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Gestational changes in oxytocin- and endothelin-1-induced contractility of pregnant rat myometrium

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Abstract

The mechanical effects of KCl, oxytocin and endothelin-1 on pregnant rat myometrium were examined using intact strips and β-escin-treated skinned strips. Myometrial tissues from delivering rats were more sensitive to 10.7 mM K^+ compared to mid and late gestation. Maximum contractions induced by K^+ were obtained at concentrations of 118 mM at mid and late gestation and during delivery. The maximum amplitude of contractions induced by oxytocin and endothelin-1 compared to the 118 mM K^+-induced contraction increased during the progress of gestation. Maximum contractions induced by oxytocin and endothelin-1 were greater than those induced by 118 mM K^+ at delivery, and maximum contractions by oxytocin were larger than those by endothelin-1 during delivery. In 10 μM nifedipine and Ca^{2+}-free (containing 2 mM EGTA) solutions, 118 mM K^+ contractions were completely abolished; however, both oxytocin and endothelin-1 produced contractions. In Ca^{2+}-free solutions, contractions by oxytocin were larger than those by endothelin-1. In skinned myometrial strips, guanosine 5′-*O*-thiotriphosphate (GTP, 1 μM–1 mM), guanosine 5′-*O*-(γ-thiotriphosphate) (GTPγS, 0.1–100 μM) and oxytocin (1 nM–0.1 μM) with 10 μM GTP, but not endothelin-1 with 10 μM GTP increased Ca^{2+} sensitivity of contractile force. These results suggests that (1) the membrane permeability for Ca^{2+} and the number of receptors of oxytocin and endothelin-1 increase during the progress of gestation, (2) both oxytocin and endothelin-1 produce contractions by activation of voltage-dependent Ca^{2+} channels and Ca^{2+} release from internal storage sites, (3) oxytocin, but not endothelin-1, coupled to receptors increases Ca^{2+} sensitivity to contractile proteins.

Keywords: Oxytocin; Endothelin; Pharmacomechanical coupling; GTP; Myometrium

1. Introduction

Oxytocin receptors increase at term during gestation, thus, it is suggested that oxytocin has an important role in the initiation of labor (Alexandrova and Soloff, 1980; Fuchs et al., 1983). Oxytocin increases intracellular Ca^{2+} levels by influencing the influx of Ca^{2+} (Kuriyama and Suzuki, 1976) and by stimulating the production of inositol-trisphosphate ($InsP_3$) to release Ca^{2+} from storage sites (Marc et al., 1986; Carsten and Miller, 1985). Like oxytocin, endothelin is also a peptide which has been isolated from the supernatant fraction of cultured vascular endothelial cells (Yanagisawa et al., 1988). It has been reported that endothelin-1 is a more potent stimulator than other endothelins to induces rhythmic contraction of the rat uterus at lower concentrations and produce sustained contractions at higher concentrations. These studies also indicate that the pregnant myometrium is more sensitive to endothelin-1 compared to the non-pregnant myometrium (Sakata and Karaki, 1992; Yallampalli and Garfield, 1994). Thus, endothelin, like oxytocin, is suggested to be an important modulator of uterine contractility. Ca^{2+} channel blockers have been shown to inhibit endothelin-1-induced contractions suggesting the involvement of Ca^{2+} influx mechanism in the action of endothelin-1 (Kozuka et al., 1989). It has been reported that endothelin-1 increases phosphoinositide hydrolysis in the rat uterus (Bousso-Mittler et al., 1989) but that endothelin-1 does not release Ca^{2+} from internal storage sites in pregnant rat myometrium (Sakata and Karaki, 1992). In addition, endothelin-1 is reported to increase Ca^{2+} sensitivity for contractile force in rabbit mesenteric artery in permeabilized muscle strips (Nishimura et al., 1992).

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However, endothelin-1 did not increase Ca^{2+} sensitivity for contractile force in studies of intact pregnant rat myometrium using Ca^{2+} indicators (Sakata and Karaki, 1992). Thus, the mechanism of endothelin-1-induced myometrial contraction has not yet been clearly defined.

In order to clarify the mechanism of endothelin-1-induced contraction, we examined the contractile properties of endothelin-1 and compared them to those of oxytocin in intact and β-escin-treated skinned strips of pregnant rat myometrium. The results are discussed in relationship to the source of Ca^{2+} for contraction and the Ca^{2+} sensitization of contractile proteins.

2. Material and methods

2.1. Tissue preparations

Pregnant female rats of the Sprague-Dawley strain (10–16 weeks after birth, 180–240 g) were used in this experiment. In delivering rats most young were born between the morning and evening of the 22nd day of gestation. Animals were used at mid gestation (14–16 days), late gestation (17–19 days) and during delivery. The dams were killed by CO_2 inhalation. Excised uteri were dissected immediately from the mesometrium side in the longitudinal direction and fetuses and placentas were carefully removed. An overdose of halothane was given to kill the fetuses. Myometrial tissues were prepared in Krebs solution at room temperature while bubbling with 95% O_2 and 5% CO_2. To prepare the longitudinal muscle strips, the circular muscle layer and the endometrium were carefully removed, with the aid of a dissection microscope.

