Comparative Effects of Mg, Ca, Sr, and Verapamil on the Uterine Longitudinal Muscle of Spayed and Estrogen-treated Rats

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Abstract A comparison was made of effects of Mg, Ca, Sr, or verapamil on the mechanical and electrical activities in the uterine longitudinal muscles of spayed and estrogen-treated rats. The muscle strips taken from spayed rat exhibited spontaneous rhythmic activity in the Locke solution which did not contain Mg, whereas spontaneous activity was less frequent in the preparation taken from estrogen-treated rat. The resting potentials were $-54$ and $-61$ mV in the spayed and the estrogen-treated preparations, respectively. An initial spike potential followed by plateau potential with abortive spikes on the top was generated in both spayed and estrogen-treated preparations. In the spayed preparation, the frequency of rhythmic contractions was reduced, and the base-line tension was lowered when 0.6 mM Mg was added to the solution. The base-line tension was elevated progressively when the external Ca concentration was raised, and reached a maximal value up to 10 mM. The amplitude of phasic contraction was progressively increased by increasing Ca concentrations in the range from 1.25 to 5 mM, and was reduced by Ca higher than 10 mM. In the estrogen-treated preparation, the amplitude of phasic contraction was increased by increasing Ca concentrations in the range from 1.25 up to 17.5 mM. When the amplitude of phasic contraction was increased, the duration of plateau potential became protracted. Substitution of the external Ca with Sr caused an increase in the spike activity generated on the top of plateau potential. However, the amplitude of phasic contraction was diminished in both the spayed and the estrogen-treated preparations. Verapamil (2 μM) caused a stronger depression of electrical and mechanical activity in the spayed preparation. Results were discussed in relation to the genomic effects of estradiol on the membrane properties so as to change the interaction with divalent cations.

Key words: rat uterus, Mg, Ca, Sr, verapamil.
Estrogen is incorporated in the cell nucleus, and results in the primary effects acting on cell metabolism and biosynthesis (KATZENELLENBOGEN and GORSKI, 1975), and the succeeding series of biochemical changes culminating in the characteristic morphological, physiological, and pharmacological responses of uterine tissue. The contractile capacity of rat myometrium is progressively increased up to the last stage of gestation (IZUMI, 1985), probably due to the anabolic effect of estrogen.

The shape of the action potential of uterine muscle was also changed by the treatment of spayed rat with estrogen (KURIYAMA and SUZUKI, 1976; OSA et al., 1981), which may modify the Ca-influx to produce phasic contraction. It is generally thought that the uterus of spayed animals is quiescent, and spontaneous rhythmic contractions are induced in the uterus of estrogen-treated animals (REYNOLDS, 1965), although in certain conditions, e.g. when estradiol-17β is given in vivo and intrauterine pressure cycles are measured, the spontaneous activity in the rat uterus becomes reduced with latency of several h to a few days (FUCHS, 1974; DOWNING et al., 1978). We have often encountered cases where uterine longitudinal muscle strips taken from estrogen-treated rats were quiescent in vitro, whereas those taken from spayed rats exhibited spontaneous activities.

Present experiments were at first undertaken in an attempt to find out a mechanism by which spontaneous rhythmic contractions are generated in the longitudinal muscle of spayed rat. For this purpose, effects of the external divalent cations (Mg, Ca, and Sr), and verapamil were compared between the preparations taken from spayed and estrogen-treated rats. A hypothesis is put forward that passive Ca leak occurs more intensely in the spayed preparation. Effects of verapamil on the contractures induced by high K and carbachol were also studied.

