Stretch-induced enhancement of contractions in uterine smooth muscle of rats

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1. We studied the effect of servo-controlled stretch of smooth muscle strips from rat uterus on tension and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$, using fura-2 as an indicator) at 30 °C.

2. When quiescent uterine muscle strips were stretched at a ramp time of 0·5 s by multiples of 5% of the resting muscle length (L_0) up to 40%, forty-two out of sixty muscle strips responded with a transient active contraction and a $[Ca^{2+}]_i$ increase. The minimum excursion of stretch required for contraction was 26·3 ± 7·5% of L_0 (mean ± S.D.). The peak response had an all-or-none property and was almost independent of the duration of stretch.

3. Stretches of 30 or 35% of L_0 induced contraction in most cases when rapidly applied in 0·2–0·5 s, but slowly applied stretch (ramp duration of 5–10 s) rarely induced contraction.

4. The stretch-induced response was inhibited by the removal of extracellular Ca^{2+} or by the addition of 10 nM nicardipine. However, it was unaffected by 1 μM tetrodotoxin, 1 μM atropine or by 10 μM cyclopiazonic acid, an inhibitor of Ca^{2+}-ATPase in intracellular Ca^{2+} stores.

5. When a stretch of 15–35% of L_0 was applied during the relaxation phase of 10 nM oxytocin-induced rhythmic contractions, the first contraction after the stretch occurred earlier than that expected from the control rhythm. However, the frequency of the subsequent rhythm returned to almost the control level even during continued application of stretch, although the half-width of rhythmic contractions was increased during stretch.

6. The present study demonstrates that stretch of uterine muscle induces a transient contraction due to Ca^{2+} influx, which is myogenic and dependent on the excursion and velocity of stretch. The all-or-none property of the stretch-induced contractions suggests initiation of Ca^{2+} spikes. Furthermore, stretch modulates the oxytocin-induced rhythmic contractions.

It has been shown that various types of smooth muscles can be stimulated by stretch. Bozler (1947) showed that if the external force applied to dog ureter muscle was sufficiently strong, stretch induced a conducted all-or-none electrical response. In guinea-pig taenia caeci and other smooth muscles, stretch caused depolarization, initiation of spiking or an increase in spike frequency and, as a result, an increase in tension (Bülbring, 1955; Burnstock & Prosser, 1960). Kleinhaus & Kao (1969) first showed in myometrium that stretch initiated spike discharges which caused contractions. It has been suggested that stretch-activated (SA) channels are involved in the stretch-induced contractions. SA channel activities were first reported by Guharay & Sachs (1984) in cultured embryonic chick skeletal muscle cells. Later, they were found in many other types of cells: oocytes and red blood cells (Yang & Sachs, 1987; Hamill & MacBride, 1992), lens epithelial cells (Cooper, Tang, Rae & Eisenberg, 1986), hair cells (Ohmori, 1984; Hudspeth, 1985) and smooth muscle cells (Kirber, Walsh & Singer, 1988; Hisada, Ordway, Kirber, Singer & Walsh, 1991; Wellner & Isenberg, 1993). It has been shown that the inward currents flowing through SA channels tend to depolarize the cell. This depolarization in turn activates voltage-sensitive Ca^{2+} channels in smooth muscle cells (Walsh & Singer, 1980; Clapp, Vivaudou, Walsh & Singer, 1987; Vivaudou, Clapp, Walsh & Singer, 1988; Wellner & Isenberg, 1994). These results suggest that the SA channels are the mechanoelectrical transducer which is responsible

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for the initiation of stretch-induced responses in smooth muscle.

