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Estradiol inhibits Ca^{2+} and K^+ channels in smooth muscle cells from pregnant rat myometrium

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Abstract

The purpose of this study was to investigate the actions of 17β-estradiol on the electrical activity of pregnant rat myometrium. The longitudinal layer of the myometrium was dissected from pregnant rats (17 to 19 days of gestation), and single cells were isolated by enzymatic digestion. Calcium currents and potassium currents were recorded by the whole-cell voltage-clamp method, and the single calcium-dependent potassium current was recorded by the outside-out patch-clamp method. The effects of 17β-estradiol on these currents were investigated. When a myometrial cell was held at −50 mV, depolarization to a potential more positive than −30 mV produced an inward current followed by a slowly developing outward current. Application of tetraethylammonium inhibited the outward current while the inward current was completely abolished in a calcium-free solution. Estradiol at high concentrations (> 3 μM) inhibited both inward and outward currents in a voltage-dependent manner. Removal of estradiol restored the amplitude of the outward but not of the inward current. Estradiol (30 μM) also inhibited the activity of single calcium-dependent potassium channels without changing single channel conductance. In conclusion, estradiol at high concentrations inhibited: (1) voltage-dependent calcium, (2) calcium-dependent potassium and (3) voltage-dependent potassium currents. These actions of estradiol would prevent action potential generation and after-hyperpolarizations. Suppression of the after-hyperpolarization might further prevent spike generation due to slowing of the calcium channel's recovery from the inactivated state.

Keywords: Estrogen; Rat myometrium; Action potential; Ca^{2+} channel; K^+ channel

1. Introduction

Differences in membrane properties between the stages of pregnancy in uterine smooth muscle cells are well documented. These differences may be mainly due to the hormonal changes during gestation. The uterus has to be quiescent during most of gestation to allow the fetus to grow, and change to be very active just before labor. The detailed mechanism of such dramatic changes in uterine activity is still obscure, estrogen is believed to be one of the most important factors that affect uterine contractility during pregnancy. Steroid hormones usually exert their effect by acting on cytosolic receptors through a genomic process, but acute actions of steroid hormones were also reported as follows. Osa and Ogasawara (1984) studied changes in the electrical and mechanical responses to ovarian hormone during various stages of pregnancy in rats. They concluded that estradiol suppressed not only spontaneously generated burst discharges and muscle contractions but also those evoked by electrical stimulation with the occurrence of slight membrane depolarization. For A7r5 cells, a cultured cell line from the rat aorta, Zhang et al. (1994) reported that estradiol suppressed the voltage-dependent calcium (Ca^{2+}) current. Collins et al. (1993) proposed that estradiol may act as a Ca^{2+} channel blocker in smooth muscle cells. Myometrium has tetrodotoxin-sensitive sodium (Na^+), dihydropyridine-sensitive Ca^{2+} and tetraethylammonium-sensitive potassium (K^+) currents (Jmari et al., 1987; Pressman et al., 1988; Inoue et al., 1990; Miyoshi et al., 1991; Young and Herdon-Smith, 1991). Recently, Yamamoto (1995) reported that, in pregnant rat myometrium, estradiol inhibited the barium (Ba^{2+}) current through Ca^{2+} channels in a voltage-dependent fashion. However, it is not clear whether suppression of voltage-dependent Ca^{2+} channels by estradiol is the sole mechanism for spike inhibition. It is very important to clarify the acute actions of estradiol on channel activity of

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pregnant uterus. It has been reported that chronic administration of estradiol to rats suppressed the expression of K^+ channels possibly via the genomic action of this steroid (Erulkar et al., 1994), but there are no reports about an acute inhibitory effect of estradiol on K^+ channels in pregnant rat myometrial cells. In this study, we used the voltage-clamp method to investigate the acute estrogen effects on the channel activity of single smooth muscle cells from the pregnant rat myometrium, and found that inhibition of both Ca^{2+} and K^+ currents is involved in the suppression of myometrial electrical activity by estradiol.

2. Materials and methods

2.1. Cell isolation

Pregnant Wistar rats (weighing 200–250 g, 17 to 19 days of gestation) were anesthetized with diethylether and bled from the carotid artery. Excised uteri were dissected immediately from the mesometrium side in the longitudinal direction, and fetuses and placentas were carefully removed. Single smooth muscle cells of myometrium were obtained by an enzymatic dispersion procedure similar to those reported previously (Okabe et al., 1987; Inoue et al., 1990). This stage of gestation in rats corresponds to late pregnancy in humans at which stage the serum level of estrogen is high. Furthermore, Osa and Ogasawara (1984) reported that the acute administration of estradiol suppressed rat uterine activity later than 17 days till 21 day of gestation to the same extent, therefore, we used rats at this stage of gestation. Briefly, the longitudinal muscle layer was carefully isolated under a binocular microscope, using fine scissors. Small segments of the longitudinal muscle were incubated for 40 min at 35°C in a Ca^{2+}-free physiological salt solution (PSS) containing 0.1% collagenase (Wako Pure Chemical, Osaka, Japan). Single cells were dispersed by gentle agitation with a glass pipette and cells were resuspended in fresh PSS containing 0.1% trypsin inhibitor (type IIS, Sigma, St. Louis, MO, USA) and 0.1% bovine serum albumin (essentially fatty acid-free: Sigma) at 10°C. The cells obtained contracted transiently in response to bath application of various agonists, such as acetylcholine (0.3 μM) or adenosine triphosphate (0.5 μM). All experiments were performed at 30°C.