METHODS

Young virgin rats (Wistar-strain), weighing about 200 g, were ovariectomized and used for experiments after about 2 weeks. One-shot injections of estradiol-17β benzoate (50 µg) dissolved in sesame oil were given subcutaneously in the morning, and animals were sacrificed in the morning after 1–3 days. Unless otherwise stated, animals treated with estrogen for 2 days were used. In order to measure the contractile response, longitudinal muscle strips of about 0.4 mm in width and 2 mm in length were dissected out, and were mounted in an organ bath of 0.2 ml capacity. Muscle strips were suspended vertically under the resting tension of about 0.1 g. Stimulating current of 300 ms duration was applied by Ag-AgCl electrodes placed in the vicinity of the ends of a muscle strip. The experiment was started after the equilibration of the freshly excised muscle strip in the control medium (36°C) for at least 2–3 h. The recording was by means of isometric transducer (SB-1T, Nihon Kohden) and displayed by pen recorder (VP-6523A, National). Intracellular recording of the membrane activity was made by a conventional microelectrode filled with 3 M KCl. In the latter case, longitudinal muscle strips of 0.5 mm width and
4 mm length were mounted in the partition chamber described by Abe and Tomita (1968).

The control bathing solution was a modified Locke solution, and the ionic composition was (mM): NaCl 130.6; NaHCO₃ 8; KCl 5.9; CaCl₂ 2.5; and glucose 11.5. Then MgCl₂ (0.6 mM) was added, or 2.5 mM Sr was substituted for 2.5 mM Ca. The external Ca concentration was changed between 1.25 and 17.5 mM without changing other ionic components, adding a stock solution of 1 M CaCl₂ in an appropriate quantity. In some experiments, Ca was increased to 10 mM by replacement of equiosmolar NaCl. High K solution (40 mM K) was prepared by replacement of 34.1 mM NaCl with equimolar KCl. Low Na solution was prepared by replacement of NaCl with sucrose. Solutions were aerated with 95% O₂ + 5% CO₂ (pH of 7.3). Drugs used were verapamil (Knoll A. G.), carbachol (Katayama), and indomethacin (Sigma).

RESULTS

Effects of Mg, Ca, and Sr on the spontaneous activity

The longitudinal muscle of the spayed rat uterus exhibited spontaneous contractions in the Locke solution which did not contain Mg ions (Fig. 1A). The frequency was 1.2 ± 0.1 min⁻¹ (S.E.M., n = 29) after 2 to 3 h stabilizing incubation. When 0.6 mM Mg was applied, the base-line tension or muscle tone declined below

Fig. 1. Comparison between effects of 0.6 mM Mg (broken line above) and 12.5 mM Ca (continuous line) on the contractions in the longitudinal muscles taken from spayed (A), and estrogen-treated (B for 1 day, C for 2 days) rats. Control solution did not contain Mg. Dots indicate the electrical stimulation (300 ms).

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the level before, and the frequency of spontaneous contractions decreased. The electric stimulation usually produced very small contraction, but occasionally evoked a contraction whose amplitude was larger than controls. When 12.5 mM Ca was applied, the muscle tone was gradually elevated, and the spontaneous activity was decreased. Contractions having very small amplitude were evoked when electric stimuli were applied. Full-size contractions were occasionally generated.

Figure 1B shows the contractions generated in the longitudinal muscle taken from the uterus of spayed rat given 50 µg estradiol-17β for 1 day. The frequency of spontaneous contractions was reduced. The muscle tone declined or was elevated by the application of 0.6 mM Mg or 12.5 mM Ca, respectively, but the extent was smaller compared with the effects of the ions on the muscle taken from the spayed animal (cf. Fig. 1A). Spontaneous activity was nearly absent in the muscle taken from the spayed rat treated with estradiol for 2 days, and electric stimulation evoked phasic contractions (Fig. 1C). When 0.6 mM Mg was applied, the amplitude of evoked contraction was depressed, but only a little. On the other hand, the muscle tone did not decline as has been observed for record A. When 12.5 mM Ca was applied, phasic contraction having larger amplitude was generated. Responses of the muscle

![Graphical representation of the muscle contractions](image-url)

**Fig. 2.** Changes by 0.6 mM Mg of action potentials in the spayed (A) and estrogen-treated (B) preparations. Aa and Ba show the control activity. Ab and Ac were recorded 5 and 15 min after exposure to Mg, respectively. In record Bb, 0.6 mM Mg was applied at the time indicated by the arrow, and Bc is the response taken 7 min after the exposure to Mg. Records Ba and Bc are displayed by 2 sweep speeds. Dots indicate the electrical stimulation.
taken from the rat treated with estradiol for 3 days were very similar to those shown in record C.