The uterus is subjected to stretch during pregnancy, and the stretch is thought to be one of the important factors controlling the initiation of labour, as well as growth and involution of uterus (Wray, 1982, 1983, 1993). To understand the physiological effects of stretch on the functions of uterine smooth muscle, we developed a method to study changes in $[Ca^{2+}]_i$ and isometric tension following controlled length alteration of longitudinal muscle strips during a resting condition and oxytocin-evoked rhythmic contractions. Stretch may induce passive tension and may also affect active tension via length–tension relationship. Ambiguity in estimating $[Ca^{2+}]_i$ from tension prompted us to carry out simultaneous measurement of $[Ca^{2+}]_i$ and tension, which would allow us to isolate the effects of stretch on $[Ca^{2+}]_i$ regulatory mechanisms. We show here that stretch induces all-or-none contractions in quiescent muscle and modifies oxytocin-induced rhythmic contractions, and that the effects are dependent on the magnitude, velocity and timing of muscle length change.

A preliminary account of this work has appeared elsewhere in abstract form (Kasai, Iino, Taketani & Endo, 1994*a*).

METHODS

Muscle preparation

Female Wistar rats, weighing about 200–300 g, were stunned and exsanguinated. In most experiments we used non-pregnant rats; however, in some experiments we also used pregnant animals (day 20 or 21 of pregnancy). Uteri were excised, and longitudinal muscle bundles (~1 mm long, 200–300 μm wide, and ~150 μm thick) were carefully dissected in physiological salt solution (PSS) with the composition given below. We obtained one or two preparations from each animal. The dissection was carried out at room temperature, and the experiments were performed at 30 °C.

Measurement of tension and fluorescence intensity

To load the smooth muscle strips with a fluorescent Ca^{2+} indicator, fura-2, muscle bundles were incubated in 20 μM fura-2AM in PSS with 0·005% Pluronic F-127 for 5 h at 30 °C. Then each end of the muscle bundle was tied with a silk filament, and the preparation was attached horizontally to a pair of stainless-steel hooks. One of the hooks was connected to an isometric tension transducer (AE801; Akers, Horten, Norway) and the other was driven by a servomotor for length control of the muscle preparation (Fig. 1*A*). The tension signal was amplified using a bridge circuit and a DC amplifier (AM30; Unipulse, Koshigaya, Japan).

The muscle strips were placed in an experimental trough (capacity, ~300 μl), mounted on a fluorimeter (CAF-100; JASCO, Tokyo, Japan), and were illuminated alternately by 340 and 380 nm

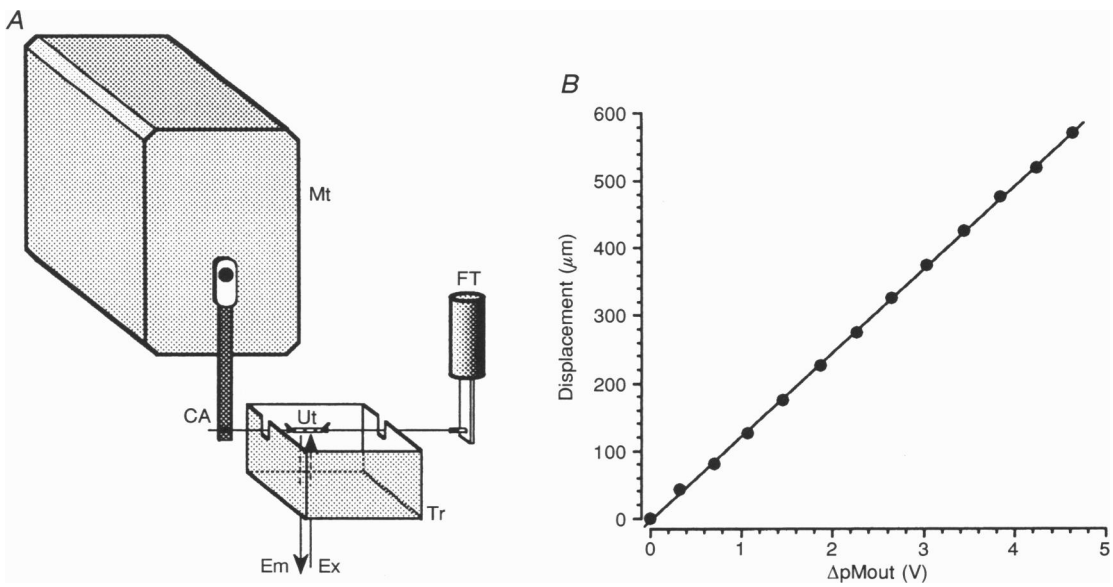

Figure 1. Schematic representation of the experimental set-up and calibration of motor