2.2. Recording of mechanical responses

The procedures are similar to those used previously (Iino, 1981; Itoh et al., 1981, 1986; Izumi, 1985; Kanmura et al., 1988). A segment of tissue (0.05–0.07 mm in width, 0.02–0.03 mm in thickness and about 0.7 mm in length) was mounted horizontally in an experimental recording chamber with a capacity of 0.9 ml, and the tissue was superfused with Krebs solution. Both ends of the preparation were tied with fine silk fibers which were then fixed to pieces (about 1 mm × 2 mm) of Scotch double-sided adhesive tape (3M Co., St. Paul, MN, USA) and isometric tension was recorded with a strain gauge transducer (U-gauge, Shinko, Tokyo, Japan). A resting tension of less than 1 mg was applied to obtain the maximum contractile force produced by K^+, oxytocin and endothelin-1 to eliminate possible involvement of elastic components on the tension measurements. The test solution was applied by jetting from one end of the chamber and the solution in the chamber was siphoned from the other end (Itoh et al., 1981; Izumi, 1985). To eliminate artifacts due to the sudden change in level of the solution in the chamber, recovery of the recording pen to the original level was checked prior to the start of the experiment (Itoh et al., 1981). As oxytocin and endothelin-1 induced rhythmic contractions at lower concentrations (oxytocin, under 1 nM; endothelin, under 10 nM) in late gestation and during delivery and the amplitudes of rhythmic contractions were almost the same at these concentrations, these agonists were applied cumulatively at lower concentrations. At higher concentrations, as these agonists produce phasic followed by sustained contractions, so they were applied at the interval of 5 min to obtain the maximum responses. To permit membrane permeabilization and 'chemical clamping of $[Ca^{2+}]_i$', the strips were skinned by exposure to 20 μM β-escin for 30 min in the relaxing solution, using the method described previously (Kobayashi et al., 1989). The Ca^{2+}-induced contractions of these skinned tissues were determined at different concentrations of Ca^{2+}, using 4 mM EGTA buffer solutions (Itoh et al., 1981; Izumi, 1985). To avoid Ca^{2+} uptake by the mitochondria of the skinned fibers, the mitochondrial inhibitor NaN_3 (5 mM) was present throughout the experiments (Kanmura et al., 1988). Ionomycin (1 μM) was added to deplete intracellular Ca^{2+} stores for investigation of the effects of Ca^{2+} on the contractile proteins (Fujiwara et al., 1989). As deterioration of the skinned muscle occurred at temperature over 25° C, the temperature in the intact and skinned muscle tissues was kept at 25° C (Iino, 1981; Itoh et al., 1981; Izumi, 1985). The effects of GTP and GTPγS on 0.3 μM Ca^{2+}-induced contractions were expressed as ED_{50} values, where ED_{50} was the molar concentration producing 50% of the maximum drug response. They were obtained by fitting the data for each dose-response curve using commercially available software (Kaleide Graph, Abelbeck Software, PA, USA) for Macintosh Computer (Apple Co.).

2.3. Solutions and drugs

The ionic composition of the modified Krebs solution was as follows (mM): Na^+, 137.4; K^+, 5.9; Mg^{2+}, 1.2; Ca^{2+}, 2.6; HCO_3^-, 15.5; $H_2PO_4^-$, 1.2; Cl^-, 134.2; and glucose, 11.5. A mixture of 95% O_2 with 5% CO_2 was bubbled into the solution. High-$[K]_o$ solution was prepared by replacing NaCl with KCl isosmotically. The pH of the solution was 7.3–7.4. The ionic composition of the relaxing solution was as follows: 100 mM K methanesulphonate, 20 mM Tris-maleate, 5.1 mM Mg methanesulphonate, 5.2 mM ATP, 4 mM ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*-tetraacetic acid (EGTA) and 10 mM phosphocreatine. Solutions of various Ca^{2+} concentrations were prepared by adding appropriate amounts of Ca methanesulphonate to 4

mM EGTA and the amount of K methanesulphonate was changed to keep the ionic strength (0.2 M). The pH of the relaxing and various Ca^{2+} solutions was kept at 7.1 at 25° C with KOH. The apparent binding constant of EGTA for Ca^{2+} was considered to be 1×10^6 M^{-1}. The calculated free Mg^{2+} was 1 mM and $MgATP^{2-}$ was 4 mM as described previously (Itoh et al., 1986).

Drugs used in the present experiments were as follows: oxytocin, endothelin-1, adenosine 5′-triphosphate (ATP), ethylene-glycol-bis-(−)-aminoethyl-ether)-*N*,*N*,*N*,*N*-tetraacetic acid (EGTA), verapamil, nifedipine (used protected from light), indomethacin (used protected from light), β-escin, guanosine 5′-*O*-thiotriphosphate (GTP), guanosine 5′-(γ-thiotriphosphate) (GTPγS), guanosine 5′-*O*-(β-thiodiphosphate) (GDPβS), phosphocreatine, sodium azide (NaN_3) and ionomycin all purchased from Sigma. Nifedipine and indomethacin were originally dissolved in ethyl alcohol, then added in Krebs solution. β-Escin and ionomycin were originally dissolved in dimethyl sulfoxide (DMSO), then added in relaxing solution. The final concentrations of ethyl alchol and DMSO were below 0.1%. All other chemicals were of the highest reagent grade.

