Oxytocin-induced phasic and tonic contractions are modulated by the contractile machinery rather than the quantity of oxytocin receptor

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Oxytocin-induced phasic and tonic contractions are modulated by the contractile machinery rather than the quantity of oxytocin receptor. Am J Physiol Endocrinol Metab 292: E992–E999, 2007. First published December 5, 2006; doi:10.1152/ajpendo.00492.2006.—To investigate the relationship between the oxytocin (OT) receptor (OTR) quantity and the contractile features systematically, we measured the mRNA expression levels of OTR and L-type Ca2+ channel α1C-subunit (α1C) and examined the regulatory mechanisms of OT-induced phasic or tonic contractions of the longitudinal smooth muscles in mouse uteri. The mRNA expression of OTR in 19.0 G (19.0 days of gestation) was greater than those in nonpregnant phases, and that of α1C in estrus and 19.0 G was higher than in diestrus. OT-induced contractions sparsely occurred in diestrus. The OT-induced all-or-none-type phasic contractions at low concentrations were abolished by verapamil in both estrus and 19.0 G. However, the magnitude in 19.0 G was much greater than that in estrus. OT-induced tonic contractions had similar pD2 values in both estrus and diestrus. The OT-induced tonic contractions at low concentrations were abolished by verapamil in both estrus and 19.0 G. Cyclopiazonic acid-induced tonic contractions were reciprocally decreased with the increase in the OT-induced ones in diestrus. The OT-induced all-or-none-type phasic contractions at low concentrations were abolished by verapamil in both estrus and 19.0 G. OT-induced tonic contractions had similar pD2 values in both estrus and 19.0 G. However, the magnitude in 19.0 G was much greater than that in estrus. The large tonic contractions also occurred in PGF2α receptor (FP) knockout mice in 19.0 G despite a small amount of OTR. Verapamil and Y-27632 partially inhibited the tonic contractions in 19.0 G. Cyclopiazonic acid-induced tonic contractions were reciprocally decreased with the increase in the OT-induced ones in 19.0 G. These results indicate that the phasic contractions are dependent on α1C. The tonic contractions in 19.0 G are dependent on both Ca2+ influxes via L-type Ca2+ channels and store-operated Ca2+ channels, and the force is augmented by the Rho signal pathway, which increases the Ca2+ sensitivity. Thus the uterine contractions are mainly controlled by the modification of contractile signal machinery rather than simply by the OTR quantity.

OXYTOCIN (OT) is a well-known neurohypophysial hormone that stimulates uterine contractions to facilitate parturition (11). The near-term myometrium is extremely sensitive to OT, and the increased responsiveness to OT occurs in parallel with an increase in the number of uterine OT binding sites in rats (6, 42), humans (7), rabbits (28, 29), and cows (8). A corresponding increase in uterine OT receptor (OTR) mRNA in late pregnancy and at parturition has been reported in rats (23, 26, 38), humans (21), cows (13), and sheep (51, 55). In human uterus at term, the sensitivity to OT is 200- to 1,000-fold greater than that during the menstrual cycle (1). Thus, although there has not yet been conclusive evidence, it has been believed that the sensitivity to OT depends on the OTR quantity.

OT induces phasic or tonic contractions in myometrium. Phasic contractions are essential for the successful progression of labor, whereas tonic contractions may contribute to prevent atomic bleeding after parturition. During the course of parturition, prolonged tonic contractions might be harmful for fetuses because an increase of the intrauterine pressure would disturb the fetomaternal circulation (53). This is understandable when one appreciates that contractions occlude blood vessels within the myometrium (24). The relationship between the two types of contractions and the OTR quantity is also unknown. The mechanisms and signal pathways mediating the induction of the phasic and tonic contractions vary greatly in smooth muscles in different tissues and species (16). There is a great advantage to examining the contractile response to OT in mouse myometrium. Using other species besides mice, it is difficult to determine the genuine OT-induced uterine contractions because of a possible cross-reactivity of OT with arginine vasopressin V1a receptor (V1aR), which coexists in myometrium in humans and other species. In mouse uteri, we revealed that V1aR was not detectable (17) and that OT did not induce any contractions in myometrium of otr knockout mice (50).