The electrical activity of the longitudinal muscle of spayed rat uterus consisted of a spike potential followed by plateau potential with abortive spikes on the top (Fig. 2Aa). Other varieties of action potential are shown in succeeding figures. The mean of the resting membrane potential was $-54.4 \pm 0.6$ mV (S.E.M., $n=30$). When 0.6 mM Mg was applied, spontaneous activity was abolished, and the evoked action potential consisted of an initial spike followed by a plateau potential with short duration (Fig. 2Ab). The hyperpolarization of the membrane was not noticed. When the exposure to 0.6 mM Mg was prolonged, spontaneous activity was still absent, but an electric stimulus caused a train of spike potentials followed by short plateau potential, or by well-developed plateau potentials (Fig. 2Ac). This restoration of the action potential was not accompanied with the change in the

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**Fig. 3.** Effects of the external Ca concentrations raised in steps (designated on the top) on the contractions in spayed (A) and estrogen-treated (B) preparations. Dots indicate electrical stimulation. Record C shows the dependence of the base-line tension in the spayed (▲) and the estrogen-treated (●) preparations on the external Ca concentrations (abscissa). The base-line tension in the presence of 2.5 mM Ca is taken as zero, and the amplitude of phasic contraction in the presence of 2.5 mM Ca is normalized as 100%. Shift of base-line tension is shown by percent (ordinate). The base-line tension was measured 15–20 min after the exposure to the Locke solution which contained each Ca concentration. Each point shows the mean ± S.E.M. ($n=14–19$).
membrane potential.

The muscle of the rat treated with estradiol for 2 days gave rise to a spike followed by a plateau potential accompanied with spikes on the top (Fig. 2Ba). The initial spike potential exhibited an overshoot. The mean of the resting potential was $-61.0 \pm 0.6$ mV (S.E.M., $n=30$). An application of $0.6$ mM Mg caused a decrease in the duration of plateau potential (Fig. 2Bb, Bc); however, the extent was much smaller compared with the muscle taken from the spayed rat (cf. Fig. 2Ab). The amplitude of initial spike potential was unaffected.

Comparative effects of raising the external Ca concentration on the mechanical activity of the muscles of spayed or estrogen-treated rats are shown in Fig. 3. In the spayed preparation (Fig. 3A), spontaneous activity was transiently suppressed, then resumed, and the amplitude of phasic contractions became larger, when the Ca concentration was raised from 1.25 to 2.5 mM. The amplitude of phasic contractions was more increased when 5 mM Ca was applied, whereas it became smaller than before when 7.5 mM Ca was administrated. Spontaneous activity was depressed by the application of 10 mM Ca during the early period, during which electrical stimuli caused contractions having very small amplitude. The muscle tone was more and more elevated as the external Ca concentration was raised to 10 mM. When 12.5 mM Ca was applied, spontaneous activity was completely suppressed, and the electric stimulation was unable to elicit a full-size contraction in this case. The muscle tone declined more than before; however it was still at a more elevated level than in the presence of 1.25 mM Ca.

In the estrogen-treated preparation (Fig. 3B), the main effect of higher Ca concentrations applied in steps was an increase in the amplitude of phasic

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Fig. 4. Effects of raising the external Ca concentration to 5 mM (A, B, C) and 12.5 mM Ca (D, E) on the membrane activities in the spayed preparations. Ca concentration was increased from 2.5 to 5 mM (A), and from 5 to 12.5 mM (D) at the time indicated by arrows. B and C were recorded 5 and 15 min, and E 7 min, respectively, after raising Ca. Dots indicate electrical stimulation.

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contractions. The muscle tone was elevated by higher Ca, but slightly. Figure 3C shows the effects of external Ca concentration on the base-line tension. Herein, the base-line tension in the presence of 2.5 mM Ca was taken as zero, and the shift of muscle tone was given by percent of the amplitude of the phasic contraction in the presence of 2.5 mM Ca. Greater dependence of the muscle tone on the external Ca in the preparations taken from spayed animals can be deduced.