2.2. Electrical recordings

The dispersed cells were put into a small chamber (0.2 ml in volume) on the stage of an inverted microscope

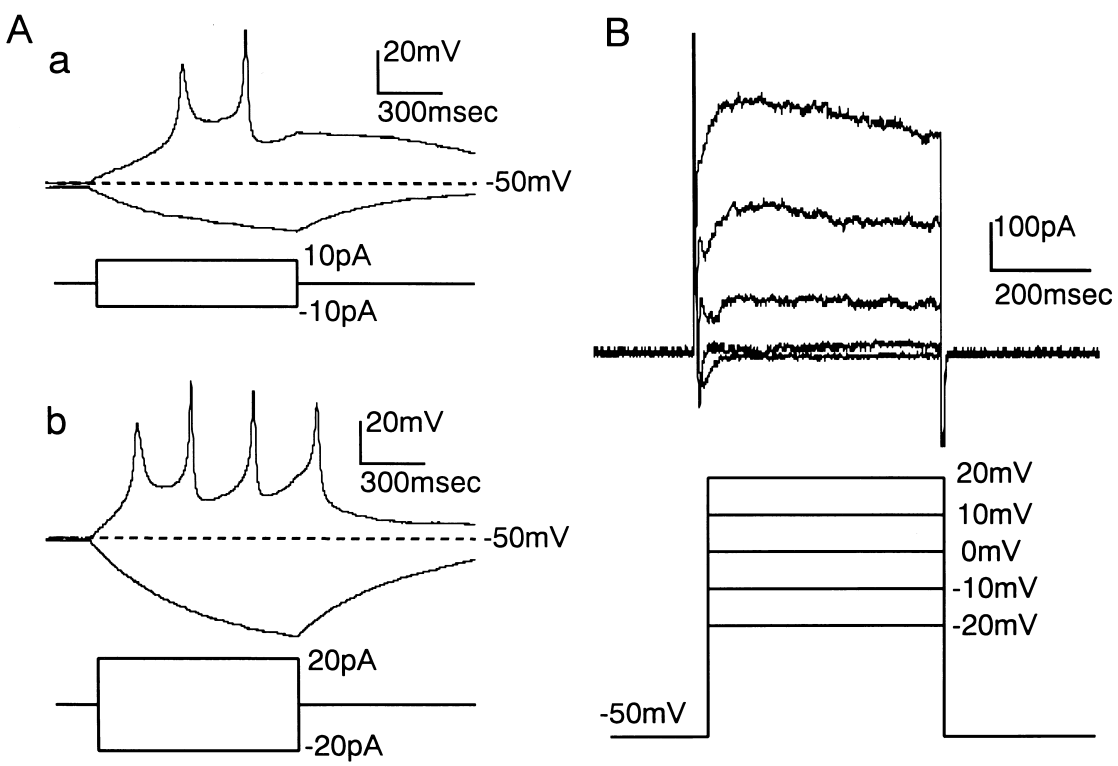

Fig. 1. Action potential and membrane currents recorded from single smooth muscle cell of pregnant rat longitudinal myometrium. The pipette was filled with high-K^+ solution (EGTA = 0.3 mM), and the bath was superfused with the bathing solution. To eliminate Na^+ current, tetrodotoxin (3 μM) was present in the bath solution. (Aa) Action potential evoked by current stimulation of 10 pA (1 s in duration). (Ab) Action potential evoked by current stimulation of 20 pA (1 s in duration). The resting membrane potential of this cell was −49 mV. (B) Traces of the whole-cell membrane currents evoked by depolarizing pulses to −20, −10, 0, 10 and 20 mV from the holding potential of −50 mV. The duration of the depolarizing pulse was 500 ms. (A) and (B) were recorded from the same cell.

(TMD-Diaphot, Nikon, Tokyo, Japan). The electrodes were advanced towards the cell with a three-dimensional micromanipulator (Manipulator-E, Leitz, Wetzlar, FRG). Patch electrodes (2–5 MΩ) were made using a double-stepped electrode puller (PP83, Narishige, Tokyo, Japan). Whole-cell voltage-clamp and current-clamp experiments were carried out with a standard patch-clamp amplifier (Axopatch 1D, Axon, CA, USA). Leak current was not subtracted in the whole-cell voltage-clamp experiments. The value of membrane capacitance was measured independently by using a short ramp pulse. To ensure isopotentiality during the voltage-clamp procedure, we excluded the cells whose capacitance was over 100 pF from this study. The mean membrane capacitance of the cells used was 74.6 ± 15.6 pF (mean $\pm$ S.D.; $n = 36$). Unitary K^+ current was recorded in the outside-out configuration (Hamill et al., 1981). Currents were filtered at 1 kHz and digitized at a sampling frequency of 2–5 kHz. Series resistance compensation (70–90%) was used to reduce the error in voltage at which the cell was clamped. Electrical responses were monitored on a high-gain oscilloscope and stored digitally on a personal computer (VC 10, Nihon Khoden, Tokyo, Japan; DeskPro, Compaq, Houston, TX, USA). Data were analyzed by pClamp software (version 5.0, Axon).

2.3. Solution

The ionic composition of the bathing solution was (in mM): NaCl 137, KCl 6, $CaCl_2$ 2.5, $MgCl_2$ 0.5 and glucose 10. A high-K^+ solution or high-Cs^+ solution of the following ionic composition was present in the pipette (in mM): High-K^+ solution; KCl 140, $MgCl_2$ 3, Na_2ATP (adenosine triphosphate disodium) 2 and EGTA (ethyleneglycol bis(b-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid) 0.3 (for Figs. 1 and 6) or 10 (for Figs. 2, 4 and 5); High Cs^+ solution; CsCl 140, $MgCl_2$ 3, Na_2ATP 2 and EGTA 10 (for Figs. 3 and 5). The high-K^+ solution was used as the pipette solution in order to elicit both inward and outward current simultaneously and the high-Cs^+ solution was used to isolate only the inward current by eliminating outward K^+ currents. The pH of the solutions was adjusted to 7.3–7.4 using 5 mM HEPES (*N*-2-hydroxyethylpiperazine) titrated with Tris (tris-(hydroxymethyl)aminomethane).