Early investigations described in vitro contractions and membrane activity in the mouse myometrium (37, 47). The influences of gestation (27), phosphatase inhibition (41), and intracellular Ca2+ concentration (30) have subsequently been reported. In this study, we analyzed the OT-induced phasic and tonic contractions in relation to the concentrations of OT to initiate the contractions, the pD2 values, and maximum responses in nonpregnant and pregnant mouse myometrium. Additionally, we examined the relationships between the OTR quantity and contractile response to clarify the regulation mechanisms of the two types of contractions.

EXPERIMENTAL PROCEDURES

Tissues and preparations. Female C57BL/6 and PGF2α receptor (FP) knockout (FP KO; see Ref. 46) mice (8–13 wk) were used. All experiments complied with the Guidelines for the Care and Use of Laboratory Animals in Tohoku University. The cycles were determined by vaginal smears, and mice in either the estrous or diestrous phase were used. Mice were anesthetized with ether, and the myometrium was dissected from the horns and stored in PBS. The uterine horn was cut into 0.5- to 1.0-mm-thick pieces and left in a 95% O2-5% CO2 incubator at 37°C for 30 min. The effective concentration for oxytocin (OT) to initiate uterine contractions is determined by concentrations ranging from 10−10 to 10−4 M. Therefore, concentrations of OT ranging from 10−8 to 10−4 M were used to examine the concentration-response relationship. The means ± SE of the data are presented. The concentration-response curves of OT for phasic and tonic contractions were fitted to a logistic function of the form

y = y0 + [A − y0] / [1 + (x/d)^(1/b)]

where y0 is the baseline response, A is the maximum response, d is the concentration at which the response is one-half A, and b is the slope of the curve. The parameters of these curves, the number of observations, and the statistical significance were calculated with commercially available software (GraphPad Prism, GraphPad Software, Inc.). Where appropriate, a two-way ANOVA was used followed by a Bonferroni’s multiple comparison test. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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phase were used. To obtain pregnant mouse myometrium, a female mouse was mated with a male overnight. The next morning, if a copulation plug was detected, it was designated as 0.5 days of gestation (0.5 G). Myometrium of pregnant mice were obtained at 19.0 G. The animals were killed by cervical dislocation, and the uteri were isolated.

Quantitative RT-PCR. We analyzed the expression levels of otr, the L-type Ca\(^{2+}\) channel \(\alpha_{1C}\) subunit (\(\alpha_{1C}\)), and calpains, including calpain 1, calpain 2, and calpain 4. Total RNA was extracted from longitudinal smooth muscle of uteri according to Chomczynski and Sacchi (3), and cDNA was produced from two micrograms of total RNA according to a previous study (17). For real-time quantitative PCR, 1 \(\mu\)l of the cDNA was added to 20 \(\mu\)l of reaction volume containing 10 \(\mu\)l DyNaSy SYBR Green qPCR kit (FINNZYMES) and 1 \(\mu\)l of 12.5 \(\mu\)M primers (forward and reverse). For each sample, a parallel reaction was set up with acidic ribosomal phosphoprotein PO (\(arbp\)) primers. The \(arbp\) was used as an endogenous control. The primer sequences used were as follows: \(arbp\) forward, ATAACCCCT-GAAGTGCTCGACAT; \(arbp\) reverse, GGGAGTGTTGACT-CAGTCTCCA; otr forward, TTCCTCCGTGCAGATGTGGAG; otr reverse, AGGAGGAGGGAGGAGGTT; \(\alpha_{1C}\) forward, CCAA-CATCAACAATGCCAAC; \(\alpha_{1C}\) reverse, TCAGCTCTTCTCATCCT; calpain 1 forward, CGGTTGGAGGAGGTGGATGA; calpain 1 reverse, ATCCAGCTTCAGTGACACAG; calpain 2 forward, CACCTCCACTGTTGACTCTATAA; calpain 2 reverse, ATCCCTCTCATTCCTGCTTT; calpain 4 forward, TCCACCTGGAATGAGCATCCTCT; and calpain 4 reverse, ATCTGTCCGTT-1C subunit. The amount of mRNA was normalized to the respective \(arbp\) values. Each reaction was performed in duplicate.