2.4. Statistics

The results obtained are expressed as the mean ± standard error of the mean (S.E.M.). The *n* values represent the number of specimens. The statistical significance of the difference between mean values was assessed by a Student's *t*-test, and probability values of $P < 0.05$ were considered to be significant.

3. Results

3.1. Effects of K^+, oxytocin and endothelin-1 on contractions of intact myometrial strips

Thin muscle preparations were obtained from the longitudinal muscle layer of pregnant rat myometrium at three different stages of pregnancy: mid (day 14–16 gestation), late (17–19 gestation) and during delivery at term. KCl 118 mM was used to produce maximum contractions of preparations from each stage of pregnancy. Submaximal responses were obtained using KCl 10.7 mM. Contractions evoked by this concentration, expressed as a fraction of the response obtained using KCl 118 mM differed in tissues from the three groups, i.e., the values were 0.39 ± 0.08 at the mid gestation ($n = 7$), 0.49 ± 0.06 at the late gestation ($n = 10$) and 0.78 ± 0.08 at delivery ($n = 9$). Thus, preparations taken at delivery were more sensitive to the low concentration of KCl than those taken at other times ($P < 0.01$).

Spontaneous contractions were irregular at the mid and late stage and were regular for long periods during delivery. Oxytocin (10 pM to 1 nM) caused concentration-dependent increases in the frequency of sponta-

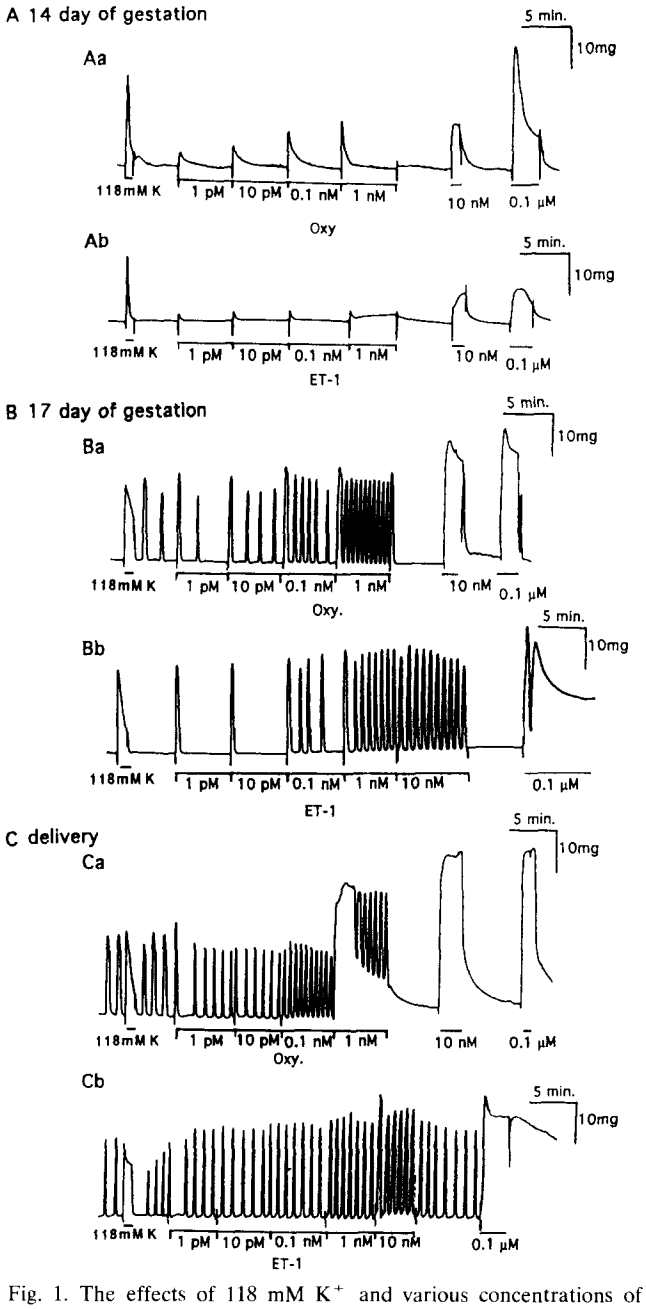

Fig. 1. The effects of 118 mM K^+ and various concentrations of oxytocin (Oxy) on intact longitudinal muscle strips of rat myometrium at mid gestation (day 14) (Aa), late gestation (day 17) (Ba) and during delivery (Ca) and the effects of various concentrations of endothelin-1 (ET-1) on rat myometrial strips in the mid gestation (Ab), the late gestation (Bb) and at delivery (Cb). Vertical lines on contractile patterns represent change in bath solutions. As shown in lines beneath contractile tracings, indicating period of the agent's application, oxytocin or endothelin-1 was cumulatively applied at every 5 min below the concentration of 10 nM and high concentrations (10 nM–0.1 μM) of agents were added at intervals of 5 min for shorter periods. These results are typical to those from 7–10 animals.

