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Vasopressin-induced contraction of uterus is mediated solely by the oxytocin receptor in mice, but not in humans

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Abstract

In the non-pregnant mouse myometrium, both arginine vasopressin and oxytocin induced contractions ($pD_2 = 8.55 \pm 0.13$ and 9.23 ± 0.09, respectively). The effect of oxytocin was the most potent, while the maximum contractions induced by these two peptides were almost of the same magnitude. Both vasopressin- and oxytocin-induced contractions were strongly inhibited by an oxytocin receptor antagonist, CL-12-42 ($d(CH_2)_5[Tyr(Me)^2,Thr^4,Tyr\text{-}NH_2^9]OVT$), and weakly inhibited by a vasopressin V_{1a} receptor antagonist, SR49059 ((2*S*)1-[(2*R*,3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide). Similar results were obtained in the pregnant mouse myometrium. These results suggest that not only oxytocin- but also vasopressin-induced contraction is mediated by the activation of oxytocin receptors in the mouse myometrium. A reverse transcription polymerase chain reaction study failed to reveal mRNA of the vasopressin V_{1a} receptor in the mouse myometrium. In contrast, in the non-pregnant human myometrium, vasopressin-induced contraction was inhibited by SR49059. Oxytocin showed no effect on the myometrium. These results suggest that there are significant differences in the functional receptors and contractile responses to vasopressin and oxytocin in the human and mouse uteri.

Keywords: Vasopressin; Vasopressin V_{1a} receptor; Oxytocin; Oxytocin receptor; Contraction; Uterus

1. Introduction

Oxytocin and arginine vasopressin are two closely related neurohypophysial nonapeptides, both of which have a six-amino acid cyclic part and a three-residue tail. Only two of the nine-amino acid residues are different, and so far one oxytocin receptor and three vasopressin receptors (V_{1a}, V_{1b} and V_2) have been cloned (Birnbaumer et al., 1992; Kimura et al., 1992; Lolait et al., 1992, 1995; Morel et al., 1992; Sugimoto et al., 1994; Thibonnier et al., 1994). Both oxytocin and vasopressin bind to the oxytocin receptor and vasopressin acts as a partial agonist on the receptor (Chini et al., 1996; Kimura et al., 1994; Postina et al., 1996). On the other hand, vasopressin receptors are quite selective for vasopressin (Burbach et al., 1995).

In the human and rat uterine myometrium, the oxytocin and vasopressin V_{1a} receptors are the predominant receptor subtypes among the four receptors that have been cloned (Clerget et al., 1997; Helmer et al., 1998). Oxytocin induces a marked contraction in the pregnant uterus and it is therefore thought that the oxytocin/oxytocin receptor system must be essential for parturition. The role of oxytocin in the non-pregnant uterus is not clear. Maggi et al. (1992) showed that expression of the oxytocin receptor is regulated by the estrus cycle in the human uterus and suggested that the receptor might play a role in increasing uterine activity during menstruation.

Vasopressin also induces contraction in the human, rabbit, rat, and mouse uteri (Bossmar et al., 1996; Chan et al., 1996; Mackler et al., 1999; Maggi et al., 1988). Though the physiological role of vasopressin in the uterus is unclear, in a pathophysiological study, the plasma vasopressin level was elevated in women with primary dysmenorrhoea (Akerlund et al., 1979) and a vasopressin V_{1a} receptor antagonist, SR49059 ((2*S*)1-[(2*R*,3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide), was

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useful for preventing dysmenorrheic pain (Brouard et al., 2000). Furthermore, there are some reports that local injection of vasopressin is effective to decrease hemorrhage in myomectomy operations (Corson et al., 1994; Fletcher et al., 1996; Frederick et al., 1994; Kimura et al., 2002).

For rat uterus, Chan et al. (1996) suggested that vasopressin-induced contraction was mainly mediated by oxytocin receptors, although vasopressin V_{1a} receptor expression was apparently observed in rat myometrium. In the non-pregnant human uterus, however, vasopressin induces a much greater reaction than oxytocin (Maggi et al., 1990, 1992). These results suggest that the expression of receptors and/or functional receptor subtypes may differ among species.