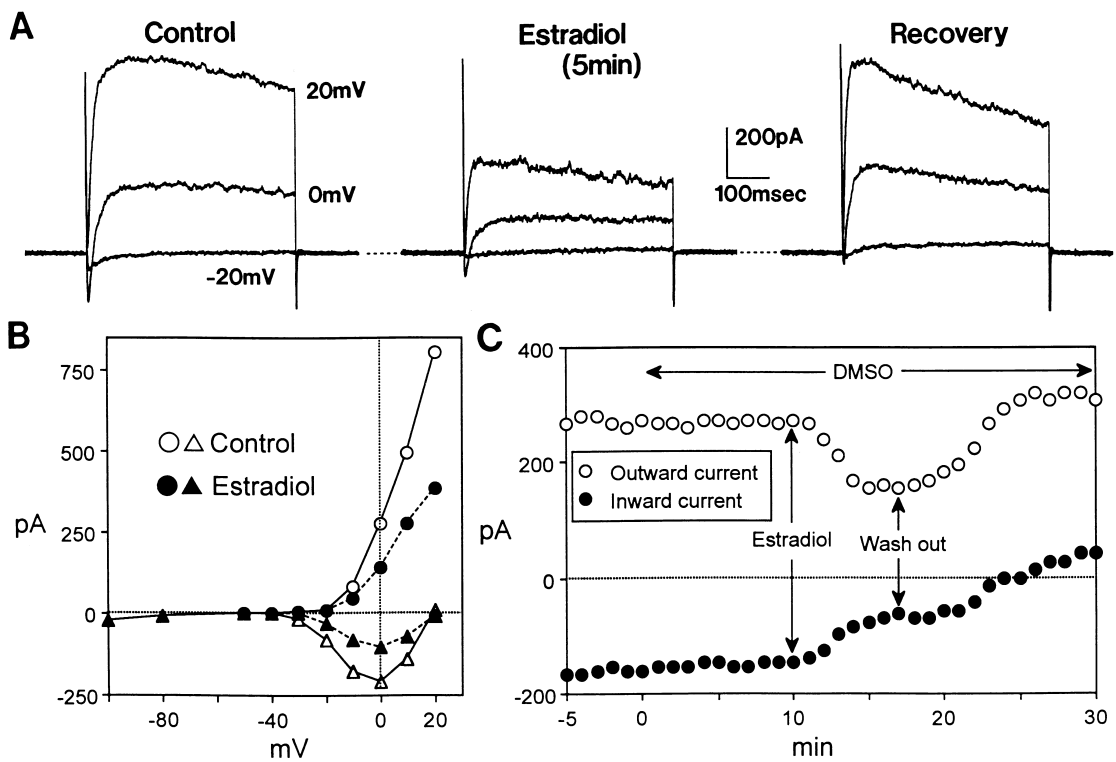

Fig. 2. Effects of estradiol (30 μM) on membrane currents evoked by depolarizing pulses in rat uterine myocytes. The holding potential was −50 mV and various amplitudes of depolarizing pulses were applied (500-ms duration). The pipette contained high-K^+ solution (EGTA = 10 mM) and the bath was superfused with bathing solution (see Section 2). (A) Traces of the membrane currents evoked by depolarizing pulses to −20, 0, and 20 mV before, during application (5 min) and after removal (20 min) of estradiol. (B) Current–voltage relationships of peak inward and outward currents in the presence and absence of estradiol. Open circles and open triangles are amplitudes of inward and outward currents recorded in bathing solution. Closed circles and closed triangles are in the presence of estradiol (30 μM). (C) Time course of amplitudes of peak inward (closed circles) and outward (open circles) currents before, during and after application of estradiol. The currents were evoked by pulses to 0 mV from a holding potential of −50 mV. Note that dimethylsulfoxide (DMSO; 0.03%) used as a solvent had no effect on either current. (A), (B) and (C) were obtained from the same cell.