neous contractions in myometrial smooth muscle strips from late gestation and during delivery. More frequent contractions were obtained from 0.1 nM at day 17 to 1 pM during delivery (Fig. 1Ba and 1Ca). However, rhythmic contractions were not evoked at mid gestation (Fig. 1Aa). Endothelin-1 also increased the frequency of contractions at concentrations from 0.1 nM to 10 nM at late gestation and during delivery (Fig. 1Bb and 1Cb), but rhythmic contractions were also not evoked at mid gestation (Fig. 1Ab). Transient followed by tonic mechanical responses were produced by oxytocin at concentrations over 1 pM at mid gestation and over 10 nM at late gestation and during delivery. Endothelin-1 also produced a transient followed by a tonic contraction at concentrations of 1 pM at mid gestation and 0.1 μM at other stages of gestation. The maximum contractions induced by oxytocin or endothelin-1 were both obtained at concentrations of 0.1 μM at all stages. Table 1 shows the relative tensions of 0.1 μM oxytocin- and 0.1 μM endothelin-1-induced contractions compared to the 118 mM K^+-induced contractions in each stage. The relative tension induced by 0.1 μM oxytocin and 0.1 μM endothelin-1 at delivery was significantly larger than those at late gestation ($P < 0.05$) and those of late gestation was significantly larger than those at mid gestation ($P < 0.05$). Thus, the maximum relative tension induced by oxytocin and endothelin-1 compared to the 118 mM K^+-induced contractions increased during the progress of gestation. Furthermore, the maximum contractions induced by oxytocin were significantly larger than those induced by endothelin-1 at the delivery ($P < 0.05$).

3.2. *Effects of oxytocin and endothelin-1 in the presence of Ca^{2+} channel blockers (verapamil and nifedipine) and Ca^{2+}-free solution at delivery*

Fig. 2 shows the effects of Ca^{2+} channel blockers and Ca^{2+}-free solutions on oxytocin- and endothelin-1-induced contractions of rat longitudinal muscle strips at delivery. Five minutes pre-treatment with 10 μM nifedipine abolished spontaneous contractions and 118 mM K^+-induced contractions. However, the phasic parts of oxytocin-induced contractions (0.1 nM–0.1 μM) and endothelin-1-stimulated contractions (10 nM–0.1 μM) were resistant to pre-treatment with 10 μM nifedipine (Fig. 2A and 2B). The recovery from

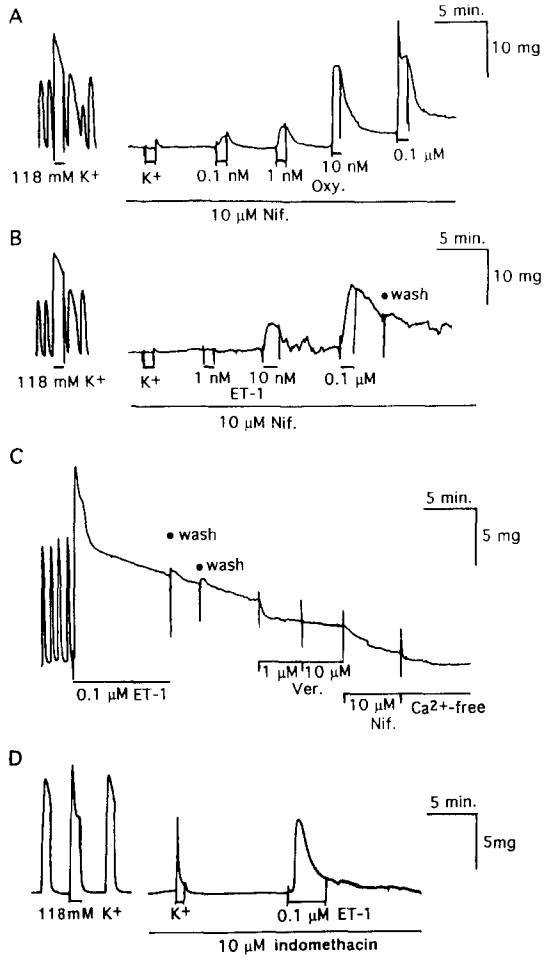

Fig. 2. Representative contractile tracings of the effects of 118 mM K^+, oxytocin (Oxy) and endothelin-1 (ET-1) on longitudinal muscle strips of rat myometrium at delivery in the presence of 10 μM nifedipine (Nif, A and B). 10 μM nifedipine was added 5 min before application of 118 mM K^+. Various concentrations of oxytocin and endothelin-1 were applied for 1 min at intervals of 5 min. Verapamil (Ver, 1–10 μM), nifedipine (10 μM) and Ca^{2+}-free solution (2 mM EGTA containing) were also applied to the relaxing phase of 0.1 μM endothelin-1-induced contractions (C). D shows the effects of 10 μM indomethacin on 118 mM K^+- and 0.1 μM endothelin-1-induced contractions. These results are typical to those from five animals.