Measurements of tension. The myometrium of longitudinal smooth muscle was prepared for the in vitro contractile experiment. Contractions were recorded isometrically using a micro-easy-magnus system (UC-5A; UFER, Kyoto, Japan) as described in a previous study (17, 18). In brief, the myometrial strip was equilibrated under a resting tension of 2 mN in normal physiological salt solution containing (in mM) 140.0 NaCl, 5.0 KCl, 1.2 MgCl\(_2\), 1.8 CaCl\(_2\), 23.8 NaHCO\(_3\), and 11.1 glucose. The 65 mM KCl solution contained (in mM) 80.0 NaCl, 65 KCl, 1.2 MgCl\(_2\), 1.8 CaCl\(_2\), 23.8 NaHCO\(_3\), and 11.1 glucose. The 35 mM KCl solution was prepared by mixing 5 and 65 mM solution equally. These solutions were saturated with a 95% O\(_2\) and 5% CO\(_2\) mixture at 37°C, pH 7.4. Each strip was repeatedly exposed to 35 mM KCl solution for 15 min until contractile responses became stable. All results are expressed as percent of the maximum response to 35 mM KCl solution. This concentration of KCl was sufficient to evoke the maximum contraction (37). Tonic contractions were induced in all tissues from diestrus (n = 5) or 19.0 G (n = 5), respectively. The plate read was performed after denaturation at 83°C for 30 s. The annealing temperature of \(E993\) was 57 or 66.8°C, respectively. The plate read was performed after denaturation at 83°C for 1 min for the products of \(otr\), \(\alpha_{1C}\), and calpain 1 to detect the specific product. The amount of \(otr\), \(\alpha_{1C}\), or calpain mRNA was normalized to the respective \(arbp\) values. Each reaction was performed in duplicate.

RESULTS

Analysis of the expression level of \(otr\), L-type Ca\(^{2+}\) channel \(\alpha_{1C}\)-subunit, and calpain mRNA in mouse uteri. The mRNA expression levels of the \(otr\) were much greater in 19.0 G (n = 5) than in diestrus (n = 5) or estrus (n = 5) in longitudinal smooth muscle (P < 0.05; Fig. 1A). There was no difference between estrus and diestrus (Fig. 1A). The mRNA expression levels of the L-type Ca\(^{2+}\) channel \(\alpha_{1C}\)-subunit (\(\alpha_{1C}\)), which is a verapamil-sensitive subunit, were about 5-fold or 28-fold higher in estrus (n = 5) or 19.0 G (n = 5), respectively, than those in diestrus (n = 8, P < 0.05; Fig. 1B). We also examined the mRNA expression levels of calpains. Calpains are thought to induce the cleavage of the COOH terminus of \(\alpha_{1C}\), resulting in the active form (9, 10, 12). The expression levels of calpain acid (CPA) was used to deplete sarcoplasmic reticulum (SR) Ca\(^{2+}\) by inhibiting the SR Ca\(^{2+}\)-ATPase.

Statistics. The results of the experiments are expressed as means ± SE. ANOVA and Student’s t-test were used for statistical analysis of the results, and a P value < 0.05 was accepted as being significantly different.

Fig. 1. Expression level of oxytocin receptor (ot; A) and \(\alpha_{1C}\)-subunit (\(\alpha_{1C}\); B) in longitudinal smooth muscle of mouse uteri. mRNA expression levels of \(otr\) and L-type Ca\(^{2+}\) channel \(\alpha_{1C}\) were examined in longitudinal smooth muscle of mouse uteri. Real-time quantitative PCR for \(otr\) and \(\alpha_{1C}\) mRNA was performed in diestrus (open bar, n = 5), estrus (gray bar, n = 5), and 19 days gestation (19.0 G; filled bar, n = 5). Acidic ribosomal phosphoprotein PO (\(arbp\)) was used as an endogenous control. The amount of mRNA is normalized to the respective \(arbp\) values. Each reaction was performed in duplicate. Each bar represents the mean ± SE, and significant differences were examined by ANOVA and t-test (P < 0.05 vs. diestrus).
1, 2, or 4 were \( \sim 10.8 \, (n = 5, \, P < 0.001) \), \( 6.8 \, (n = 5, \, P < 0.01) \), or \( 4.1 \, (n = 5, \, P < 0.001) \)-fold higher in 19.0 G than in estrus, respectively, whereas there was no difference in diestrus and estrus.

