PSS (Table 1).

In the control group, the maximum contractile effect of oxytocin on myometrium was significantly greater than for misoprostol, but was not significantly different to that of carboprost. However, the contractile potency (pEC50) of oxytocin on myometrium was significantly greater than that of carboprost. In the experimental group, the potency of oxytocin on myometrial contraction was significantly greater than that of both misoprostol and carboprost. However, there was no significant difference in the maximum contractile effect between the three groups.

After completing the contraction tests stimulated by uterotonics, the myometrial strips for the experimental group were evaluated for OXTR expression at the mRNA and protein levels. Compared to continuous oxytocin exposure, OXTR expression increased significantly following equilibration in PSS. There was no significant difference in OXTR expression in myometrium between the misoprostol, carboprost and oxytocin treatment groups. Moreover, there was no statistical difference in OXTR expression between the PSS treatment and misoprostol or carboprost treatment groups (Fig. 5).

8. Discussion

Following pretreatment *in vitro* with 10^{-6} M oxytocin for 2 hours, the contractile response of myometrium to oxytocin decreased significantly, although mRNA expression for OXTR, FP and EP3 did not change. After oxytocin pretreatment, the sensitivity of myometrium to carboprost increased, but the maximal contraction induced by carboprost did not change. Misoprostol had an obvious contractile effect on myometrium pretreated with oxytocin, but was inactive for myometrial contraction in the control group. Following the desensitization of OXTR, continuous oxytocin expo-