A, experimental set-up. The uterine smooth muscle strip (Ut) was mounted horizontally between metal hooks in an experimental trough (Tr). The preparation and hooks were submerged in solution through narrow slits on both sides of the trough. Surface tension prevented flow of solution out of the slits. One of the hooks was connected to a servomotor (Mt) via a carbon-fibre arm (CA). The other hook was attached to a force transducer (FT). The motor and force transducer were mounted separately on manipulators for position adjustment. The excitation (Ex) and emission (Em) lights were guided through the glass bottom of the trough. Inlet and outlet tubings for the solution in the trough are not shown. Temperature-controlled circulating water around the experimental trough was used to maintain the temperature of solution in the trough at 30 °C. *B*, relationship between change in output voltage of the motor position sensor (ΔpMout) and the horizontal excursion of the tip of the hook measured under a binocular microscope (× 20 objective lens).

wavelength light at 50 Hz (Fig. 1A). Fluorescence signals from the muscle strips were collected by a photomultiplier tube through a 500 nm interference filter. When $[Ca^{2+}]_i$ rose, the changes in the fluorescence intensity (photomultiplier current) at 340 and 380 nm excitations showed mirror-image features, as expected from the property of the dye. Therefore, the ratio of the two fluorescence intensities (F_{340}/F_{380}) was used as an indicator of $[Ca^{2+}]_i$. The diameter of the excitation light beam was sufficiently large (~2·8 mm) to illuminate the entire length of the muscle preparations so that with proper positioning, negligible artifact in the fluorescence signal was observed during stretch. However, in some experiments, small movement artifacts were noted (Fig. 2A).

To change bathing solutions, the solution in the trough was first aspirated through an electromagnetic valve and a new solution was injected using a peristaltic pump (MHRE-200; Watson-Marlow, Falmouth, UK). The procedure was controlled by a personal computer (PC9801DS; NEC, Tokyo, Japan) and was accomplished within 5 s. The two fluorescence intensity signals, isometric tension and muscle length signals (see below) were collected at 5 or 10 Hz using a 12-bit analog-to-digital converter board (AnalogPro II; Canopus, Kobe, Japan) in the computer and stored on floppy disks for subsequent analysis and plotting using another computer (Quadra 800; Apple, USA).

Application of length change

A thin stainless-steel hook (diameter, 100 μm) was shellacked at 90 deg onto a carbon-fibre arm connected firmly to a driver shaft of a galvanometer motor with a position sensor (G120DT; General Scanning Inc., Watertown, MA, USA; Fig. 1A). The motor position was controlled with feedback from the output of the position sensor using a driver amplifier (MmPic 20A; Nihon Telescom, Tokyo, Japan). The motor was capable of producing a step position change in 0·5 ms in response to a rectangular command signal; however, for the present experiments it was driven at much slower speed with ramp command signals: between 50 and 400 μm of excursion in 0·1–10 s. The lengths of the motor arm and the metal hook were 14 and 6 mm, respectively. With this setting and horizontal excursion of 400 μm in 1 mm long muscle, the vertical movement of the hook was less than 1% of the total muscle length. The horizontal excursion of the tip of the metal hook was linearly related to the output of the motor position sensor, which was used as the muscle length signal (Fig. 1B). The programmed command signal to the motor was applied by another personal computer (PC9801VF; NEC) through a 12-bit digital-to-analog converter board (DAJ-98; Canopus). The duration of the stretch was varied between 0·1 and 240 s.

Before each experiment, muscle strips were stretched to L_0, which was 125 and 250% of the slack length of non-pregnant and pregnant uterus, respectively. Muscle length *in situ* was estimated from several muscle strips using the previously reported method (Iino, 1981). Briefly, immediately after laparotomy, a pair of charcoal markings separated by 10 mm were made on the surface of the uterus. Then the uterine muscle was excised, from which thin muscle bundles were dissected. By measuring the distance between the charcoal markings on the thin bundle, we were able to estimate the *in situ* length of the bundle. L_0 was close to the *in situ* length of the muscle in both non-pregnant and pregnant rats.