The action potential of the spayed preparation illustrated by Fig. 4A was composed of twin ones, or the plateau potential was dichotomized. Each action potential consisted of initial spike and irregular spikes on the top of plateau. When the external Ca concentration was raised from 2.5 to 5 mM (Fig. 4A, B, C), the duration of plateau potential became longer. Neither the membrane potential nor the amplitude of initial spike was markedly changed. The amplitude of the plateau potential was augmented. Figure 4D and E show the electrical activity in the presence of 12.5 mM Ca. The interval between spontaneous activities became protracted (Fig. 4D), and these were finally abolished (Fig. 4E). When electric stimulation was applied, the responses were abortive spikes in some cases or generation of train of plateau potentials having short duration.

Figure 5 shows the electric activity of the estrogen-treated preparations in the presence of Ca at 2.5 mM (Fig. 5A), 5 mM (Fig. 5A, B, C), and 12.5 mM (Fig. 5C, D). The muscle exhibited spontaneous activity in this preparation (Fig. 5A, B). The control action-potential consisted of an initial spike potential, then a plateau and spike potentials generated on it. When 5 mM Ca was applied, a marked effect was a
prolongation of plateau potential. The amplitude of the initial spike as well as the ones generated on the plateau was generally augmented. Spontaneous activity stopped when 12.5 mM Ca was applied (Fig. 5C, D), but action potentials were evoked by electric stimulation. The plateau potential was more prolonged, and the amplitude of spike potentials was more augmented.

The membrane potential was nearly the same in the 5 mM Ca solution as in the control solution in both the spayed and the estrogen-treated preparations. Membranes were depolarized by about 6–7 mV in the 12.5 mM Ca solution (Figs. 4E, 5D).

It has been known for smooth muscles that Sr can be substituted for Ca in the generation of contraction and action potential (taenia coli, BÜLBRING and TOMITA, 1970; INOMATA and KAO, 1979; portal vein, ÜVELIUS et al., 1974; HOTTA and YAMAMOTO, 1983). The effects of Sr were thus tested for uterine muscle. When the external 2.5 mM CaCl₂ was replaced by equimolar SrCl₂, the muscle tone declined, the frequency of spontaneous activity decreased, and the amplitude of phasic contraction was reduced in the spayed preparation (Fig. 6A). The amplitude of phasic contractions generated in the Sr-solution was 76.5 ± 3.4% (+ S.E.M., n = 11) of that generated in the control media. When 0.6 mM Mg was added to the Sr-solution, spontaneous activity disappeared, and contractions having small amplitude were generated by electrical stimulation. When the preparation was exposed to the control solution, the muscle tone was again elevated, and spontaneous activity took place. Application of 0.6 mM Mg caused a decline of base-line tension and a depression of phasic contraction, phasic contraction with large amplitude being occasionally produced by electric stimulation. The estrogen-treated preparation

![Fig. 6. Effects of replacing Ca with 2.5 mM Sr (continuous line) and applying 0.6 mM Mg (broken line) on the contractions of the longitudinal muscles taken from spayed (A), and estrogen-treated (B) rats. Dots indicate the electrical stimulation.](image-url)

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used for the experiment shown in Fig. 6B exhibited spontaneous contractions. The muscle tone was not changed, but the spontaneous activity was abolished, when the external Ca was replaced by Sr. The amplitude of phasic contraction generated in the Sr-solution was $78.7 \pm 2.9\%$ (± S.E.M., $n = 7$) of that generated in the control solution. The amplitude of phasic contraction was slightly depressed by addition of 0.6 mM Mg to either the Sr-solution or control media.

The depression of phasic contraction by 0.6 mM Mg in the solutions containing 2.5 mM Ca or Sr was compared between the spayed and estrogen-treated preparations (Fig. 7). Each point shows percent response of the amplitude of phasic contraction in the presence of 0.6 mM Mg in the Sr-solution (ordinate) or in the

![Graph](Vol. 36, No. 5, 1986)
normal media (abscissa), examined for the same muscle strip. It is concluded that the extent of depression caused by 0.6 mM Mg was smaller in the estrogen-treated preparations than in the spayed preparations, either in the Sr- or Ca-solution.