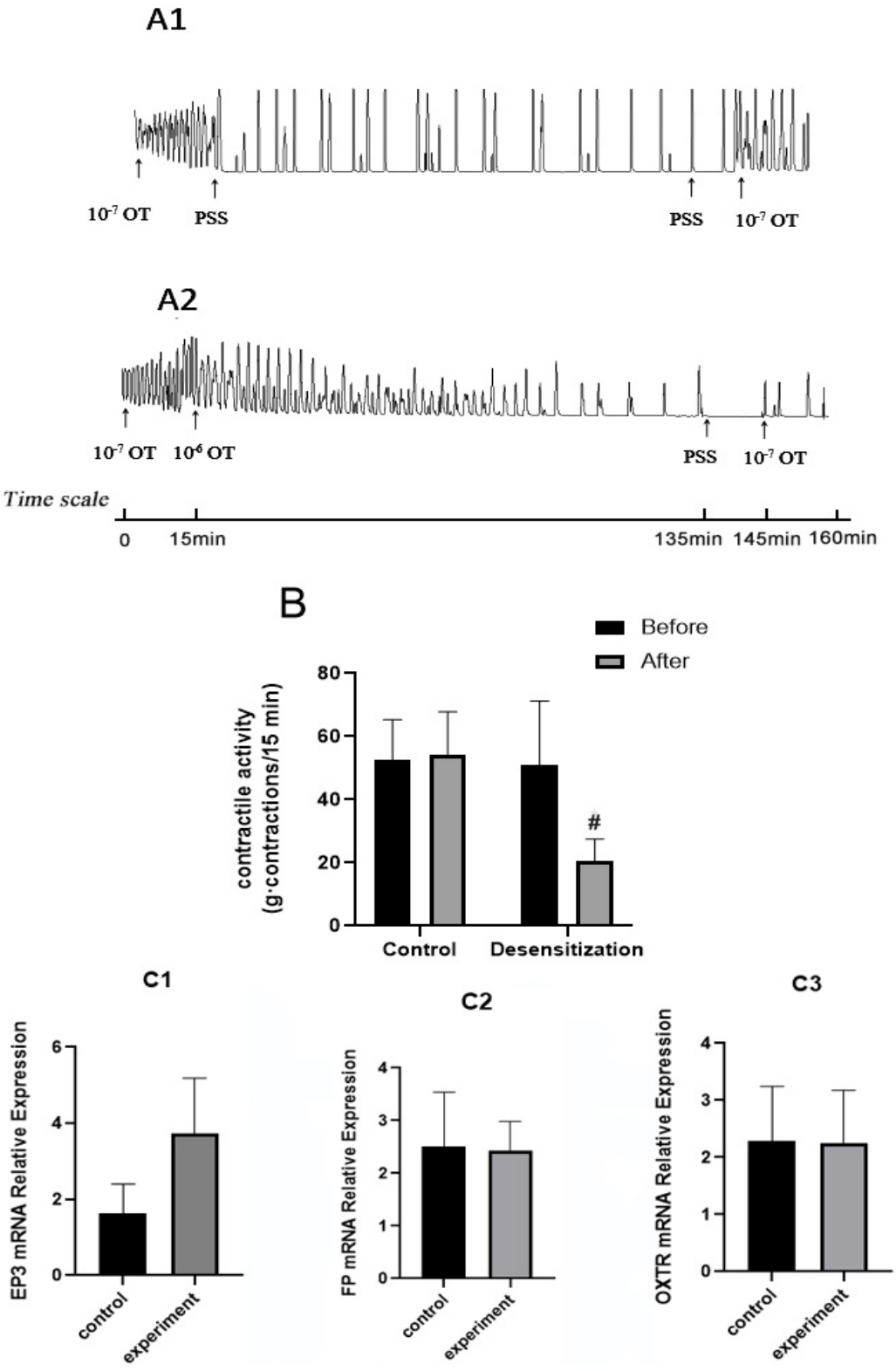

Fig. 3. Experiment 1: Receptor mRNA expression in myometrium from the experimental group (with oxytocin pretreatment) and from the control group (without oxytocin pretreatment). (A) Representative isometric tension recordings for the control group (A1) and for the experimental group (A2). The time scale was suitable for Fig. A1 and Fig. A2. (B) Myometrial contractile activity before and after desensitization with 10^{-6} M oxytocin or PSS for 2 hrs of contraction, stimulated by 10^{-7} M oxytocin. # statistically significant, $P = 0.005$ (paired-samples T test). (C) mRNA expression levels for the PGE2 receptor (EP3; C1), PGF2α receptor (FP; C2), and oxytocin receptor (OXTR; C3) were measured and normalized to the housekeeping gene GAPDH. No statistical differences in the mRNA levels of these receptors were observed between the control and experiment groups. Data are mean $\pm$ SD (whiskers). OT, oxytocin; PSS, physiological salt solution.