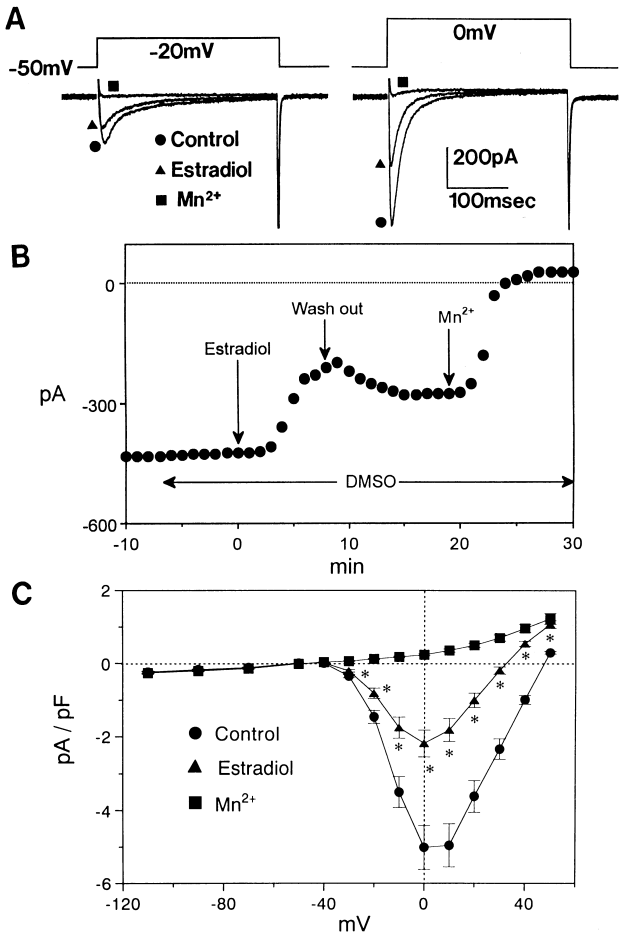

Fig. 3. Effects of estradiol (30 μM) on inward Ca^{2+} currents evoked by depolarizing pulses in rat myometrium. The holding potential was −50 mV and depolarizing pulses were applied (300 ms duration). The pipette contained high-Cs^{+} solution (EGTA = 10 mM) and the bath was superfused with bathing solution. To eliminate Na^{+} current, tetrodotoxin (3 μM) was present in the bath solution. (A) Traces of the membrane currents evoked by depolarizing pulses to −20 and 0 mV before (circles) and during (triangles) application of estradiol. Currents recorded in the presence of 2 mM Mn^{2+} are also shown (squares). (B) Time course of the amplitudes of peak inward current before, during and after application of estradiol and Mn^{2+}. Currents were evoked by depolarizations to 0 mV from a holding potential of −50 mV. Note that dimethylsulfoxide (DMSO; 0.03%) used as a solvent had no effect on inward current. (C) Current–voltage relationships of averaged peak inward current in the presence and absence of estradiol or Mn^{2+}. Closed circles are amplitudes of inward current recorded in the bathing solution and closed triangles and closed squares are in the presence of estradiol and Mn^{2+}, respectively (mean ± S.D.; $n = 5$). Asterisks mean a significant difference between the averaged peak amplitudes of inward Ca^{2+} current in the absence and presence of estradiol ($p < 0.05$).

2.4. Drugs

The following drugs were used: 17β-estradiol, tetrodotoxin (all from Sigma) and tetraethylammonium (Tokyo Kasei, Tokyo, Japan). Estradiol was dissolved in dimethylsulfoxide. The final concentration of dimethylsulfoxide in bathing solution was less than 0.03% and had no effect on the membrane currents.

2.5. Statistical analysis

The results are expressed as mean values with standard deviation (S.D.). Statistical significance was assessed with Student's *t*-test and *p*-values less than 0.05 were considered to be significant.

3. Results

Fig. 1A shows action potentials evoked by current stimulation recorded from a single longitudinal smooth muscle cell of pregnant rat myometrium in current-clamp mode. When high-K^{+} solution was in the pipette (EGTA = 0.3 mM) and bathing solution was superfused in the bath, the resting membrane potential of rat myometrial cells was −48.2 ± 3.5 mV (mean ± S.D.; $n = 11$). Depolarizing current stimulation of 10 pA evoked action potentials (Fig. 1Aa), and more trained action potentials could be evoked when 20-pA current stimulation was applied through the patch-clamp amplifier (Fig. 1Ab). A hyperpolarizing current could not evoke an action potential. When the same cell was switched form current-clamp to voltage-clamp mode, and depolarizing pulses were applied from the holding potential of −50 mV, there was a transient inward current followed by transient and sustained outward currents (Fig. 1B). These results suggest that the cells obtained were viable. Based on the results of this current-clamp study, we set the holding potential of rat myometrial cell at −50 mV in the subsequent voltage-clamp experiments.

Fig. 2A shows the effects of 30 μM 17β-estradiol on membrane currents evoked by depolarizing pulses to −20, 0 and 20 mV from a holding potential of −50 mV. When a high-K^{+} solution was in the pipette (EGTA = 10 mM), and the bathing solution containing Ca^{2+} was superfused, a transient inward current followed by a slowly developing outward current could be recorded (Fig. 2A, control). Estradiol inhibited both inward and outward currents evoked by these depolarizing pulses (Fig. 2A, estradiol and Fig. 2B). Removal of estradiol restored the peak amplitude of the outward current, however, it did not restore the inward current (Fig. 2A, recovery and Fig. 2C). In these experiments, 10 mM estradiol was dissolved in 100% dimethylsulfoxide, and cells were exposed to 0.03% dimethylsulfoxide when 30 μM estradiol was superfused. As shown in Fig. 2C, 0.03% dimethylsulfoxide did not affect the amplitudes of inward or outward currents in the myometrial cells of pregnant rats.

When pipettes with high-Cs^{+} solution were used, the outward current was abolished, leaving an inward current, suggesting that the outward current was mainly carried by K^{+}. When 30 μM estradiol was applied in the presence of 3 μM tetrodotoxin in the bath (in order to inhibit Na^{+} current), the peak amplitude of the inward current was