Changes in membrane activity when 2.5 mM Ca was replaced by 2.5 mM Sr are shown for the spayed preparation (Fig. 8). In the presence of Sr the amplitude of spike potential increased and the duration of plateau potential became longer (Fig. 8A, B), the features of which are more clearly presented on faster sweep (Fig. 8C, D). When 0.6 mM Mg was added, spontaneous activity consisted of tiny potentials, and electric stimulation failed to evoke the action potential (Fig. 8E).

Membrane activity of the estrogen-treated preparation which was changed by the replacement with Sr is shown in Fig. 9A–D. The tissue was spontaneously active in this case (Fig. 9A). Action potentials exhibiting a larger amplitude of spike potentials were sometimes generated under the substitution with Sr (Fig. 9B, D vs. C). When 0.6 mM Mg was added, spontaneous activity stopped, and the action potential exhibiting a plateau potential of shorter duration was generated by electric stimulation (Fig. 9E).

The membrane potential was hyperpolarized by about 5 mV in the Sr-solution in both the spayed and the estrogen-treated preparations.

Fig. 8. Effects of replacing Ca with 2.5 mM Sr (A, B), and further application of 0.6 mM Mg (E) on the membrane activity in the spayed preparation. Replacement with Sr is indicated by arrow (A), and B and E are the records taken after 4 and 25 min exposure to Sr. C and D show the electrical activity displayed on fast sweep in control solution and 19 min after the exposure to Sr, respectively. In E, 0.6 mM Mg was applied at the time indicated by arrow. Dots indicate the electrical stimulation. Time scales are shown for 2 sweep speeds.
Effects of verapamil, carbachol, and 40 mM K on the electrical and mechanical activity

It has been shown that D600, a Ca antagonist, depresses the generation of action potential and phasic contraction of parturient rat uterus at concentrations of $10^{-8}$ to $10^{-7}$ M (Reiner and Marshall, 1975). Figure 10 illustrates the effects of 0.6 mM Mg and 2 μM verapamil for the spayed (Fig. 10A) and estrogen-treated (Fig. 10B) preparations. The base-line tension declined by the application of Mg in the spayed preparation. The amplitude of phasic contractions evoked by electrical stimulation was variable in the presence of Mg. The muscle tone declined by verapamil, and the depression of phasic contraction was greater by verapamil than by Mg at these concentrations. The phasic contraction was very slightly depressed by 0.6 mM Mg in the estrogen-treated preparation (Fig. 10B). Verapamil caused a weaker depression than in the spayed preparation (Fig. 10B vs. A). The base-line tension was unaffected. The means of percent response of phasic contraction in the presence of 2 μM verapamil, as referred to the amplitude of phasic contractions in the control media, was $9.5 \pm 1.7\%$ (± S.E.M., n = 9) for the spayed preparations, and $52.0 \pm 2.7\%$ (± S.E.M., n = 10) for the estrogen-treated preparations. During wash with the control media after the application of verapamil, spontaneous activity of high frequency was generated in the spayed preparation (Fig. 10A).

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**Fig. 9.** Effects of replacing Ca with 2.5 mM Sr (A, B), and further application of 0.6 mM Mg (E) on the membrane activity in the estrogen-treated preparation. Replacement with Sr is indicated by arrow (A). B and E are the records taken after 4 and 15 min exposure to Sr. C and D show the electrical activity displayed on fast sweep in control solution and 11 min after the exposure to Sr, respectively. In E, 0.6 mM Mg was applied at the time indicated by the arrow. Dots indicate the electrical stimulation. Time scales are shown for 2 sweep speeds.
spontaneous contractions exhibiting smaller amplitude occurred for further 2 h observation.