the contraction tone after removal of a high concentration (0.1 μM) of endothelin-1 needed a prolonged time in spite of repeated washings (Fig. 2B and 2C). Ca^{2+} channel blockers accelerated relaxation from contractions induced by endothelin-1 in the myometrium; nifedipine was more effective than verapamil. Ca^{2+}-free

Table 1
The maximum amplitudes of contractions induced by oxytocin and endothelin-1 compared to the amplitude of 118 mM K^+ contractions

	Oxytocin (0.1 μM)	Endothelin-1 (0.1 μM)	Number of animals
Mid gestation	1.01 ± 0.14	0.38 ± 0.11	7
Late gestation	1.42 ± 0.10	1.10 ± 0.04	10
During delivery	1.85 ± 0.11	1.38 ± 0.11	9

The amplitudes of responses to 118 mM K^+ at each stage of gestation were used as 1.0 to normalize other values. Values represent the means ± S.E.M., n = 7–10 animals.

solution (containing 2 mM EGTA) was further effective in relaxing the contractions (Fig. 2C). With 30 min pre-treatment with 10 μM indomethacin, 118 mM K^+- and endothelin-1-induced contractions were reduced in amplitudes and the tone almost vanished after removal of endothelin-1 (Fig. 2D). 118 mM K^+-induced contractions were also abolished in Ca^{2+}-free (2 mM EGTA containing) solution as in the presence of 10 μM nifedipine (Fig. 3). However, both oxytocin (10 nM–0.1 μM) and endothelin-1 (10 nM–0.1 μM) evoked contractions in Ca^{2+}-free solution. Oxytocin contractions (0.1 μM) and endothelin-1 contractions (0.1 μM) in Ca^{2+}-free solutions were 0.53 ± 0.10 times (oxytocin) and 0.18 ± 0.03 times (endothelin-1) the amplitudes of 118 mM K^+-induced contractions in the presence of extracellular Ca^{2+}. Oxytocin produced larger contractions ($P < 0.01$) than endothelin-1 in Ca^{2+}-free solution.

3.3. Effects of GTP, GTPγS, oxytocin and endothelin-1 in skinned muscle strips at delivery

In a relaxing solution containing high K^+ with ATP, application of Ca^{2+} did not produce any contraction. When muscle strips were treated with 20 μM β-escin (to make skinned muscle cells), 0.3 μM Ca^{2+} produced bi-phasic contractions: a large phasic, followed by a maintained tonic contraction, presumably by the formation of a Ca^{2+}-calmodulin complex to activate myosin light chain kinase (MLCK). Relaxation occurred after solutions were changed to 4 mM EGTA containing Ca^{2+}-free solutions. In the skinned strips of pregnant myometrial strips at delivery, GTP (1 μM–1 mM) or GTPγS (0.1–100 μM) alone enhanced the tonic contraction induced by 0.3 μM Ca^{2+}, in a concentration-dependent manner (Fig. 4). The maximum increases in tension by GTP or GTPγS were obtained at concentrations of 1 mM and 100 μM, and maximum tensions were enhanced to 2.57 ± 0.17 times control and 3.59 ± 0.22 times control, respectively. Fig. 4B summarizes dose (GTP or GTPγS)-response (increase of tension of 0.3 μM Ca^{2+}-induced contractions of skinned strips) relationships. The ED_{50} value of GTP was 0.51 ± 0.06 mM and that of GTPγS was 1.07 ± 0.04 μM.

Fig. 5 shows the effects of GTP, oxytocin and endothelin-1 on the contractions evoked by 0.3 μM Ca^{2+} in skinned myometrial strips at delivery. GTP (10 μM) enhanced the tonic contraction of 0.3 μM Ca^{2+}-induced contractions. Oxytocin (1 nM–0.1 μM) further increased the Ca^{2+}-induced tonic contraction concentration dependently (Fig. 5Aa). GDPβS (1 mM, an inhibitor of activation of G-proteins) blocked the increase in action of oxytocin with GTP (data not shown). However, similar effects of endothelin-1 on the 0.3 μM Ca^{2+}-induced contractions were not observed (Fig. 5Ab). Fig. 5B summarizes the tension of 0.3 μM Ca^{2+}-induced contractions and the tension levels increased