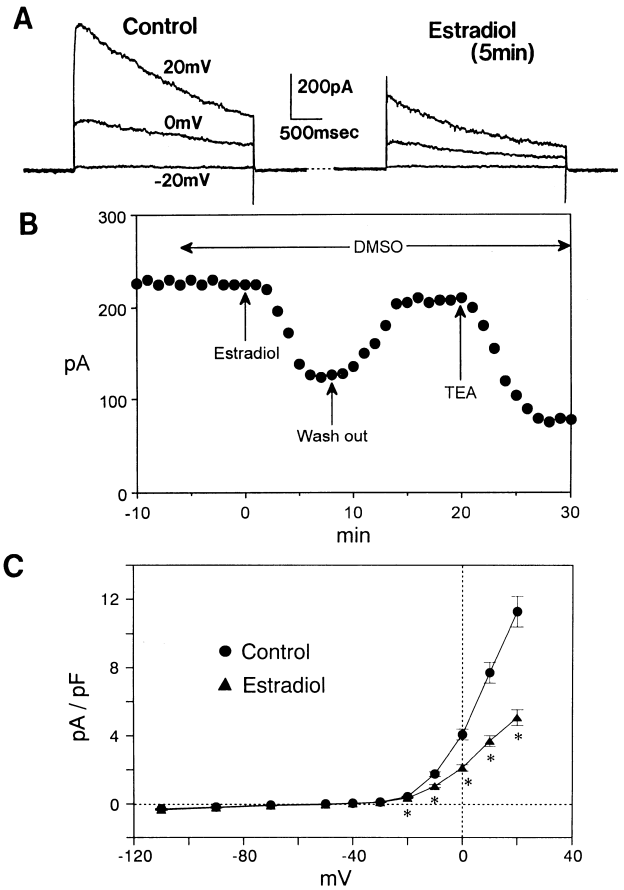

Fig. 4. Effects of estradiol (30 μM) on outward K^+ currents evoked by depolarizing pulses in the rat myometrium. The holding potential was −50 mV and depolarizing pulses were applied (2-s duration). The pipette contained high-K^+ solution (EGTA = 10 mM) and the bath was superfused with Ca^{2+}-free (2 mM Mn^{2+}) bathing solution. To eliminate Na^+ current, tetrodotoxin (3 μM) was present in the bathing solution. (A) Membrane currents evoked by depolarizing pulses to −20, 0 and 20 mV before (left traces) and 5 min during (right traces) application of estradiol. (B) Time course of the amplitudes of peak outward (closed circles) current before, during and after application of estradiol or tetraethylammonium (TEA). Currents were evoked by pulses to 0 mV from a holding potential of −50 mV. Note that dimethylsulfoxide (DMSO; 0.03%) used as a solvent had no effect on the outward current. (A) and (B) were obtained from the same cell. (C) Current–voltage relationships of averaged peak outward current in the presence (closed triangle) and absence (closed circle) of estradiol (mean ± S.D.; $n = 5$). Asterisks mean a significant difference between the averaged peak amplitudes of outward K^+ current in the absence and presence of estradiol ($p < 0.05$).

inhibited (Fig. 3A). The inhibitory effect of estradiol was more prominent on currents evoked by depolarizing pulses to 0 mV compared to −20 mV. Removal of estradiol partially restored the amplitude of the inward current (Fig. 3B). Further application of 2 mM Mn^{2+} to the bath completely blocked the inward current (Fig. 3A and 3B). These results suggest that this inward current was mainly carried by Ca^{2+}. Dimethylsulfoxide (0.03%) alone had no effects on amplitude or time course of the inward current. Fig. 3C shows the averaged current–voltage relationships of the peak inward Ca^{2+} current from longitudinal muscle cells of pregnant rats in the presence and absence of 30 μM estradiol or 2 mM Mn^{2+} ($n = 5$). The averaged peak amplitudes of the inward Ca^{2+} current in the presence of 30 μM estradiol were significantly smaller than those in the absence of estradiol at any depolarizing stimulations more than −40 to +50 mV. The inhibition of Ca^{2+} current by estradiol was more prominent at more depolarizing potentials, suggesting that the inhibition of Ca^{2+} current by estradiol may be voltage-dependent (Fig. 3C).

Using pipettes with the high-K^+ solution (EGTA = 10 mM) and Ca^{2+}-free (2 mM Mn^{2+}) bath solution (in order to eliminate inward Ca^{2+} current) with 3 μM tetrodotoxin, large outward currents but no inward currents were seen (Fig. 4A). Under these conditions, Ca^{2+} currents and Ca^{2+}-dependent K^+ currents were inhibited, leaving voltage-dependent K^+ currents. This outward current (voltage-dependent K^+ current) was inhibited to approximately 30% of the control by either 10 mM 4-aminopyridine (data not shown) or 3 mM tetraethylammonium (Fig. 4B). Estradiol (30 μM) reduced the peak amplitude of the outward current by half, and removal of estradiol restored this current (Fig. 4B). Fig. 4C shows the averaged current–voltage relationships of the peak delayed outward K^+ current from longitudinal muscle cells of pregnant rats in the presence and absence of 30 μM estradiol. The averaged peak amplitudes of the delayed K^+ current in the presence of 30 μM estradiol were significantly smaller than those in the absence of estradiol at any depolarizing stimulations more than −20 to +20 mV. The inhibition of the outward currents by estradiol was more prominent with

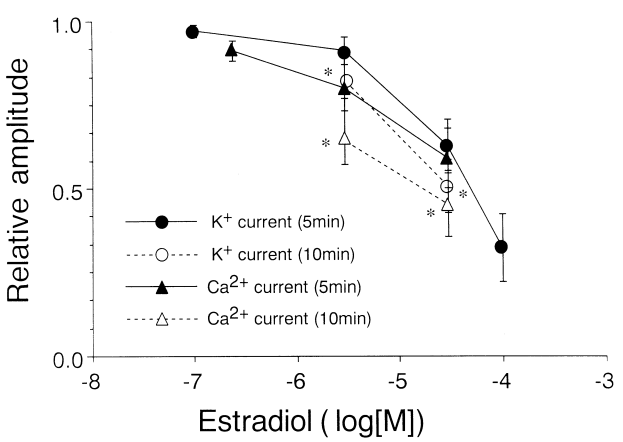

Fig. 5. Dose–response relationship between estradiol concentration and relative amplitude of the membrane currents recorded at 5 and 10 min after application of estradiol. The peak inward and outward currents at the membrane potential of 0 mV in the absence of estradiol were normalized as 1.0. Closed triangles and closed circles show the relative amplitudes of inward Ca^{2+} and outward K^+ currents observed at 5 min after application of estradiol, respectively. Open triangles and open circles show the relative amplitudes of inward Ca^{2+} and outward K^+ currents observed at 10 min after application of drug. Each symbol indicates the mean amplitude of three to eight observations with S.D. Asterisks mean a significant difference between the relative amplitudes at 5 and 10 min after application of estradiol at each concentration ($p < 0.05$).