The electrical activity of the spayed preparation considered of twin action potentials in the case shown in Fig. 11Aa; each action potential had an initial spike potential and abortive spike on the top of plateau potential. When 2 µM verapamil was applied, the frequency of spontaneous activity decreased, and both spike and plateau potentials were progressively depressed (Fig. 11Aa, Ab). Spontaneous activity finally stopped, and electric stimulation elicited action potential of depressed amplitude and short duration (Fig. 11Ac). In the estrogen-treated preparation (Fig. 11B), the duration of plateau potential became shorter at first by the application of 2 µM verapamil (Fig. 11Ba). As a steady effect (Fig. 11Bb, Bc), the depression of plateau potential was pronounced, while leaving the initial spike potential nearly unaffected.

When 40 mM K was applied, contractures were produced (Fig. 12). A phasic component of K-contracture was dominant in the spayed preparation (Fig. 12A), compared with the response in the estrogen-treated preparation (Fig. 12B). Contraction was evoked in addition, when 5 mM carbachol was applied at the time when the K-contracture underwent a tonic phase. During the continuous application of 2 µM verapamil, the same procedure was repeated for the spayed (Fig. 12A) and estrogen-treated (Fig. 12B) preparations. The development of K-contracture was more depressed than the contracture induced by carbachol. With this regard, no distinguishable difference was noticed between the spayed and

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**Fig. 10.** Comparison between effects of 0.6 mM Mg (broken line) and 2 µM verapamil on the contractions in the longitudinal muscles taken from spayed (A) and estrogen-treated (B) rats. Verapamil was applied during the period interpolated by arrows. Dots indicate electrical stimulation.
Fig. 11. Effects of 2 µM verapamil on the membrane activities in the spayed (Aa, Ab, Ac) and estrogen-treated (Ba, Bb, Bc) preparations. Verapamil was applied at the time indicated by arrows (Aa, Ba). Aa, Ab, Ac and Ba, Bb, Bc are the successive records, respectively. Dots indicate electrical stimulation.

Fig. 12. Comparison between effects of 2 µM verapamil on the K-contracture in the longitudinal muscles taken from spayed (A) and estrogen-treated (B) rats. High K (40 mM) was administrated during the periods indicated by continuous lines and 5 µM carbachol by broken lines. Verapamil was applied at the time indicated by arrows. Dots indicate electrical stimulation.
estrogen-treated preparations. It is thus concluded, in accordance with the current view (Bolton, 1979; Van Breemen et al., 1982), that verapamil depresses the voltage-dependent Ca channel more than the receptor-operated channel.

Effects of low Na on the contractions in the estrogen-treated preparation

The estrogen-treated preparation illustrated in Fig. 13 was quiescent, and phasic contractions were evoked only when electric stimulation was applied (Fig. 13A). When the external Na concentration was reduced from 138.6 to 73.3 mM, phasic contractions were spontaneously generated. When the external Na was further reduced to 36.7 mM, the base-line tension was elevated, and frequency of spontaneous activity was accelerated. Record B is the continuation of A. When 0.6 mM Mg was added, the muscle tone declined, and the frequency of spontaneous activity decreased. The external Ca concentration was raised from 2.5 to 10 mM, then spontaneous activity stopped, and the muscle tone was elevated. Electric stimulation evoked phasic contraction, but the amplitude was smaller. When the external Na concentration was increased to the control value (138.6 mM), the muscle tone declined. Trains of phasic contractions were generated spontaneously during the early exposure period to control media, then the muscle became again quiescent.

The membrane potential remained nearly the same as in the control media.
during 2 h observation in the low Na (36.7 mM) solution.

Effects of indomethacin on the spontaneous contractions of spayed preparations

The longitudinal muscles taken from spayed rats were, so far, characterized by frequent rhythmic contractions in the Locke solution. When 5 µM indomethacin, a dose which is thought sufficient to abolish a synthesis of prostaglandins, was applied the amplitude of phasic contractions was gradually decreased, and reached a stabilized state after about 20 min application, while the contractions were as frequent as the control. The basal tension declined slightly. Therefore, an indispensable prerequisite of endogenous prostaglandins for the generation of spontaneous activity in the spayed muscle can be ruled out.