In the present experiments, we examined the functional receptors involved in oxytocin- and vasopressin-induced contractions in the mouse uterus, and compared them with those in the human myometrium.

2. Materials and methods

2.1. Tissues and preparations

Female C57BL/6J mice (11–13 weeks) were used in the experiments. All experiments complied with the Guidelines for Care and Use of Laboratory Animals in Tohoku University. In the study of non-pregnant mouse uterus, the estrus cycle was monitored by taking vaginal smears and mice in the estrus stage were used. In the study of pregnant mouse uterus, female mice were mated with male mice overnight. The morning when a copulation plug was detected was designated as 0.5 day of gestation. The normal length of pregnancy in mice is 19.5 days. Uteri of pregnant mice were obtained from 18.5 days of gestation. The non-pregnant and pregnant mice were killed by cervical dislocation and the uteri were isolated. Myometrium was isolated from the uteri in a longitudinal direction.

Uteri from five non-pregnant women, aged 40–48 years, with regular menstruation were used in the experiments. Hysterectomy was performed due to benign fibroids or cervical intraepithelial neoplasia. At operation, three women were in the follicular phase and two were in the luteal phase. All patients provided informed consent and this study was approved by the ethics committee of Osaka Medical Center for Cancer and Cardiovascular Diseases. Immediately after hysterectomy, the normal part of the myometrium was dissected from the endometrium and serosa and placed in Krebs–Ringer solution. The segments were carried to the laboratory in the solution at 4 °C.

2.2. Measurements of contractile tension

Muscle contraction was recorded isometrically using a micro-easy-magnus system (UC-5A, UFER, Kyoto, Japan). A small strip (0.7–1.0 mm wide and 3–4 mm long) of myometrium was connected to a force–displacement transducer (UL-10GR, Minebea, Japan) and a recorder. Then it was equilibrated under a resting tension of 2 mN in normal physiological salt solution containing: 140.0 mM NaCl, 5.0 mM KCl, 1.2 mM $MgCl_2$, 1.8 mM $CaCl_2$, 23.8 mM $NaHCO_3$ and 11.1 mM glucose. The 35 mM KCl solution was made by replacing NaCl by equimolar KCl. These solutions were saturated with 95% O_2 and 5% CO_2 mixture at 37 °C and pH 7.4. Each strip was repeatedly exposed to 35 mM KCl until the contractile responses became stable. Receptor antagonists were added 5 min before the addition of vasopressin or oxytocin. All results are expressed as percentages of the response to 35 mM KCl; 35 mM was sufficient to evoke the maximum contraction induced by KCl. The K_b values of the antagonist were calculated according to the Schild equation (Arunlakshana and Schild, 1959).

2.3. Reverse transcription polymerase chain reaction (RT-PCR) analysis of vasopressin V_{1a} receptor

Uteri were obtained from non-pregnant female mice in estrus and total RNA was extracted according to Chomczynski and Sacchi (1987). Two micrograms of total RNA were reverse transcribed in a 20-μl reaction volume containing 200 U of Superscript II reverse transcriptase (Invitrogen), 4 μl of 5 × first strand buffer (Invitrogen), 0.5 μg of oligo-dT (Amersham Biotech), 1 μl of 10 mM dNTP mix (Sigma) and 2 μl of 100 mM dithiothreitol (Invitrogen) at 42 °C for 50 min and 2 U of RNase H (Invitrogen) for 37 °C 20 min. One microliter of the cDNA product was used to amplify the cDNAs for the vasopressin V_{1a} receptor or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by polymerase chain reaction. The primer sequences used for the PCR reactions were: V_{1a}-F: TGGGTACCTGCTATGGCTTC; V_{1a}-R: TTCCACGTCCCAGTGCTGTT, GAPDH-F: ATGGTGAAGGTCGGTGTGAACG, GAPDH-R: AAACATGGGGGCATCGGCAGAA. For V_{1a} and GAPDH, the PCR reaction was performed in a 100-μl reaction volume containing 1 μl of the cDNA mixture, 50 pmol of forward and reverse primers, 10 μl of 10 × PCR buffer (Sigma), 2 μl of 10 mM dNTP mix (Sigma) and 2.5 U of Taq polymerase (Sigma). After 1 min of denaturation at 95 °C, 40 cycles of amplifications consisting of 95 °C, 1 min; 60 °C, 30 s; 72 °C, 30 s were performed. Aliquots (10 μl) were obtained at 25, 28, 31, 34, 37 and 40 cycles. The two primers' sequences for V_{1a} were designed not to originate from the same exon. For the negative control, the same polymerase chain reaction was performed using the same total RNA without reverse transcription.