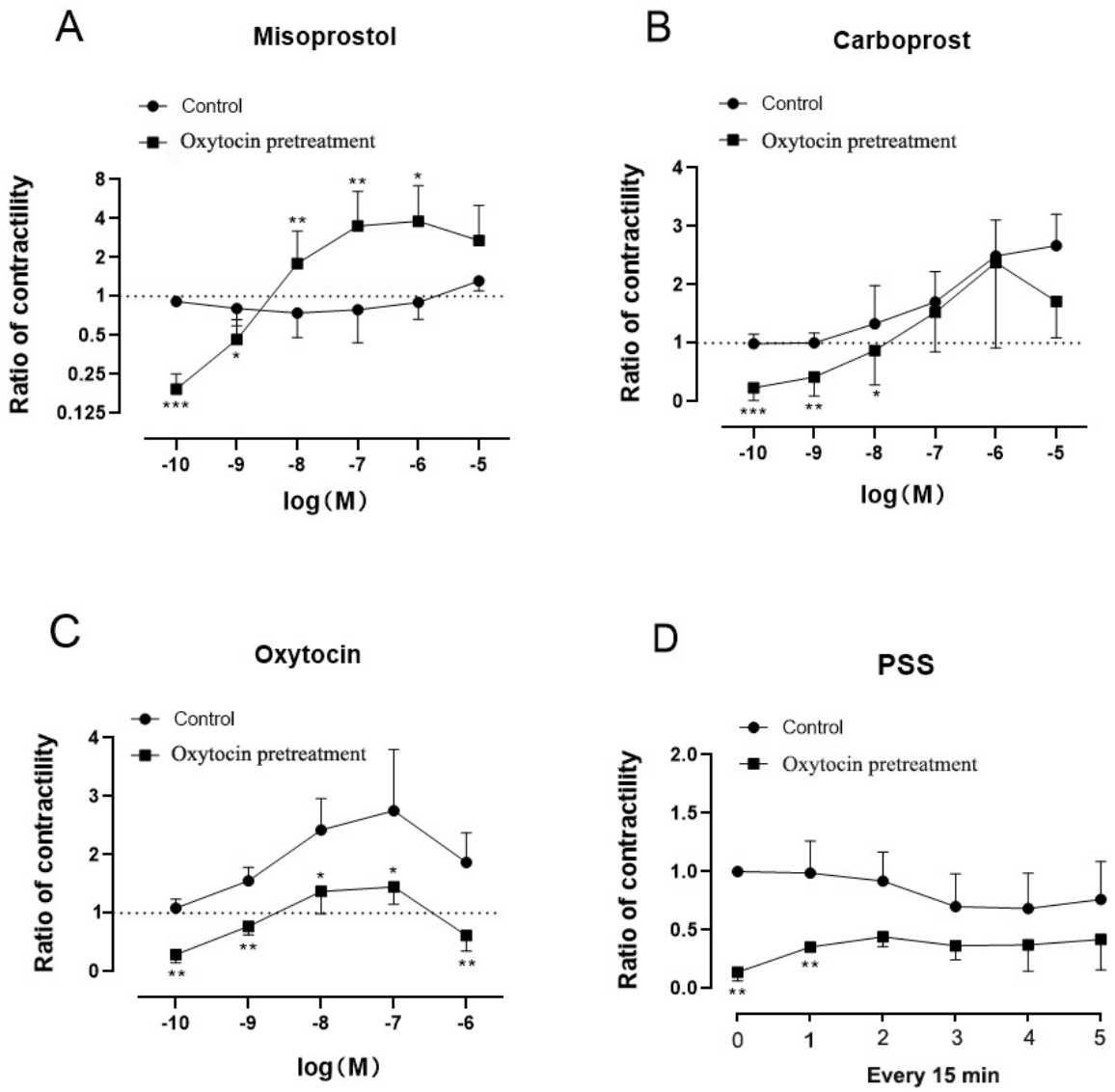

Fig. 4. Experiment 2. Concentration-response curves for misoprostol (A), carboprost (B), oxytocin (C) and PSS (D) in late-pregnancy rat myometrium, with or without oxytocin pretreatment. Contractile responses are shown as ratios of contractility. The ratio of contractility was calculated as the contractile activity at each drug concentration divided by the baseline spontaneous activity. Data are mean ± SD (whiskers). The difference in ratio between the control group (n = 6) and oxytocin pretreatment group (n = 6) was compared using *T*-test at each drug concentration (at each period in the PSS group). "*" represents $0.01 < P < 0.05$, "**" represents $0.001 < P < 0.01$, "***" represents $P < 0.001$.

sure reduced OXTR expression compared to equilibrium in PSS. Furthermore, there were no statistically significant differences between the PSS, carboprost and misoprostol groups for OXTR mRNA expression.

Oxytocin acts directly on phosphoinositidase C-linked G protein coupled receptors (OXTR) to increase cytosolic Ca^{2+} to strengthen myometrial contraction. Many literatures as well as our experimental results demonstrated that oxytocin exposure would induce desensitization of OXTR. In the research of Phaneuf *et al.* [21], oxytocin exposure decreased the binding of oxytocin to cell membranes, however flow cytometry experiments demonstrated that OXTR were not internalized during this treatment. The second messengers calcium, inositol phosphates (InsPs) and cyclic nucleotides play decisive roles in uterine contractility [22]. In a previous study, oxytocin-induced desensitization did not change the ability of PGF2α to increase intracellular free calcium, did but change such ability for oxytocin [17]. The present study confirms that FP expression was not affected by oxytocin pretreatment, and that the myometrial contractile potency of carboprost became stronger. One possible explanation is that oxytocin-induced desensitization acts only on OXTR levels without affecting post-receptor signaling. Earlier studies involving human and rat myometrial tissues also showed that pretreatment with oxytocin decreased the response to subsequent oxytocin exposure, but the myometrium consistently responded to PGF2α stimulation [17, 18].

Table 1. Myometrial contractile activity to uterotonics in rat isolated myometrial strips with or without oxytocin pretreatment.

	Oxytocin	Misoprostol	Carboprost
Control group			
n1	6	6	6
Emax(ratio)	2.82 ± 0.98	1.32 ± 0.22 [a]	2.74 ± 0.44
pEC50	9.53 ± 0.37	10.23 ± 0.25	6.81 ± 0.25 [a]
Experiment group			
n2	6	6	6
Emax(ratio)	1.62 ± 0.27	4.44 ± 3.60	2.40 ± 1.25
pEC50	9.00 ± 0.51	8.27 ± 0.29 [a]	7.74 ± 0.56 [a]
***P1* value**	0.02 [b]	0.02 [b]	0.23
***P2* value**	0.11	0.00 [b]	0.03 [b]

The Emax(ratio) and pEC50 were compared among three uterotonics in each group ("a" means $P < 0.02$, one-way analysis of variance, Dunnett's post hoc comparison to oxytocin). Emax(ratio) = the maximum myometrial contractility of responding to uterotonics divided by baseline contractile activity; pEC50 = negative logarithm of the concentration of uterotonic agent required to elicit 50% maximum response. ***P1* value:** compare the difference of Emax(ratio) between myometrial strips with and without oxytocin pretreatment to the same drug. ***P2* value:** compare the difference of pEC50 between myometrial strips with and without oxytocin pretreatment to the same drug. "b" means ***P1* value** and ***P2* value** < 0.05, which were accepted as statistically significant. Data represent mean $\pm$ SD; n = number of myometrial strips from separate rats.