Stretches were applied by multiples of 5% of L_0 up to 40%, both in the quiescent condition in PSS and during oxytocin-evoked rhythmic contractions. In most experiments stretch was applied in 0·5 s. We also applied faster and slower stretches depending on the design of experiments. In some preparations spontaneous rhythmic contractions were observed. However, since we dissected out only a small part of the longitudinal muscle, spontaneous contractions were unstable and often faded away in several tens of minutes. Therefore, we were not able to systematically study the effect of stretch on spontaneous contractions. Even in quiescent muscles oxytocin-induced rhythmic contractions were always observed.

Materials

The solutions used were as follows (mM): physiological salt solution (PSS): NaCl, 150; KCl, 4; $CaCl_2$, 2; $MgCl_2$, 1; Hepes, 5; glucose, 5·6. High-K^+ solution: NaCl, 74; KCl, 80; $CaCl_2$, 2; $MgCl_2$, 1; Hepes, 5; glucose, 5·6. High-K^+ Ca^{2+}-free solution: NaCl, 74; KCl, 70; $MgCl_2$, 3; Hepes, 5; EGTA, 5; glucose, 5·6. The pH of all solutions was adjusted to 7·4 with NaOH.

Chemicals used were fura-2 AM from Dojindo Labs (Kumamoto, Japan), Pluronic F-127 from BASF (Parsippany, NJ, USA), cyclopiazonic acid (CPA) and nicardipine from Sigma, tetrodotoxin (TTX) from Calbiochem (USA), and oxytocin from Peptide Institute Inc. (Osaka, Japan). All the other reagents were of the highest grade available.

RESULTS

Stretch-induced contractions in uterine smooth muscle

Figure 2A shows a typical effect of stretch on the tension and $[Ca^{2+}]_i$ of uterine smooth muscle in PSS. In A, the same muscle strip was stretched by 15, 20 and 25% of L_0 in 0·5 s and subjected to continuous stretch of the same magnitude for 1·5 s before being released so that its length returned to the original value in the next 0·5 s. When stretched by 15% from L_0, $[Ca^{2+}]_i$ remained at the resting level and contraction did not occur. However, when stretched by 20%, a rise in $[Ca^{2+}]_i$ and an accompanying contraction were observed. When stretched by 25%, the peak value of the rise in $[Ca^{2+}]_i$ seemed to be the same as that with 20% stretch. The results suggest that there is a threshold level of stretch for activation, and that the response has an all-or-none property.

Similar results were observed in many other strips. When uterine muscle strips including that in Fig. 2A were stretched in 0·5 s by multiples of 5% of L_0 up to 40%, forty-two out of sixty (70%) muscle strips responded with a transient contraction and a $[Ca^{2+}]_i$ increase. The average minimum excursion of stretch required for inducing contraction was 26·3 ± 7·5% of L_0 (mean ± S.D., $n = 42$). The magnitude of stretch-induced contractions was 69 ± 25% (mean ± S.D., $n = 23$) of that for 80 mM K^+-induced contractions, and the $[Ca^{2+}]_i$ increase was 83 ± 13% (mean ± S.D., $n = 18$) of the high-K^+ response.

The peak value of the rise in $[Ca^{2+}]_i$ was normalized by that of a successful response with the minimum magnitude of stretch (threshold) and plotted against the excursion minus