DISCUSSION

The addition of Mg or verapamil, elevation of external Ca concentration, replacement of Ca with Sr altered the mechanical activity of the longitudinal muscles of spayed rat uterus. Not only the phasic contraction, but also the base-line tension were affected. Changes in phasic contractions were accompanied by those in the electrical activity. Effects of the above agents were in some cases differentiated between the spayed and the estrogen-treated preparations. If the nature of different responses was reasonably explained, the genomic influence of estrogen on the cell membrane of uterine muscle would become better understood.

Action potential in the longitudinal muscle of both the spayed and the estrogen-treated animals consists of the initial spike followed by plateau potential accompanied with spike potentials on the top (Figs. 2, 4, 5, 8, 9, 11). The difference in the shape of action potential is clearly recognized; the action potential of the estrogen-treated preparation is characterized by a relatively large initial spike and the following plateau potential which was not intermitted by repolarizations. A Na-origin for plateau potentials has been assumed in smooth muscles in normal or altered ionic environments (ureter, KURIYAMA and TOMITA, 1970; SHUBA, 1977; taenia coli, BULBRING and TOMITA, 1970; portal vein, HOTT and YAMAMOTO, 1983). In the present study, when the phasic contraction was potentiated by 5 mM Ca, the plateau potential was prolonged (Figs. 3–5). On the contrary, when the phasic contraction was depressed by 0.6 mM Mg, the plateau became shorter (Figs. 1, 2). Furthermore, the plateau potential was more susceptible to 2 µM verapamil (Fig. 11), at the concentration of the agent exerting as a Ca antagonist rather than a local anaesthetic drug. It has also been shown that the plateau at first, then the spike potential is suppressed by the application of 0.5 mM Mn (OsA et al., 1981). Although details of ionic nature were not examined in the present study, the above features favor a view of the plateau potential being a Ca-origin in the longitudinal muscle of rat uterus.

Substitution of Ca with Sr caused a prolongation of plateau potential in the guinea pig ureter (KURIYAMA and TOMITA, 1970), and rat portal vein in which the
phasic contraction was enhanced at the same time (Hotta and Yamamoto, 1983). In the present experiments, the plateau potential was prolonged, and in addition the generation of spike potential was accelerated particularly in the spayed preparation (Figs. 8, 9). On this basis of observation, and assuming that both the spike potential and plateau potential are of Ca-origin, "spike channel" and a "plateau channel" may be differentiated. The Sr may be more permeable through the channel than Ca. Alternatively, intracellular Ca would be replaced by Sr, and thus a condition was produced whereby the generation of Sr-spike was accelerated. This is because Sr seems to be less potent than Ca at activating the K conductance in molluscan neurones (Gorman and Hermann, 1979). The acceleration of spike generation by Sr has been found in rat portal vein at the concentrations of 10–20 mM (Uvelius et al., 1974; Hotta and Yamamoto, 1983). Nevertheless with uncertain ionic mechanisms, the spike activity was facilitated, whereas the phasic contraction was depressed under the substitution of external Ca with Sr (Figs. 6, 8, 9). It is probable that the capability of Sr to bind with myosin is lower than Ca, as has been shown for chicken gizzard (Ebash and Endo, 1968).

With some exceptions such as Ca-free contraction of uterine muscle (Sakai et al., 1981; Mironneau et al., 1984; Aschoori et al., 1985), it is generally accepted that smooth muscle contraction is a function of intracellular Ca concentration (Endo et al., 1977; Ochiai et al., 1981). Intracellular Ca of smooth muscles is by some means, e.g. via activation of Ca-channel, subject to extracellular Ca. Present results have shown that the base-line tension of the spayed preparation was elevated by raising external Ca concentration, whereas the elevation was less marked in the estrogen-treated preparation (Fig. 3). When the external Ca was depleted the base-line tension declined in the spayed preparation, whereas it remained the same as in the control solution in the estrogen-treated preparation (unpublished observation). The different effects of high Ca on the base-line tension may be due to: (1) Ca sequestering site is poorly developed, (2) a background Ca-influx occurs, and/or (3) a mechanism by which intracellular Ca is extruded is less developed in the spayed preparation.