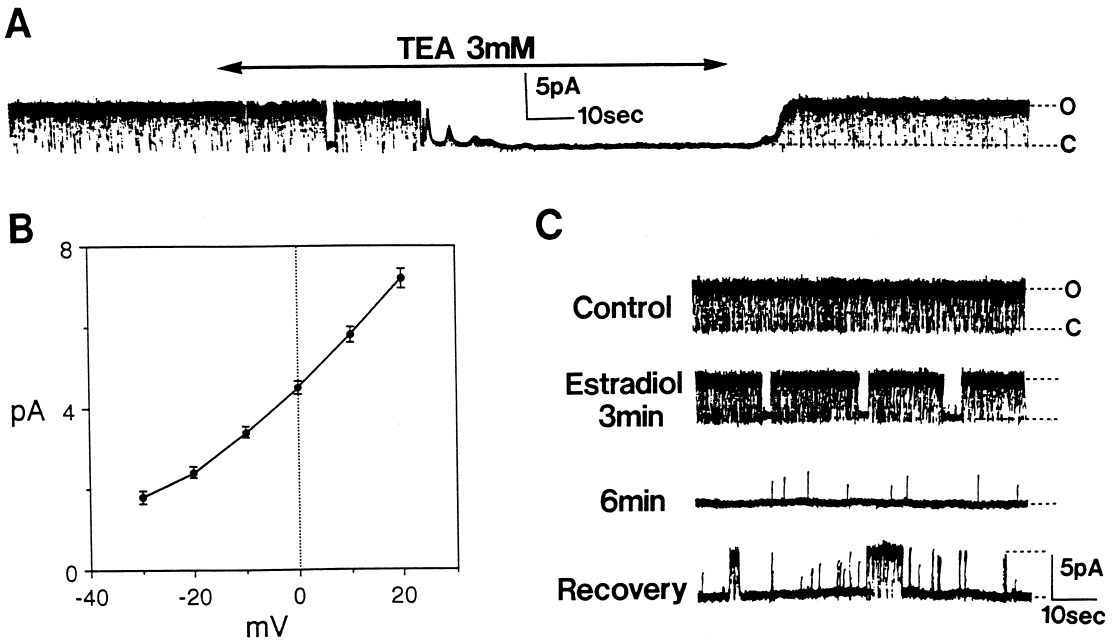

Fig. 6. Effects of tetraethylammonium (TEA) and estradiol (30 μM) on single-channel K^+ current (outside-out patch). (A) Effects of tetraethylammonium (TEA; 3 mM) on the unitary K^+ current recorded from an outside-out membrane patch. Dotted lines at C and O indicate the current level of closed and open state, respectively. (B) Unitary K^+ current conductance as a function of membrane potential. The single-channel conductance was 112.5 ± 7.6 pS (mean ± S.D.; $n = 4$). (C) Effects of estradiol (30 μM) on single-channel K^+ current. Traces before, 2 and 5 min after application, and 10 min after removal of estradiol are shown. Dotted lines at C and O indicate the current level of closed and open state, respectively.

a more depolarizing stimulation, suggesting voltage-dependence of K^+ current inhibition by estradiol (Fig. 4C).

Fig. 5 shows the relationships between estradiol concentration and relative amplitudes of inward and outward currents obtained at 5 and 10 min after the application of estradiol. The current inhibition by estradiol could be exerted at concentrations higher than 3 μM. Estradiol inhibited both currents to the same extent in a dose-dependent manner. The inhibitory effects of estradiol on Ca^{2+} and K^+ current were significantly more prominent at 10 min than those at 5 min. When 30 μM estradiol was present in the bath solution, relative amplitudes of Ca^{2+} and K^+ currents at 5 min after application of estradiol were 0.59 ± 0.09 ($n = 7$) and 0.63 ± 0.08 ($n = 8$), respectively (mean ± S.D.). On the other hand, at 10 min after application of estradiol, the relative amplitudes of Ca^{2+} and K^+ currents were 0.45 ± 0.10 ($n = 3$) and 0.50 ± 0.08 ($n = 3$), respectively (mean ± S.D.). Therefore, dose–response curves were shifted to the left.

It is known that tetraethylammonium inhibits the Ca^{2+}-dependent K^+ and the voltage-dependent K^+ channels in smooth muscle cells (Inoue et al., 1985; Ohya et al., 1987). When a unitary K^+ current was recorded in outside-out membrane patches from the pregnant rat myometrium, a large unitary K^+ current with a single channel conductance of 112.5 ± 7.6 pS (mean ± S.D.; $n = 4$) was observed (membrane potential of 0 mV, Fig. 6A and B). Application of tetraethylammonium (3 mM) completely blocked the amplitude of this current. Upon removal of tetraethylammonium from the bath, the amplitude of the unitary K^+ current was gradually increased and restored to the control level (Fig. 6A). Estradiol (30 μM) inhibited this channel activity within 5 min after application, but clearly did not change single channel conductance of K^+ currents in this outside-out patch (Fig. 6C).

4. Discussion

In this study, we found that acute administration of estradiol inhibits not only Ca^{2+} current but also two types of K^+ currents, i.e., voltage-dependent delayed K^+ and Ca^{2+}-dependent K^+ currents. As far as we know, this is the first report showing the acute effect of estradiol on two types of potassium channels in pregnant rat myometrium. Inhibition of the Ba^{2+} current by estradiol which passes through voltage-dependent Ca^{2+} channels has been reported for pregnant rat myometrium (Yamamoto, 1995) as well as cardiac and other smooth muscle cells (Jiang et al., 1992; Zhang et al., 1994; Ogata et al., 1996). For myometrium, Yamamoto (1995) reported that estrogen and the synthetic estrogen, diethylstillbesterol, blocked Ba^{2+} currents in a voltage-dependent manner. He also reported that estradiol shifted the voltage-dependent inactivation curve of Ba^{2+} current to the left. This might indicate that estradiol inhibits the Ca^{2+} channels by a mechanism similar to that of organic Ca^{2+} channel blockers (Collins et al., 1993). However, in this study, we found that estradiol at high concentrations inhibited both Ca^{2+} and K^+ currents with the same potency (Fig. 5). This suggests that estradiol

has a Ca^{2+} channel blocking action different from that of organic Ca^{2+} channel blockers. Inhibition of the Ca^{2+} current by estradiol would inhibit membrane depolarization, so suppressing action potential generation. The significance of the inhibition of K^+ currents on action potential generation is not clear. When the outward K^+ current was inhibited, membrane potential would be depolarized, therefore the inward Ca^{2+} current would be activated. However, Ca^{2+} channels might shift from activated state to inactivated state if membrane depolarization persists (Hess et al., 1984). After-hyperpolarization is thus necessary to activate Ca^{2+} channel repetitively. It may be speculated that inhibition of after-hyperpolarizations would suppress spike generation, due to slowing of the Ca^{2+} channel recovery from the inactivated state. These actions may explain the inhibitory effects of estrogen on uterine contractility, that were reported by Osa and Ogasawara (1984).