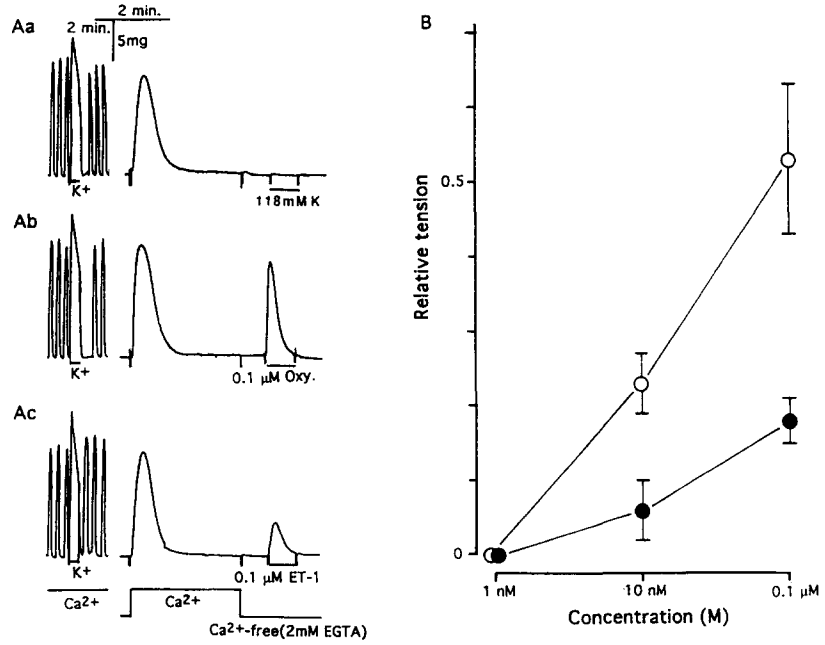

Fig. 3. Representative contractile tracings of the effects of 118 mM K^+ (Aa), oxytocin (Oxy, Ab) and endothelin-1 (ET-1, Ac) on longitudinal muscle strips of rat myometrium at delivery in Ca^{2+}-free solution (containing 2 mM EGTA). After Ca^{2+} storage sites were depleted, Ca^{2+} was loaded to store sites in Ca^{2+} containing Krebs solution for 5 min. Then either 118 mM K^+, oxytocin or endothelin-1 was applied 1 min after exposure to Ca^{2+}-free solutions. B summarizes the amplitudes of contractions induced by oxytocin (○) and endothelin-1 (●) in Ca^{2+}-free solution compared to the amplitudes of 118 mM K^+-induced contraction in 2.6 mM Ca^{2+}-containing (Krebs) solution. The amplitudes of responses to 118 mM K^+ in each strip were used as 1.0 to normalize other values. Values represent the means; vertical bars indicate S.E.M., $n = 7$ animals.

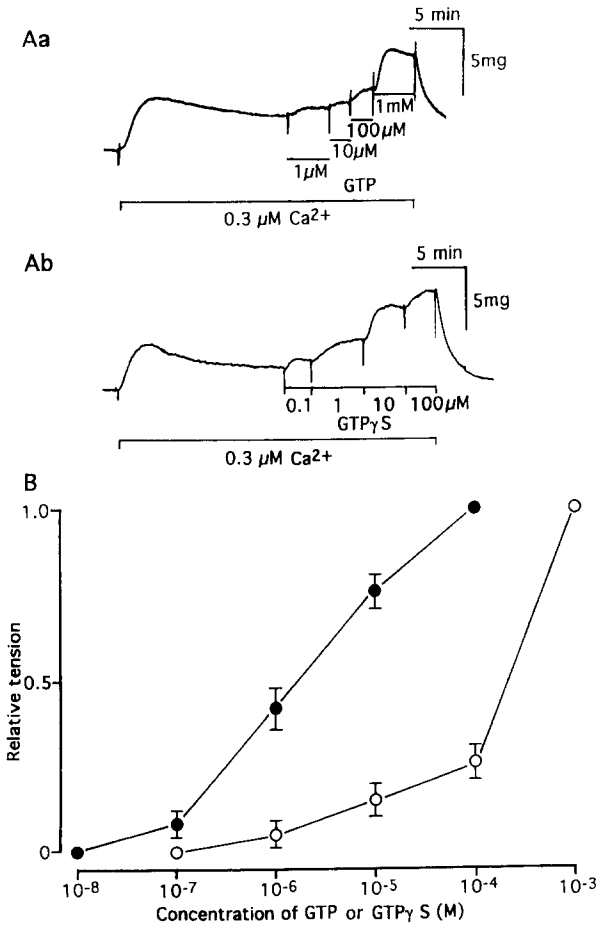

Fig. 4. The effects of GTP (Aa) and GTPγS (Ab) on the tonic level of 0.3 μM Ca^{2+}-induced contractions in β-escin-treated and skinned myometrial strips at delivery. Various concentrations of GTP (1 μM–1 mM) and GTPγS (0.1–100 μM) were applied to the 0.3 μM Ca^{2+}-induced contractions (Aa and Ab) cumulatively until the tension reached the peak of contraction. B shows the dose-response curves for GTP (○) and GTPγS (●). The distances between the steady tonic level of 0.3 μM Ca^{2+}-induced contractions and the maximum responses by GTP or GTPγS were used as 1.0 to normalize other values. Values are the means; vertical bars indicate S.E.M., $n = 9$ animals.

by 10 μM GTP, 0.1 μM oxytocin with 10 μM GTP and 0.1 μM endothelin-1 with 10 μM GTP. The tonic tension level by 0.3 μM Ca^{2+} was used as 1.0 to normalize other values. The tension level with 10 μM GTP compared to the control was 1.37 ± 0.08, whereas the tension level of 0.1 μM oxytocin with 10 μM GTP was 1.79 ± 0.09 and that of 0.1 μM endothelin-1 with 10 μM GTP was 1.39 ± 0.04. Oxytocin but not endothelin-1 significantly increased the 0.3 μM Ca^{2+}-induced contractions in the presence of 10 μM GTP ($P < 0.01$).