**OT-induced contractile responses of mouse uteri.** The contractile responses of myometrium in diestrus \( (n = 6) \), estrus \( (n = 8) \), and 19.0 G \( (n = 7) \) were analyzed. Representative recordings are shown in Fig. 2. No spontaneous contractions were observed in any tissues. Contractile responses by OT were obtained under normal physiological salt solution. In this condition, the phasic and tonic contractions induced by OT were clearly distinguished. After the administration of OT \((0.01–100 \, \text{nM})\), phasic contractions were sparsely induced in some preparations of diestrus (Fig. 2A). The phasic contractions and weak tonic contractions were induced at low concentrations of OT in estrus (Fig. 2C). Large tonic contractions occurred predominantly and concentration dependently in addition to phasic contractions with high frequency in 19.0 G (Fig. 2E).

To analyze the differences in the phasic and tonic contractions, we obtained the cumulative concentration-response curves of the two types of contractions. To make the concentration-response curves, the amplitude of the tonic contractions was measured from the baseline, and the phasic contractions were measured by the subtraction of tonic ones from the phasic force of contractions. The summarized data are shown in Table 1. Phasic contractions were observed in four muscles in six diestrous myometria; however, tonic contractions were hardly observed (Fig. 2B). A few phasic or weak tonic contractions were initiated at the concentration of \( 105 \pm 68.4 \, \text{nM} \, (n = 4) \) or \( 4.17 \pm 1.17 \, \text{nM} \, (n = 6) \) of OT, respectively. The contractile profile of the phasic contractions was significantly different from that of the tonic ones in estrus. Phasic contractions were more dominant than tonic ones at concentrations of OT from 0.3 to 100 nM (Fig. 2D). The pD\(_2\) values of the phasic \((9.36 \pm 0.10, \, n = 8)\) and tonic \((8.20 \pm 0.07, \, n = 8)\) contractions were significantly different \((P < 0.001)\). The phasic or tonic contractions were initiated at the concentration of \( 0.38 \) or \( 1.93 \, \text{nM} \) of OT, respectively. The slope values of the phasic contractions were so high compared with those of tonic ones, showing the all-or-none nature of the phasic contractions (Table 1).

The contractile profiles of the phasic contractions were also significantly different from those of the tonic ones in 19.0 G. The subtracted values of phasic contractions were decreased since the tonic ones became predominant at the concentrations of \( 10–100 \, \text{nM} \) of OT (Fig. 2F). There were significant differences in the pD\(_2\) values in the phasic \((9.18 \pm 0.16, \, n = 7)\) and
tonic (8.46 ± 0.14, n = 7) contractions (P < 0.01). The maximum responses of the phasic (128.0 ± 25.7%) in 19.0 G were almost the same as in estrus (131.6 ± 13.4%), although tonic contractions (182.5 ± 20.5%) in 19.0 G were greater than those in estrus (30.7 ± 8.4%, P < 0.001). There were, however, no significant differences either in the pD2 values for the phasic (estrus: 9.36 ± 0.10; 19.0 G: 9.18 ± 0.16) and tonic (estrus: 8.20 ± 0.07; 19.0 G: 8.46 ± 0.14) contractions or in the slope values of the tonic contractions (estrus: 1.60 ± 0.28; 19.0 G: 1.55 ± 0.31). Interestingly, the tonic contractions in 19.0 G were initiated at a lower concentration of OT than were the phasic ones (0.135 vs. 1.18 nM, P < 0.001).

Large tonic contractions in 19.0 G of FP KO mice. Sugimoto et al. (46) reported that FP KO mice failed to labor, and the expression levels of otr did not increase in uteri even at 20.0 G. We examined the expression levels of otr and contractile responses to OT in the longitudinal smooth muscle of FP KO mice in 19.0 G. The expression levels of otr were 6.0-fold lower in the myometrium of FP KO mice (n = 5) than that of wild-type mice in 19.0 G (n = 5, P < 0.01). The expression levels of α1C in the myometrium of FP KO mice at 19.0 G (n = 5) were similar to those of the wild type in 19.0 G (n = 5) and 5.2-fold higher than those of wild-type mice at estrus (n = 5, P < 0.05).