After Csapo (1956) presented the progesterone block theory, some investigators suggested that estrogen activates uterine contraction, and progesterone blocks it (Turnbull et al., 1974; Broditsky et al., 1978). Indeed, Batra et al. (1983) reported that the plasma progesterone/estrogen ratio was decreased in women in labor compared to that in women not in labor. However, no report has shown a clear stimulating effect of estrogen on uterine contractility by subacute or chronic administration, or even by acute administration. It is possible that the chronic effect of estrogen differs from our results because steroid hormones can act on cytosolic receptors, and affect uterine contractility via a genomic process. Another difference in estrogen actions between acute and chronic administration is the concentration used. Although during pregnancy, the serum level of 17β-estradiol increases by as much as 10 times the normal in humans (Speroff et al., 1994), reaching 30 to 100 nM near term, much higher concentrations of estradiol (>1 μM) are used to obtain inhibition of uterine contraction (Osa and Ogasawara, 1984; Juberg and Loch-Caruso, 1992; Yamamoto, 1995). In the present study, estradiol at concentrations (>3 μM) higher than the normal serum level during pregnancy is necessary to inhibit Ca^{2+} and K^+ currents. Thus, the physiological importance of this acute inhibition by estradiol on uterine contraction is not clear. As longer administration (10 min) of estradiol produced a greater inhibition of the Ca^{2+} and K^+ currents as shown in Fig. 5, the period of drug administration might be a factor for the difference in the concentrations of estradiol in acute and chronic administration. White et al. (1995) suggested that protein (steroid hormone binding globulin)-bound hormone can act in vivo as a free fraction because of the evidence that steroid hormones dissociated rapidly from plasma protein. If this is the case, the actual concentration of hormone which can act on the myometrial membrane may be in reality many times greater than the plasma concentration of free hormone. Under our conditions, the bathing solution did not contain steroid binding protein, thus higher concentrations of free hormone would be necessary to mimic in vivo physiological conditions. Waldegger et al. (1996) reported that estrogen (1 μM) could not inhibit the delayed K^+ current expressed in *Xenopus* oocyte by injection of rat Kir 2.1 protein. Because the experimental procedure was different from ours, and they did not use much higher concentrations of estradiol on the delayed K^+ current, it is difficult to compare our results with their report.

Miyoshi et al. (1991) classified the membrane currents of longitudinal cells of the pregnant rat myometrium into fast Na^+ and Ca^{2+} inward, and transient and delayed outward currents. A T-type Ca^{2+} channel has not yet been identified in rat myometrium, although it was reported to exist in human pregnant myometrium (Inoue et al., 1990). In the present experiments, we excluded the fast Na^+ and transient outward currents from our whole-cell clamp study since both currents are completely inactivated at a membrane potential of -50 mV, i.e., the resting membrane potential of the rat myometrial cells. Recently, Erulkar et al. (1994) reported that the chronic administration of estradiol but not progesterone, inhibited a 4-aminopyridine-sensitive transient K^+ current in immature rat uterine myocytes when the membrane was held at -90 mV. In rat myometrial cells, the resting membrane potentials is between -50 and -60 mV (Osa and Ogasawara, 1984; Kawarabayashi, 1994). Since 4-aminopyridine-sensitive transient K^+ currents are inactivated at -40 to -50 mV, actions of estradiol on delayed K^+ current are likely to be more significant for electrical activity than those on the 4-aminopyridine-sensitive transient K^+ current. We showed that estradiol inhibits the delayed K^+ current as well as voltage-dependent Ca^{2+} current. Although we did not separate the outward current into Ca^{2+}-dependent and Ca^{2+}-independent K^+ currents in whole-cell voltage-clamp experiments, we found that tetraethyl ammonium (3 mM) only partially blocks the outward current in the absence of Ca^{2+} in the bath and in the presence of EGTA (10 mM) in the pipette, suggesting that a component of the outward current might be carried through the Ca^{2+}-independent delayed rectifier K^+ channel. Under these conditions, estradiol inhibited the remaining outward current. Estradiol also inhibited the outward current when 0.3 mM EGTA was included in the pipette solution. Under these conditions the Ca^{2+}-dependent K^+ current may be active (Kitamura et al., 1992). The unitary K^+ current recorded in 0.3 mM EGTA solution was most likely a Ca^{2+}-dependent K^+ channel (BK channel) due to its large unitary current conductance. Estradiol inhibited a channel open probability without affecting the amplitude of the unitary currents (Fig. 6). These results indicate that estradiol inhibits both Ca^{2+}-dependent and delayed rectifier K^+ currents.

Ogata et al. (1996) suggested that estrogen acts on the cell membrane receptors rather than on cytosolic receptors because its action was observed very quickly and current was restored rapidly after removal of estrogen from the

bath solution. In our experiments, the inhibitory action of estradiol on Ca^{2+} and K^+ channels was observed rapidly after application, and this inhibition could easily be removed by washout in the case of K^+, but not in the case of Ca^{2+}. It is well known that the Ca^{2+} current in enzymatically dispersed single cells runs down spontaneously in a relatively short time. Therefore, the lack of recovery of the Ca^{2+} current in this study might be partly due to this run-down phenomenon, but it is possible to speculate that a different mechanism of current inhibition between Ca^{2+} and K^+ currents might exist, such as Ca^{2+} induced-inactivation of the Ca^{2+} current. Yamamoto (1995) also reported that the Ca^{2+} current was only partially restored by wash-out after estrogen inhibition. Furthermore, tamoxifen (1 μM) also reversibly inhibited the outward current in the pregnant rat myometrium (45 ± 17% of the control), but progesterone did not (K. Okabe and Y. Inoue; unpublished observations). We speculate that under our conditions estrogen may act through a non-genomic pathway, possibly via cell membrane receptors for acute inhibition of the channel activity. Further studies are necessary to elucidate the detailed mechanism of these acute effects of estrogen on the electrical activity of pregnant myometrium, but estrogen may play some role to inhibit uterine contractility during pregnancy.