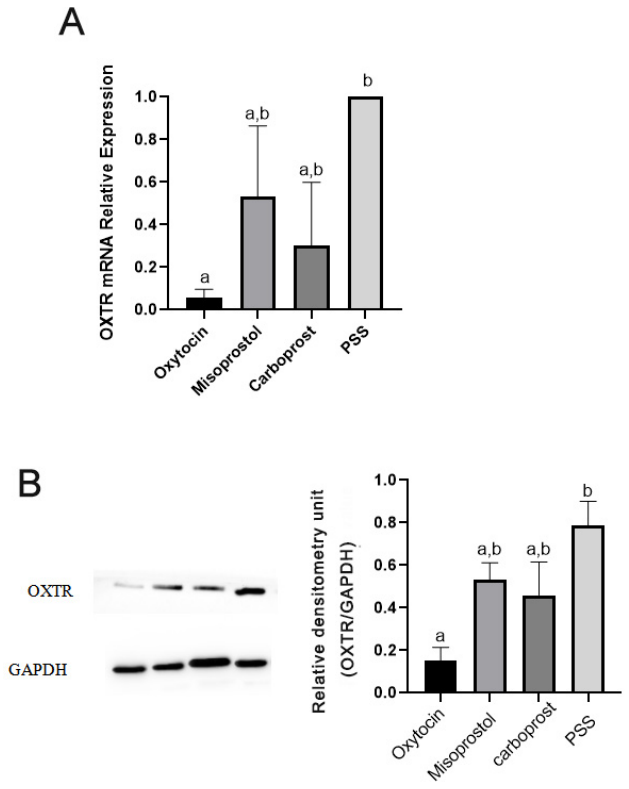

Fig. 5. Experiment 2. Oxytocin receptor (OXTR) expression. Expression of the housekeeping gene GAPDH was used as an internal control to assess the expression of OXTR mRNA following treatment of myometrium with oxytocin, misoprostol, or carboprost (A). Whole-tissue lysates were subjected to Western blotting. GAPDH served as a loading control, and blots were scanned for densitometric analysis (B). Representative Western blots are also shown. Data are mean $\pm$ SD (whiskers). Significant differences (A,B) between groups are illustrated by different lowercase letters above each bar; groups sharing the same letter did not differ (one-way analysis of variance, Bonferroni post hoc comparison). PSS, physiological salt solution.

As shown in Fig. 3, treatment with 10^{-6} M oxytocin for 2 hours caused OXTR desensitization and the response to 10^{-7} M oxytocin before and after this process showed significant differences (A2, B). However, the level of OXTR mRNA did not change (C3). In the oxytocin pretreatment group, OXTR expression decreased significantly following continuous exposure to oxytocin compared with equilibration in PSS (Fig. 5). We speculate that continuous use of oxytocin initially decreases the response of myometrium to oxytocin, while the decreased expression of OXTR mRNA needs to further prolong the time of oxytocin treatment. Although it is believed that oxytocin/OXTR signaling is essential, numerous studies have shown that OXTR expression does not correlate with the oxytocin-induced uterine contraction effect. A recent study showed that peaks in oxytocin level during labor did not correlate with the time of uterine contractions [23]. Another study showed that late-pregnancy uterine contractions in mice are mainly controlled by modification of the contractile signal machinery rather than by the level of OXTR [24]. The decrease in OXTR mRNA in myometrium accompanied by the decline in response to oxytocin might be related to the longer time of oxytocin pretreatment [21].

A previous study showed that EP3 receptor-deficient mice have normal parturition [25]. Similarly, the present study found the EP3 agonist misoprostol had no contractile effect on late-pregnancy rat myometrium in the control group. However, we also found that misoprostol had a significant myometrial contractile effect following oxytocin-pretreatment, even though EP3 mRNA did not increase. Misoprostol is a mixed EP3/EP2 receptor agonist [26]. During human pregnancy, myometrial EP3 receptors are excitatory while EP2 receptors are inhibitory [27]. Therefore, we speculate the myometrial contractile effect of misoprostol following oxytocin-pretreatment may be due to inhibition of EP2 expression or activity. In contrast to the present results, Balki *et al.* [28] showed that responses to PGF 2α and to misoprostol were not affected by labour or by prior exposure to oxytocin. A possible reason for the discordant results is that the myometrium in their research was balanced in PSS for 2 hours before administering uterotonics and was not exposed to oxytocin, thus causing the myometrium to resensitize to oxytocin [29].