2.4. Materials

Arginine vasopressin and oxytocin were purchased from Sigma. The oxytocin receptor antagonist, CL-12-42 ($d(CH_2)_5[Tyr(Me)^2,Thr^4,Tyr\text{-}NH_2^9]OVT$), was a generous gift from Dr. Maurice Manning (Medical College of Ohio). The vasopressin V_{1a} receptor antagonist, SR49059 ((2*S*)1-

[(2*R*,3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzene-sulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-carbonyl]-pyrrolidine-2-carboxamide), was kindly provided by Sanofi Recherche (France).

2.5. Statistics

Results of the experiments are expressed as the means ± S.E.M. Student's *t*-test was used for statistical analysis of the results and a *P* value less than 0.05 was considered to indicate a significant difference.

3. Results

3.1. Contractile responses to oxytocin and vasopressin in the non-pregnant mouse myometrium

Uteri from mice in the estrus stage were used in the experiment. In the preliminary experiment, neither oxytocin nor vasopressin induced contraction in the myometrium from mice in the diestrus stage (data not shown).

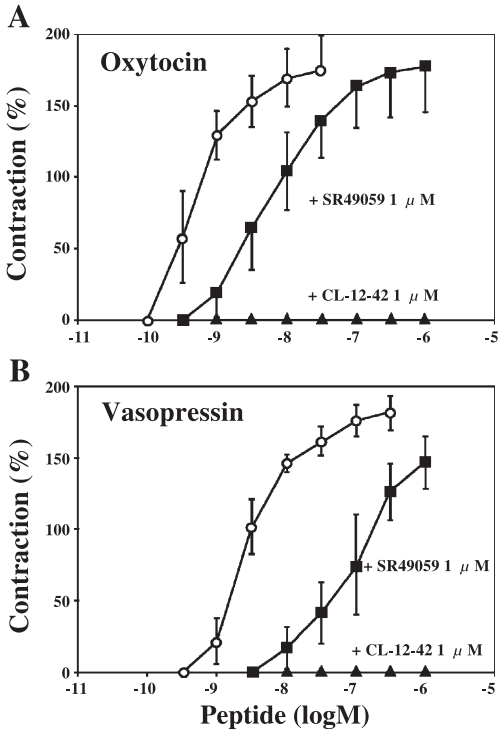

Fig. 1. Cumulative dose–response curves for oxytocin and vasopressin in non-pregnant mouse myometrium in the absence or presence of the antagonist. After the effect of 35 mM KCl became stable, oxytocin (A) or vasopressin (B) was cumulatively added to the strips. Each antagonist was added 5 min before the addition of the peptide. One hundred percent represents the magnitude of contraction induced by 35 mM KCl. The peptide-induced response in the absence (○) or presence of the antagonist (CL-12-42, 1 μM, ▲; SR49059, 1 μM, ■). Each point represents means ± S.E.M. of five to six experiments.

Table 1
Antagonistic properties and receptor affinities of CL-12-42 and SR49059

Species	Receptor	Cells/tissues	Ligands	CL-12-42	SR49059
K_b in nM					
Mouse		Uterus (P)	Oxytocin	0.39	105.6
			Vasopressin	–	–
		Uterus (NP)	Oxytocin	–	82.1
			Vasopressin	–	35.0
K_i in nM					
Human	V_{1a}	Platelets	Vasopressin	1.39 ± 2.36[a]	6.3 ± 0.6[b]
	Oxytocin	Uterus (P)	Oxytocin	–	130[b]
	Oxytocin	CHO cells[c]	Oxytocin	0.28 ± 0.30[a]	–
Rat	V_{1a}	Liver	Vasopressin	25.6 ± 10.6[d]	2.2 ± 0.4[b]
	Oxytocin	Uterus (E)	Oxytocin	0.17 ± 0.03[d]	–
	Oxytocin	Mammary gland	Oxytocin	–	1080 ± 115[b]

K_b values of antagonists were calculated according to the Schild equation (Arunlakshana and Schild, 1959). Values are given as the means ± S.E.M.