References

Batra, S., Bengtsson, L.P., Ingemarsson, I., 1983. The role of estradiol and progesterone in the regulation of myometrial activity for the onset of labor. Acta Obstet. Gynecol. Scand. 62, 207–209.

Broditsky, R.S., Reyes, F.I., Winter, J.S.D., Faiman, C., 1978. Maternal serum estrogen and progesterone concentration preceding normal labor. Obstet. Gynecol. 51, 686–691.

Collins, P., Rosano, G.M.C., Jiang, C., Lindsay, D., Sarrel, P.M., Poole-Wilson, P.A., 1993. Cardiovascular protection by oestrogen — a calcium antagonist effect?. Lancet 341, 1264–1265.

Csapo, A.I., 1956. Progesterone block. Am. J. Anat. 98, 273–291.

Erulkar, S.D., Rendt, J., Nori, R.D., Ger, B., 1994. The influence of 17 beta-oestradiol on K^+ currents in smooth muscle cells isolated from immature rat uterus. Proc. R. Soc. London, Ser. B 256, 59–65.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F., 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch. 391, 85–100.

Hess, P., Lansman, J.B., Tsien, R.W., 1984. Different modes of Ca channel gating behavior favoured by dihydropyridine Ca agonists and antagonists. Nature 311, 538–544.

Inoue, R., Kitamura, K., Kuriyama, H., 1985. Two Ca-dependent K-channels classified by the application of tetraethylammonium distribute to smooth muscle membranes of the rabbit portal vein. Pflügers Arch. 405, 173–179.

Inoue, Y., Nakao, K., Okabe, K., Izumi, H., Kanda, S., Kitamura, K., Kuriyama, H., 1990. Some electrical properties of human pregnant myometrium. Am. J. Obstet. Gynecol. 162, 1090–1098.

Jiang, C., Poole-Wilson, P.A., Sarrel, P.M., Mochizuki, S., Collins, P., MacLeod, K.T., 1992. Effects of 17β-oestradiol on contraction, Ca^{2+} current and intracellular free Ca^{2+} in guinea-pig isolated cardiac myocytes. Br. J. Pharmacol. 106, 739–745.

Jmari, K., Mironneau, C., Mironneau, J., 1987. Selectivity of calcium channels in rat uterine smooth muscle: Interactions between sodium, calcium and barium ions. J. Physiol. 384, 247–261.

Juberg, D.R., Loch-Caruso, R., 1992. Investigation of the role of estrogenic action and prostaglandin E2 in DDT-stimulated rat uterine contraction ex vivo. Toxicology 74, 161–172.

Kawarabayashi, T., 1994. Electrophysiology of the human myometrium. In: Chard, T., Grudzinskas, J.G. (Eds.), The Uterus. Cambridge Univ. Press, Cambridge, pp. 148–172.

Kitamura, K., Xiong, Z., Teramoto, N., Kuriyama, H., 1992. Roles of inositol trisphosphate and protein kinase C in the spontaneous outward current modulated by calcium release in rabbit portal vein. Pflügers Arch. 421, 539–551.

Miyoshi, H., Urabe, T., Fujiwara, A., 1991. Electrophysiological properties of membrane currents in single myometrial cells isolated from pregnant rats. Pflügers Arch. 419, 386–393.

Ogata, R., Inoue, Y., Nakano, H., Ito, Y., Kitamura, K., 1996. Oestradiol relaxes the rabbit basilar artery by inhibition of voltage-dependent Ca channels through GTP-binding protein. Br. J. Pharmacol. 117, 351–359.

Ohya, Y., Kitamura, K., Kuriyama, H., 1987. Cellular calcium regulates outward currents in rabbit intestinal smooth muscle cell. Am. J. Physiol. 252, C401–410.

Okabe, K., Kitamura, K., Kuriyama, H., 1987. Features of 4-aminopyridine sensitive outward current observed in single smooth muscle cells of the rabbit pulmonary artery. Pflügers Arch. 409, 561–568.

Osa, T., Ogasawara, T., 1984. Effects in vitro of progesterone and estradiol-17β on the contractile and electrical responses in rat myometrium. Jpn. J. Physiol. 34, 427–441.

Pressman, E.K., Tucker, J.A., Anderson, N.C., Young, R.C., 1988. Morphological and electrophysiologic characterization of isolated pregnant human myometrial cells. Am. J. Obstet. Gynecol. 159, 1273–1279.

Speroff, L., Glass, R.H., Kase, N.G., 1994. Clinical Gynecologic Endocrinology and Infertility, 5th edn. Williams & Wilkins, Baltimore, MA.

Turnbull, A.C., Flint, A.P.F., Jeremy, J.Y., Patten, P.T., Keirse, M.J.N.C., Anderson, A.B.M., 1974. Significant fall in progesterone and rise in oestradiol levels in human peripheral plasma before onset of labour. Lancet 1, 101–103.

Waldegger, S., Lang, U., Herzer, T., Suessbrich, H., Binder, K., Lepple-Wienhues, A., 1996. Inhibition of minK protein induced K^+ channels in *Xenopus* oocytes by estrogen. Naunyn-Schmiedeberg's Arch. Pharmacol. 354, 698–702.

White, R.E., Darkow, D.J., Falvo Lang, J.L., 1995. Estrogen relaxes coronary arteries by opening BK_{Ca} channels through a cGMP-dependent mechanism. Circ. Res. 77, 936–942.

Yamamoto, T., 1995. Effects of estrogens on Ca channels in myometrial cells isolated from pregnant rats. Am. J. Physiol. 268, C64–C69.

Young, R.C., Herdon-Smith, L., 1991. Characterization of sodium channels in cultured human uterine smooth muscle cells. Am. J. Obstet. Gynecol. 164, 175–181.

Zhang, F., Ram, J.L., Standley, P.R., Sowers, J.R., 1994. 17β-estradiol attenuates voltage-dependent Ca^{2+} currents in A7r5 vascular smooth muscle cell line. Am. J. Physiol. 266, C975–C980.