Following OXTR desensitization, both the contractile potency of carboprost and the maximum contractility of misoprostol increased significantly. This suggests it was reasonable and necessary to use FP and EP3 receptor agonists to enhance myometrial contraction after OXTR desensiti-

zation. Following pretreatment with oxytocin, OXTR expression was significantly higher in the PSS group compared to the oxytocin exposure group, while OXTR expression in the carboprost and misoprostol groups was not significantly different compared to the PSS group. A previous study on human myometrium also demonstrated that oxytocin combined with carboprost produced a better contractile effect compared to oxytocin alone following oxytocin pretreatment [30]. Recent literature has also reported that misoprostol plus oxytocin was a more effective strategy for preventing PPH than oxytocin alone [10, 31]. Therefore, combinations or alternate uses of oxytocin, FP and EP3 receptor agonists may be more effective at strengthening myometrial contraction.

Balki *et al.* [30] reported there was incomplete information on the plasma levels of uterotonics following parenteral administration in the setting of PPH. They suggested that oxytocin levels vary significantly during pregnancy, labor and postpartum from 10^{-12} to 10^{-8} M and may not accurately reflect the local myometrial concentration [32–35]. Because the release of oxytocin during physiological labor is pulsatile, the measured values of serum oxytocin concentration vary greatly depending on the sampling intervals. Oxytocin levels doubled in response to a doubling of the infusion rate of exogenous oxytocin [23]. Thus, accurate plasma levels of oxytocin are difficult to define. The approximate peak serum level of carboprost after intramuscular injection of 250 μg was approximately 10^{-8} M in term pregnant women [36]. Misoprostol is commonly administered into the vagina and hence the plasma level of this drug may differ significantly from the concentrations to which the uterine smooth muscle is exposed.

Although the *in vitro* drug concentrations used in the present study may not directly reflect serum levels *in vivo*, the concentration range (10^{-10} to 10^{-5} M) investigated here may well include the physiologic serum levels of these drugs. Morrison *et al.* [20] provided a detailed pharmacodynamic analysis of uterotonics on isolated myometrium from women undergoing elective cesarean delivery at term. Their results showed similar pEC50 for oxytocin and carboprost to the present study, while misoprostol was also inactive in myometrial contraction. Hence, the previous literature suggests that rat and human myometrial tissues have similar pharmacodynamic responses to uterotonics.

The present study of the receptor activation effect confirmed our hypothesis that carboprost and misoprostol enhance myometrial contraction following OXTR desensitization. However, we did not further explore the possible mechanism of OXTR desensitization, such as the oxytocin-induced effect of increased cytoplasmic free calcium concentration before and after receptor desensitization. Pretreatment with oxytocin for 2 hours increased EP3 mRNA expression, although this did not reach statistical significance. It is unclear whether prolonging the duration of oxytocin exposure would lead to significantly increased EP3 mRNA expression. Misoprostol also acts on EP2 to inhibit myometrial contraction [27], but we did not further investigate the effect of oxytocin pretreatment on EP2. *In vitro* experiments based on animal tissues have inherent limitations, such as species variation and differences between *in vivo* and *in vitro* environments.

In summary, we found that following pretreatment with 10^{-6} M oxytocin for 2 hours, the response ability of myometrium to subsequent oxytocin decreased significantly. However, the mRNA expression levels of EP3, FP and OXTR were not affected by pretreatment with oxytocin, whereas the contractile potency of carboprost and the maximal contractile effect of misoprostol both increased significantly. No significant difference in OXTR expression was observed between oxytocin and prostaglandin treatments. Misoprostol was inactive in normal myometrial contraction. These experimental results suggest that prostaglandin uterotonics, especially misoprostol, may have desirable therapeutic effects on uterine atony caused by long-time oxytocin exposure. Further studies are required to confirm this hypothesis.

Author contributions

LL and SH designed the research study. LL and JH performed the research. TW analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were approved by the Animal Ethics Committee of the Shanghai Medical College, Fudan University [Approval number: 201907007Z].

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Conflict of interest

The authors declare no conflict of interest.

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