[a] The K_i values of the antagonists were taken from Lemaire et al. (2002).

[b] The K_i values of the antagonists were taken from Serradeil-El Gal et al. (1993).

[c] Human oxytocin receptors expressed in Chinese hamster ovary (CHO) cells. Uterus isolated from pregnant (P), non-pregnant (N) and estrogen-treated (E) human/rodent.

[d] The K_i values of the antagonists were taken from Elands et al. (1988).

Cumulative addition of oxytocin or vasopressin induced concentration-dependent contraction in the non-pregnant mouse myometrium (Fig. 1A and B). There was no significant difference in the maximum contractile responses between oxytocin and vasopressin (173.2 ± 24.4%, $n=5$ and 182.4 ± 10.5%, $n=6$). The pD_2 values for oxytocin and vasopressin were 9.23 ± 0.09 ($n=5$) and 8.55 ± 0.13 ($n=6$), respectively.

As shown in Fig. 1A, pretreatment with an oxytocin receptor antagonist, CL-12-42 (Elands et al., 1988), strongly inhibited the oxytocin-induced contraction in the myometrium. On the other hand, a vasopressin V_{1a} receptor antagonist, SR49059 (Serradeil-Le Gal et al., 1993), was less potent to inhibit the contraction. In contrast to our expectations, CL-12-42 also strongly inhibited the vasopressin-induced contraction (Fig. 1B), whereas SR49059 showed weak inhibition of the vasopressin-induced contractions in the myometrium. The K_b values were calculated and are shown in Table 1.

3.2. Contractile responses to oxytocin and vasopressin in the pregnant mouse myometrium

In the pregnant mouse myometrium of day 18.5, both oxytocin and vasopressin induced contraction in a concentration-dependent manner (Fig. 2A and B). There was no significant difference in the maximum contractile responses between oxytocin and vasopressin (154.4 ± 9.0%, $n=4$ and 157.6 ± 18.7%, $n=4$). The pD_2 values for oxytocin and vasopressin were 9.34 ± 0.14 ($n=4$) and 8.47 ± 0.12 ($n=4$), respectively. Similar to the results for mouse myo-

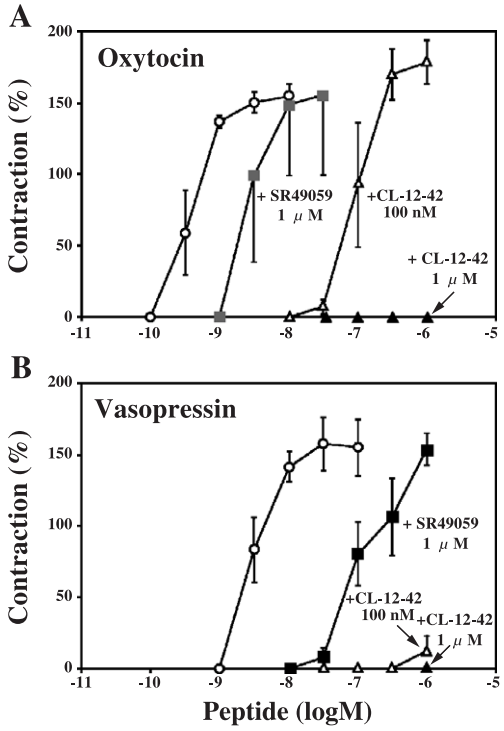

Fig. 2. Cumulative dose–response curves for oxytocin and vasopressin in pregnant mouse myometrium in the absence or presence of the antagonist. After the effect of 35 mM KCl became stable, oxytocin (A) or vasopressin (B) was cumulatively added to the strips. Each antagonist was added 5 min before the addition of the peptide. One hundred percent represents the magnitude of the contraction induced by 35 mM KCl. The peptide-induced response in the absence (○) or presence of the antagonist (CL-12-42, 100 nM, △; 1 μM, ▲; SR49059, 1 μM, ■). Each point represents means ± S.E.M. of four experiments.

metrium of the estrus phase, CL-12-42 strongly inhibited both oxytocin-and vasopressin-induced contractions (Fig. 2 and Table 1). The effects of SR49059 were also less potent to inhibit the contraction in the pregnant myometrium.