The base-line tension is well defined in uterus muscle because of rhythmic contractions. When 0.6 mM Mg was applied to the spayed preparation, the base-line tension declined (Figs. 1, 10). According to the concept of Mg as a Ca-antagonist (Altura and Altura, 1981), it can be supposed that the spayed preparation is in a state of mild contracture, upon which phasic contractures are generated, due to a background Ca influx. A similar situation with regards to the decline of muscle tone by Mg and the elevation by raising Ca was mimicked in the estrogen-treated preparation, when the external Na concentration was reduced to 36.7 mM (Fig. 13). The membrane potential is hyperpolarized by 5 mV in the estrogen-treated preparations more than in the spayed ones. The hyperpolarization does not seem to fully account for the less active spontaneous activity, because the membrane potential of the estrogen-treated preparation was not altered in the low Na (36.7 mM) solution, but the spontaneous activity was accelerated (Fig. 13). By the use of different types of
Ca-antagonist, Weiss (1981) proposes sites of Ca entry for vascular smooth muscle as follows: voltage-sensitive channel, receptor-linked channel, and resting Ca entry. Passive Ca leak is also proposed for arterial smooth muscle (Van Breemen et al., 1982). Perhaps, resting Ca entry or passive Ca leak is more operative in the spayed preparation. The Ca leak or Ca permeability is thought to increase upon removal of external Na (artery, Van Breemen et al., 1982; myometrium, Masahashi and Tomita, 1983). Then, a rather specific action of Mg to block the passive Ca leak would be suggested from the present experiment (cf. Fig. 10). The Sr may enter through the passive Ca leak, when substituted for Ca, and lower the base-line tension (Fig. 6A) because of its low affinity to the contractile element.

Verapamil was less potent in inhibiting the action potential in the estrogen-treated preparation than in the spayed preparation (Figs. 10, 11). The reason is not well understood at present. It has been postulated that the sites of action of Ca-antagonist such as D600 are located in the membrane interior and that some permeation is needed to reach these sites (Triggle, 1981). Then, it could be that the permeation of verapamil was hindered in the estrogen-treated muscle, possibly by the alteration of membrane constituents.

External Ca higher than 12.5 mM depressed the generation of phasic contraction (Fig. 3) and action potential (Fig. 4) in the spayed preparation. The depression of action potential could be accounted for by the [Ca]$^{2+}$-sensitive K conductance as shown by Mironneau and Savineau (1980) for pregnant rat uterus. It has been shown in a particular case of rat iris sphincter that the Ba-induced action potential is inhibited by addition of Ca (Imaizumi et al., 1984). Why the “stabilizing” action of Ca is weak in the estrogen-treated preparation (Fig. 5 vs. Fig. 4) is to be explored. A question is again raised that the external Ca was less permeable across the membrane of the estrogen-treated preparation, whereby an increase in the intracellular Ca concentration was prevented (cf. Tomita and Watanabe, 1973).

Because of the all-or-none nature of phasic contractions, the functional syncytium may be assumed in uterine muscle strips of rat. Then, unidentified pacemaker cells may exist in the spayed preparation. Intracellular recording would have often been made on follower cells, whereby a noticeable change in the membrane potential may not have been observed when the spontaneous activity of the spayed preparation was altered by application of Mg or verapamil (Figs. 2, 11). Whether or not a background Ca influx composes a pacemaker potential, as proposed for guinea pig taenia coli (Riemer et al., 1975), is a remaining question.

The spayed preparation is spontaneously active and the estrogen-treated preparation is less active in vitro, and the same tendency exists in vivo (Fuchs, 1974; Downing et al., 1978). The teleological significance may be that the uterus at diestrus stage is spontaneously active in order to empty the intrauterine fluid which is gained during the estrus state. Moreover, the uterine tissue of the spayed rat may endeavor to prevent an atrophy from disuse, and to assist the blood circulation. The uterus may be less active at the estrus stage in order assure the movement of semen.
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