4. Discussion

Contractions produced by high concentrations of K^+ are presumed to be induced by voltage-dependent Ca^{2+} influx because they are inhibited in the presence of voltage-dependent Ca^{2+} channel blockers, such as nifedipine and verapamil, as reported previously (Kanmura et al., 1983). The amplitude of contractions by 10.7 mM K^+ increase during delivery. These results confirm earlier data showing that membrane properties to evoke potential-dependent contractions become more sensitive during delivery, suggesting an increase of Ca^{2+} channels at term (Casteels and Kuriyama, 1965). In addition, oxytocin and endothelin-1 at low concentrations increase the frequency of spontaneous contractions and high concentrations of these agents produce large phasic followed by tonic contractions (Fig. 1). The sensitivity to evoke rhythmic contractions and the maximum amplitudes induced by oxytocin and endothelin-1 compared to the 118 mM K^+ contraction

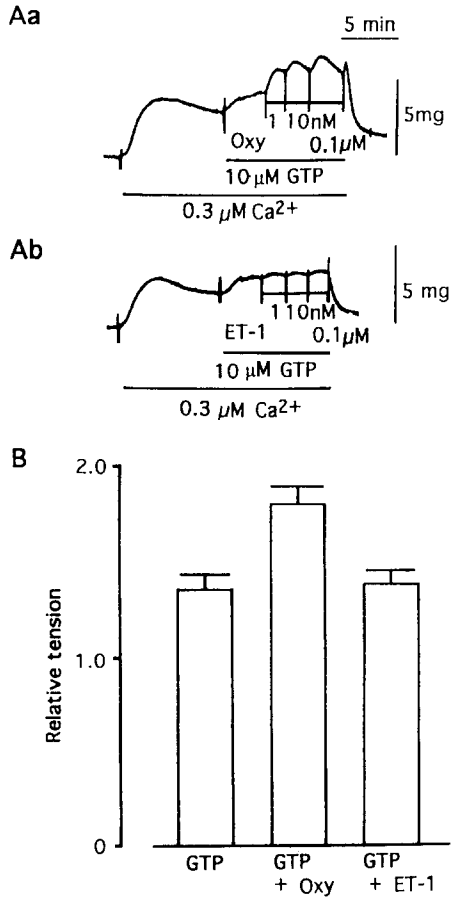

Fig. 5. The effects of oxytocin (Oxy, Aa) and endothelin-1 (ET-1, Ab) on the 0.3 μM Ca^{2+}-induced contractions in β-escin-treated and skinned myometrial strips at delivery. After 0.3 μM Ca^{2+}-induced contractions reached a steady state, oxytocin (1 nM–0.1 μM) and endothelin-1 (1 nM–0.1 μM) were applied to the contractions in the presence of 10 μM GTP. B shows the effects of 10 μM GTP and oxytocin or endothelin-1 with 10 μM GTP on the tension level of steady state in 0.3 μM Ca^{2+}-induced contractions in skinned strips. The steady-state tension levels of 0.3 μM Ca^{2+}-induced contractions were used as 1.0 to normalize other values. Values are the means; vertical bars indicate S.E.M., $n = 9$ animals.

increase during the progress of gestation (Fig. 1 and Table 1). The maximum relative tension induced by oxytocin and endothelin-1 compared to the 118 mM K^+-induced contractions also increases during the advance of gestation (Table 1). Thus, the maximum contractions induced by oxytocin and endothelin-1 per cross-sectional area may also be magnified during the progress of gestation because the amplitude of 118 mM K^+ contraction per cross-sectional area increases during the advancement of gestation (Izumi, 1985). These data are consistent with observations that the number of receptors for oxytocin and endothelin-1 increases with the progression of pregnancy (Alexandrova and Soloff, 1980; Sakata and Karaki, 1992).

It has been reported that oxytocin and endothelin-1 activate voltage-dependent Ca^{2+} channels (Kuriyama and Suzuki, 1976; Kozuka et al., 1989). Nifedipine abolished spontaneous contractions, high K^+ contractions and rhythmic contractions by oxytocin and endothelin-1 (Fig. 2). However, oxytocin and endothelin-1 produce phasic contractions in the presence of nifedipine. This might indicate that oxytocin and endothelin-1 evoke contractions via receptor-operated Ca^{2+} influx (insensitive to Ca^{2+} channel blockers) or via Ca^{2+} release from intracellular storage sites. Furthermore, pre-treatment with indomethacin was also effective in decreasing the tension induced by endothelin-1 (Fig. 2D). This suggests that endogenous prostaglandin production is related to endothelin-1 contraction as in the case of oxytocin (Moritoki et al., 1979).