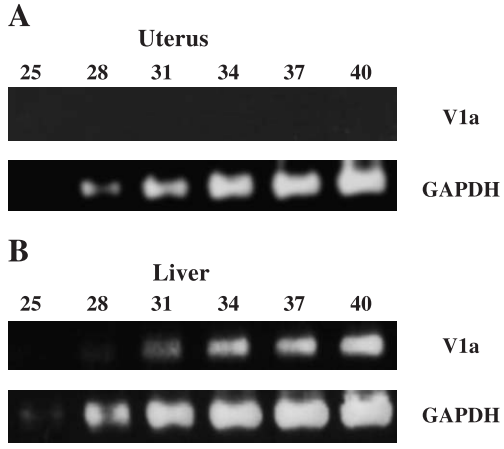

Fig. 3. RT-PCR analysis of vasopressin V_{1a} receptor and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the non-pregnant mouse uterus (A) and liver (B). The numbers above lanes indicate the cycle of PCR amplification in each sample.

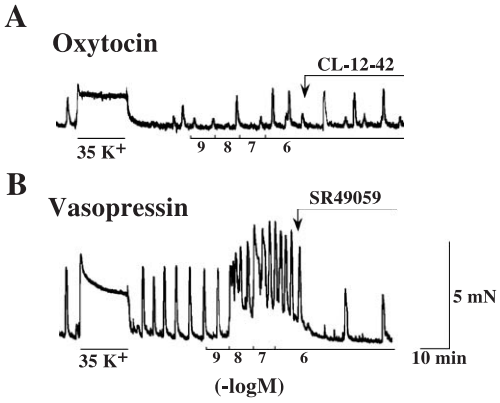

Fig. 4. Effects of oxytocin and vasopressin in the non-pregnant human myometrium. After the effect of 35 mM KCl became stable, oxytocin (A) or vasopressin (B) was cumulatively added to the strips. CL-12-42 (1 μM) or SR49059 (1 μM) was added after the contractile response to each peptide (1 μM) reached a steady level. Traced from typical results of five experiments.

3.3. RT-PCR analysis of vasopressin V_{1a} receptor in the mouse uterus

The expression of the vasopressin V_{1a} receptor was studied by RT-PCR. Messenger RNA of vasopressin V_{1a} receptor was not detected in the non-pregnant mouse uterus (Fig. 3A). In the mouse liver, mRNA of the vasopressin V_{1a} receptor was apparently detected (Fig. 3B). The amount of PCR products from glyceraldehyde-3-glyceraldehyde-3-phosphate dehydrogenase mRNA increased with the PCR cycles in these two tissues, indicating that our experiments were performed with intact RNA and before the plateau of amplification.

3.4. Contractile responses to oxytocin and vasopressin in the non-pregnant human myometrium

In the experiments with non-pregnant human myometrium, spontaneous contractions were observed in almost all strips. On the other hand, these spontaneous contractions did not occur or disappeared shortly after the beginning of the experiments with mouse myometrium.

The addition of oxytocin (1 nM–1 μM) showed no effect on the amplitude or frequency of the spontaneous contractions (Fig. 4A). The oxytocin receptor antagonist, CL-12-42 (1 μM), also showed no effect on the spontaneous contractions. Vasopressin (1 nM–1 μM) induced concentration-dependent contraction in the human myometrium (Fig. 4B). SR49059 (1 μM) completely inhibited the vasopressin-induced contraction.

4. Discussion

In both non-pregnant and pregnant mouse myometrium, the oxytocin-induced contraction was strongly inhibited by the oxytocin receptor antagonist, CL-12-42, and weakly

inhibited by the vasopressin V_{1a} receptor antagonist, SR49059 (Figs. 1A and 2A). The K_b values of CL-12-42 and SR49059 for the oxytocin-induced contraction were consistent with the K_i values of these antagonists for the oxytocin receptor (Table 1). These results showed that the oxytocin-induced contraction was mediated by the oxytocin receptor in the non-pregnant and pregnant mouse myometrium.