The OT-induced tonic contractions occurred in myometrium of FP KO mice in a concentration-dependent manner like wild-type mice in 19.0 G (Fig. 3, A and B). The maximum responses of the tonic contractions in FP KO mice (211.6 ± 12.0%, n = 7) were similar to the wild-type mice (182.5 ± 20.5%, n = 7).

Effects of Ca2+-free condition, verapamil, or Y-27632 on the OT-induced contractions. We examined the mechanism of the OT-induced phasic and tonic contractions mainly in 19.0 G of wild-type mice under a Ca2+-free condition, in the presence of an L-type Ca2+-channel blocker, verapamil, or a Rho-kinase inhibitor, Y-27632. The phasic contractions induced by 10 nM OT in estrus were abolished by the addition of verapamil (1 μM) with a reduction of the weak tonic contractions from 14.9 ± 7.5 to 9.7 ± 4.5% (n = 4; Fig. 4A). The tonic contractions induced by 10 nM OT in 19.0 G were reduced by verapamil from 182.5 ± 31.4 to 55.3 ± 13.1% (n = 6, P < 0.001; Fig. 4B).

Under the Ca2+-free condition, OT (0.01–100 nM) did not induce either type of contraction in 19.0 G (n = 4; Fig. 4C). In the presence of verapamil (1 μM), the OT-induced phasic contractions were abolished as in estrus (Fig. 4D). The maximum response of the tonic contractions was significantly decreased by verapamil from 182.5 ± 31.4 to 55.3 ± 13.1% (n = 6, P < 0.001; Fig. 4D).

The cumulative concentration-response relations for the phasic or tonic contractions of Fig. 4, C–E, and Fig. 2F are summarized in Fig. 4, F and G, and the maximum amplitude, pD2 values, and slope values are shown in Table 2. In the presence of Y-27632, the maximum responses of the phasic contractions in 19.0 G were decreased from 128.0 ± 25.7% (n = 7) to 66.8 ± 22.0% (n = 6; Fig. 4F and Table 2) and those of tonic contractions from 182.5 ± 31.4% (n = 7) to 85.0 ± 12.9% (n = 6, P < 0.01; Fig. 4G and Table 2), whereas the generation of phasic contractions was not suppressed. The pD2 values of the phasic and tonic contractions were not changed by Y-27632.

Effects of CPA on mouse uteri. Inhibition of the SR Ca2+-pump by CPA depolarizes the plasma membrane and increases intracellular Ca2+, inducing phasic and tonic contractions in rat uteri (48). Figure 5 shows the amplitudes of the phasic (left) and tonic (right) contractions induced by CPA (10 μM). CPA induced weak tonic contractions (8.1 ± 2.6%) and strong phasic ones.

### Table 1. Characterization of phasic and tonic contractions

<table>
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<tr>
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<td>30.7±8.4c</td>
<td>128.0±25.7</td>
<td>182.5±20.5d</td>
</tr>
<tr>
<td>pD2</td>
<td>9.36±0.10</td>
<td>8.20±0.07c</td>
<td>9.18±0.16</td>
<td>8.46±0.14b</td>
</tr>
<tr>
<td>Slope (nM)</td>
<td>5.92±0.38</td>
<td>1.60±0.28c</td>
<td>22.8±4.7b</td>
<td>1.55±0.31b</td>
</tr>
<tr>
<td>Initiation (100±10 pM)</td>
<td>3.82±0.13</td>
<td>19.3±18.2</td>
<td>11.8±0.14d</td>
<td>1.35±0.16d</td>
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<tr>
<td>19.0 G (n = 7)</td>
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<td></td>
<td>128.0±25.7</td>
<td>182.5±20.5d</td>
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<tr>
<td>pD2</td>
<td>9.18±0.16</td>
<td>8.46±0.14b</td>
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<td>1.35±0.16d</td>
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Data are presented as means ± SE; n, no. of mice. G, days gestation. The initiation values [the lowest concentration of oxytocin (OT) to initiate contraction] were calculated on the basis of the logarithm. Phasic vs. tonic: *P < 0.05, **P < 0.01, and ***P < 0.001; estrus vs. 19.0 G: 4*P < 0.05, 5*P < 0.01, and 6*P < 0.001 in ANOVA and t-test.
(142.9 ± 18.9%) with high frequency in estrus (n = 6; Fig. 5, A and C). On the other hand, CPA induced strong tonic contractions in 19.0 G (n = 5; Fig. 5B). The amplitude of the tonic contractions spontaneously decreased after the maximum and became stable by 15 min in 19.0 G (transient, 130.0 ± 20.8%; stable, 65.6 ± 6.8%). The remaining tonic contractions, both in estrus and 19.0 G, were not inhibited by verapamil (1 μM; data not shown).