Ca^{2+} release from intracellular storage sites has been considered for triggering contraction in uterine smooth muscle, like in various other smooth muscles (Lalanne et al., 1984; Izumi, 1985). It is recognized in myometrial tissues that oxytocin stimulates the synthesis of inositol-1,4,5-trisphosphate ($InsP_3$) through hydrolysis of phosphatidyl inositol-4,5-bisphosphate (Marc et al., 1986). $InsP_3$ was reported to release Ca^{2+} from uterine microsomes (Carsten and Miller, 1985) and produce contraction in skinned myometrial strips (Kanmura et al., 1988; Savineau et al., 1988). It has also been shown that sarafotoxin, which is structurally and functionally similar to endothelin-1, generates $InsP_3$ in the rat myometrium (Bousso-Mittler et al., 1989). Thus, it is plausible that endothelin-1, like oxytocin, releases Ca^{2+} from internal storage sites in pregnant rat myometrium as reported for human myometrium (Word et al., 1990). In our experiments, endothelin-1 produced contraction in 2 mM EGTA containing Ca^{2+}-free solution (Fig. 3), while it has been reported in pregnant rat myometrium that endothelin-1 does not produced contraction 10 min after the application of 0.5 mM EGTA containing Ca^{2+}-free solution (Sakata and Karaki, 1992). The differences between previous studies and our observations are perhaps due to the different sizes of tissue preparations. In general, the contractions by Ca^{2+} released from internal storage sites were studied using small-sized strips (Itoh et al., 1981; Lalanne et al., 1984; Kanmura et al., 1988). Our data suggests that oxytocin and endothelin-1 contribute to the increased responsiveness for contractile force compared to high concentrations of K^+, by generating $InsP_3$ which releases internal stores of Ca^{2+}.

Recently, it has been reported from studies of intact smooth muscle using Ca^{2+} indicators that the tension-Ca^{2+} relationship was more sensitive in agonist-induced contractions than K^+-induced contractions (Himpens and Casteels, 1987). In permeabilized muscle strips (skinned fibers), it has been demonstrated that agonists with GTP, GTP or GTPγS alone increase Ca^{2+} sensitivity for contractile force of smooth muscle (Kitazawa et al., 1989,1991; Fujiwara et al., 1989; Nishimura et al., 1992). In our experiments, the maximum contractions by agonists were larger than 118 mM K^+-induced contraction (Table 1) as reported previously (Izumi, 1985; Izumi et al., 1990). Thus, it is plausible that agonists such as oxytocin or endothelin-1 increase Ca^{2+} sensitivity for contractile force also in pregnant rat myometrium. In this study, GTP (1 μM–1 mM) or GTPγS (0.1–100 μM) increases the tension level induced by 0.3 μM Ca^{2+} in β-escin-treated skinned strips. Thus, these data support the concept that G-protein-mediated Ca^{2+} sensitization systems also exist in pregnant myometrium. Furthermore, oxytocin with 10 μM GTP increased 0.3 μM Ca^{2+}-induced contractions in skinned myometrial strips (Fig. 5Aa) and the action of oxytocin on 0.3 μM Ca^{2+}-induced contractions was inhibited by GDPβS, a non-hydrolyze analogue of GDP (data not shown). Thus oxytocin increases Ca^{2+} sensitivity of contractile force at delivery. However, endothelin-1 did not enhance the Ca^{2+} sensitivity (Fig. 5Ab). These data support previous observations of intact myometrial strips using fura-2 methods showing that the relationship between $[Ca^{2+}]_i$ and muscle tension in the presence of endothelin-1 is identical to that in the presence of high K^+ in the pregnant rat uterus (Sakata and Karaki, 1992). Thus at delivery, oxytocin coupled to its receptor may enhance the contractile force produced by the myofilaments with the same concentration of Ca^{2+} as prior to delivery, but endothelin-1 does not have this ability.

The maximum contractile force generated by oxytocin was greater than that produced by endothelin-1 in Krebs solution during delivery (Table 1). At delivery, the amplitudes of contractions induced by oxytocin in Ca^{2+}-free solutions were also larger than those induced by endothelin-1 (Fig. 3), which may indicate that oxytocin releases more Ca^{2+} from storage sites than endothelin-1. In addition, oxytocin, but not endothelin-1, increased the sensitivity for contractile force in the myometrium of rats during delivery (Fig. 5). This suggests that oxytocin produces larger contractions with

the same amount of Ca^{2+}. These contractile properties of oxytocin compared to endothelin-1 might be the reason that maximum oxytocin contractions were greater than endothelin-1 contractions in intact myometrial strips.

In conclusion, the contractile sensitivity of pregnant rat myometrium for endothelin-1 and oxytocin and maximum contractions by these agonists increased during the progress of gestation suggesting an increased number of receptors for these agents. The data also suggest that both oxytocin and endothelin-1 activate Ca^{2+} channels to increase voltage-dependent Ca^{2+} influx and receptor-operated Ca^{2+} influx or Ca^{2+} release from storage sites. However, the Ca^{2+}-releasing effect of oxytocin is greater than that by endothelin-1. In addition, the results indicate that oxytocin but not endothelin-1 increases Ca^{2+} sensitivity for contractile development.

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