The vasopressin V_{1a} receptor antagonist, SR49059, was less potent than CL-12-42 to inhibit the vasopressin-induced contraction in the mouse myometrium. The K_b value of SR49059 for the vasopressin-induced contraction (35 nM) was much higher than the K_i value of the antagonist for the vasopressin V_{1a} receptor (2.2–6.3 nM, Table 1). Furthermore, mRNA expression of the vasopressin V_{1a} receptor was not detected in the mouse uterus (Fig. 3). These results indicate that not only oxytocin- but also vasopressin-induced contractions are mediated by oxytocin receptors in the mouse myometrium. Our results are consistent with previous findings for rat uterus (Chan et al., 1996). In the rat uterus, however, the apparent mRNA expression of vasopressin V_{1a} and V_{1b} receptors was detected (Clerget et al., 1997; Lolait et al., 1995). On the other hand, mRNA of the vasopressin V_{1b} receptor was not detected in the mouse uterus (Ventura et al., 1999).

In the mouse myometrium, mRNA expression of the oxytocin receptor was detected (Sugimoto et al., 1997). Recently, we created oxytocin receptor gene knockout mice and found that neither oxytocin-induced nor vasopressin-induced contraction occurred in the myometrium of the knockout mice (Takayanagi et al., unpublished observation). These results strongly support the finding that vasopressin-induced contractions are mediated by oxytocin receptors but not by vasopressin receptors in the mouse myometrium.

In the mouse myometrium, a five- to seven-fold higher concentration of vasopressin was needed to induce the same contractile response as was induced by oxytocin (Figs. 1 and 2). As the binding affinity of oxytocin to the oxytocin receptor was 5–10-fold higher than that of vasopressin to the same receptor (Kimura et al., 1994, Muller et al., 1989, Postina et al., 1996), the difference in contractile potency between oxytocin and vasopressin in the mouse myometrium might be due to the difference in the binding affinity of these two peptides to the mouse oxytocin receptor. In human oxytocin receptor-expressing cells, a 100-fold higher concentration of vasopressin was needed to evoke the same reaction as that evoked by oxytocin (Chini et al., 1996; Kimura et al., 1992, 1994), and vasopressin acted as a partial agonist on the oxytocin receptor in these experiments. In the present experiments, however, the magnitudes of the maximum contractions caused by these two peptides were the same in the mouse myometrium. Therefore, vasopressin seems to be a full agonist of the mouse oxytocin receptor in the myometrium because the vasopressin-induced contraction in the mouse myometrium is considered to be mediated by the oxytocin receptor.

It is widely accepted that the expression levels of the oxytocin receptor in the myometrium increase at term. Mackler et al. (1999) also showed that oxytocin induced a greater contraction in the pregnant mouse myometrium than in the non-pregnant mouse myometrium. In the present experiments, however, there was no significant difference in the pD_2 values and maximum responses for oxytocin between non-pregnant and pregnant mouse myometrium. This discrepancy may have been due to the different state of the non-pregnant mice. Mice in the estrus phase were used in the present experiments.

It has been reported that the vasopressin V_{1a} receptor is highly expressed in the human myometrium (Helmer et al., 1998). In the non-pregnant human myometrium, vasopressin increased the frequency and the magnitude of spontaneous contractions (Fig. 4B). The contractions were inhibited by the addition of the vasopressin V_{1a} receptor antagonist, SR49059. These results are consistent with the previous finding that vasopressin-induced contraction is mediated by the vasopressin V_{1a} receptor in the human myometrium (Bossmar et al., 1995, 1997).

In the human myometrium, neither oxytocin nor the oxytocin receptor antagonist, CL-12-42, showed any effect on spontaneous contraction (Fig. 4A). It has been reported that the expression level of the oxytocin receptor is indeed very low in the non-pregnant human myometrium (Helmer et al., 1998).

In conclusion, vasopressin-and oxytocin-induced contractions are mediated by the oxytocin receptor in the mouse myometrium. On the other hand, vasopressin-induced contraction is mediated by the vasopressin V_{1a} receptor in the human myometrium.

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