To investigate whether capacitative Ca²⁺ entry from store-operated Ca²⁺ channels (SOCs) was involved in the tonic contractions induced by OT in 19.0 G, we examined the effects of CPA after the addition of OT (1–100 nM). The amplitudes of the CPA-induced tonic contractions decreased in accordance with the increase in the OT concentration- and OT-induced tonic ones (Fig. 6). The OT-induced tonic contractions were reciprocally decreased with the increase in the CPA-induced ones (data not shown). These results suggest that OT and CPA used a common source of Ca²⁺ signaling for the genesis of the tonic contractions in 19.0 G and that, after the stimulation by OT at higher concentrations, Ca²⁺ entry from SOCs is involved in the strong tonic contractions.

**DISCUSSION**

In this study, we have shown the following. First, the quantity of OTR mRNA in 19.0 G was much greater than in diestrus and estrus. However, the pD₂ values of the phasic contractions were similar in estrus and 19.0 G. Thus we think that the sensitivity to OT does not directly correspond to the OTR quantity. Interestingly, the phasic contractions were initiated at a lower concentration of OT in estrus than in 19.0 G.

The phasic contractions showed an all-or-none nature and were induced by Ca²⁺ influx via L-type Ca²⁺ channels, which

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### Table 2. Characterization of phasic and tonic contractions with verapamil or Y-27632

<table>
<thead>
<tr>
<th>Inhibitor (n = 7)</th>
<th>+Verapamil (1 μM, n = 6)</th>
<th>+Y-27632 (10 μM, n = 6)</th>
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<td></td>
<td>Phasic</td>
<td>Tonic</td>
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<tr>
<td>Maximum, %</td>
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<td>182.5±31.4a</td>
</tr>
<tr>
<td>pD₂</td>
<td>9.18±0.16</td>
<td>8.46±0.21b</td>
</tr>
<tr>
<td>Slope (μN)</td>
<td>22.8±4.76</td>
<td>1.55±0.48a</td>
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</table>

Data are presented as means ± SE; n, no. of mice. ND, phasic contractions are not detected. Phasic vs. tonic: *P < 0.05 and **P < 0.01; –inhibitor vs. +inhibitor: *P < 0.05, **P < 0.01, and ***P < 0.001 in ANOVA and t-test.
reflects the spike activity induced by OT (37, 47). This result corresponds to that in a report using rat myometrium (39). Although the L-type Ca\(^{2+}\)/H11001 channels activated by high KCl could generate the same force of contractions in both diestrus and estrus, the OT-induced phasic contractions seldom occurred in diestrus, reflecting the expression of L-type Ca\(^{2+}\) channels at a lower level that might be influenced by the levels of sex hormones estrogen and progesterone. The phasic contractions induced by OT in estrus seem to be related to the increase in the expression levels of \(\alpha_{1C}\), a major pore component of the channels, which suggested the OTR signal might be coupled efficiently with the L-type Ca\(^{2+}\) channels in estrus. The large tonic contractions in 19.0 G are derived from the Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) channels because verapamil strongly reduced the tonic ones. Furthermore, the expression levels of \(\alpha_{1C}\) as well as calpain 1, 2, and 4 were significantly higher in 19.0 G than in estrus. The pregnant myometrium near term might have sustained depolarization followed by Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) channels. In pregnant rat myometrium, a high concentration of intracellular Ca\(^{2+}\) was sustained during the tonic contractions induced by OT (20). The higher level of expression of \(\alpha_{1C}\) may be partly involved in the tonic contractions in 19.0 G.

The nature of the tonic contractions was significantly different from that of the phasic contractions. Interestingly, the tonic contractions of 19.0 G were similar to those of estrus in terms of the pD2 and the slope values, although the maximum responses in 19.0 G were higher than those in estrus. Thus it is likely that the amplitude of the tonic contractions is dependent on the OTR quantity. Increasing the receptor density causes an increase in the maximal response with little effect on the EC50 (19); this was found by measuring the maximum phosphatidylinositol response to norepinephrine in DDT1MF-2 cells transfected with various densities of \(\alpha_{1B}\)-adrenergic receptors (5). The effects of verapamil and Y-27632 on the tonic contractions showed no decrease in the pD2 values, whereas the amplitude was decreased. The results of noncompetitive antagonism suggest that there seems to be no spare receptor in OTR in mouse uterus.
Uterotonic agents have been reported to increase myometrial contractions, not only by increasing the intracellular Ca\(^{2+}\) but also by increasing the Ca\(^{2+}\) sensitivity of the myometrial force production through a receptor-coupled, G protein-mediated mechanism (14, 15). One of the mechanisms of the increase in the Ca\(^{2+}\) sensitivity is mediated by RhoA-associated kinase (ROK; see Refs. 44 and 25). Using Y-27632, a ROK inhibitor (35), it was demonstrated that ROK was involved in the augmented tonic contractions in 19.0 G. The present study is the first investigation of the relationship between ROK activity and the tonic contractions induced by OT in mouse myometrium. The increased Ca\(^{2+}\) sensitivity is also related to the OT-induced phasic contractions in human (31, 34) and rat (49) myometrium. Y-27632 reduced the force of the contractions but did not affect the generation of either phasic or tonic contractions and the pD\(_2\) values of OT, which shows Y-27632 does not have any effects on OTR activation nor the Ca\(^{2+}\) contractions in the myometrium of FP KO mice in 19.0 G.

The increased Ca\(^{2+}\) sensitivity also relates to the force of the spontaneous contractions (22, 52, 54) and the tonic contractions induced by depolarization by high KCl solution (22, 54, 57). These reports suggest that the pregnant myometrium gains the ability to augment the tonic contractions with an increase of Ca\(^{2+}\) sensitivity via Rho signals independent of the OT/OTR signals. CPA induces large tonic contractions by capacitative Ca\(^{2+}\) entry in 19.0 G, but not in estrus. In pregnant rat myometrium, large tonic contractions are also induced by CPA in parallel with increases in intracellular Ca\(^{2+}\) concentration, but not in nonpregnant myometrium (48). This difference between the estrus and pregnant myometrium might be because of the SR volume (48), which is increased with gestation (43). The results of the present study suggest that OT induces large tonic contractions by capacitative Ca\(^{2+}\) entry in pregnant mouse myometrium. Similar conclusions were reported based on intracellular Ca\(^{2+}\) measurement in an immortalized myometrial cell line derived from a pregnant woman (32, 40).

In conclusion, we have described for the first time the relationship between the OTR quantity and the features of the tonic contractions, not only by increasing the intracellular Ca\(^{2+}\) influx via SOCs.

The serum progesterone level was decreased in 19.0 G but not in estrus. In pregnant rat myometrium, large tonic contractions are also induced by CPA in parallel with increases in intracellular Ca\(^{2+}\) concentration, but not in nonpregnant myometrium (48). This difference between the estrus and pregnant myometrium might be because of the SR volume (48), which is increased with gestation (43). The results of the present study suggest that OT induces large tonic contractions by capacitative Ca\(^{2+}\) entry in pregnant mouse myometrium. Similar conclusions were reported based on intracellular Ca\(^{2+}\) measurement in an immortalized myometrial cell line derived from a pregnant woman (32, 40). Therefore, a part of the large tonic contractions in 19.0 G myometrium might be because of the elevation of Ca\(^{2+}\) influx via SOCs.

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