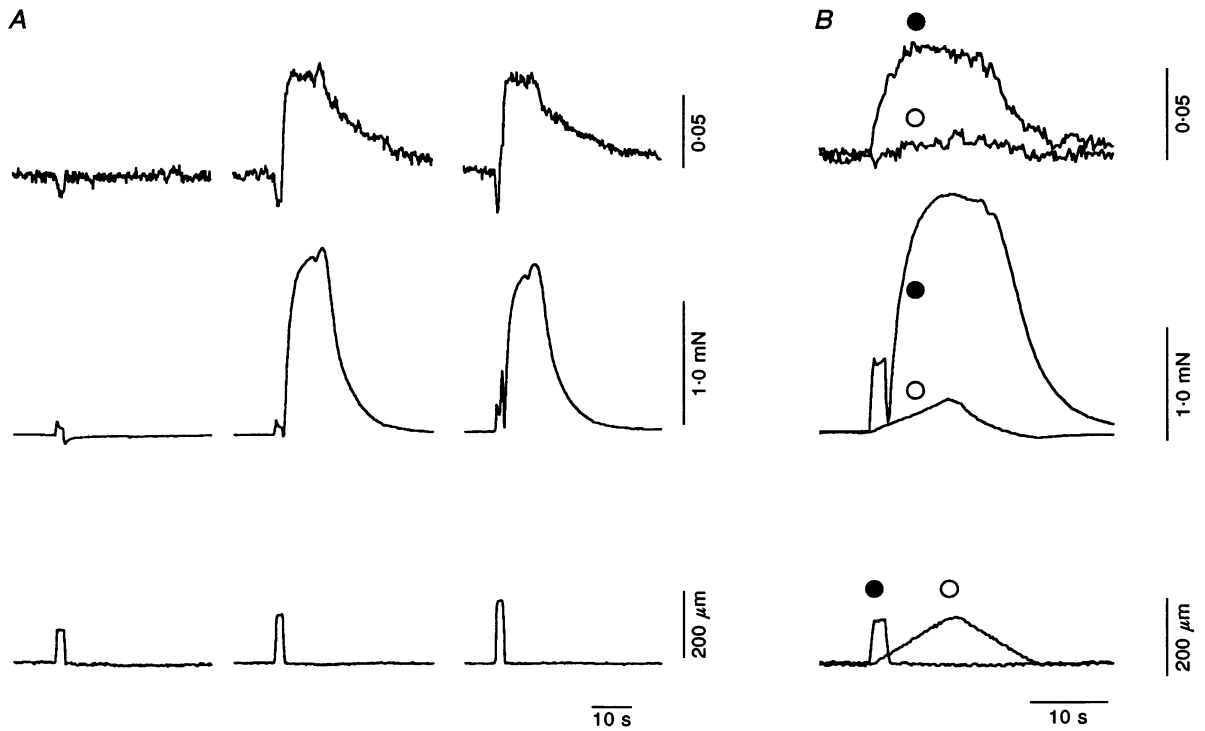

Figure 2. Stretch-induced contraction in the uterine smooth muscle and its dependence on the magnitude and velocity of stretch

Changes in $[Ca^{2+}]_i$ expressed by F_{340}/F_{380} (upper traces) and isometric tension (middle traces) were simultaneously measured with the muscle length (lower traces). *A*, the same muscle strip was stretched by 15, 20 and 25% of L_0 in 0·5 s (left to right). $L_0 = 0{\cdot}80$ mm. The dips in the $[Ca^{2+}]_i$ traces during stretch were due to movement artifacts. These results are representative of 6 muscle strips, and they show that the response had an all-or-none property. *B*, another muscle strip was stretched by 20% of L_0 in either 0·5 s (●) or 10 s (○). $L_0 = 0{\cdot}75$ mm. Representative result of 9 muscle strips.

threshold (Fig. 3). Normalized peak responses to stretches which were 5 and 10% of L_0 greater than the minimum excursion were $0{\cdot}95 \pm 0{\cdot}08$ (mean ± S.D., $n = 6$) and $1{\cdot}04 \pm 0{\cdot}11$ ($n = 4$), respectively. Similarly, the normalized peak tensions were $0{\cdot}95 \pm 0{\cdot}13$ (threshold + 5% of L_0, mean ± S.D., $n = 6$) and $0{\cdot}96 \pm 0{\cdot}14$ (threshold + 10% of L_0, mean ± S.D., $n = 4$). These results demonstrate that the stretch induces an all-or-none response. We systematically increased the size of stretch in these experiments. This was because we observed that the threshold excursion value required for contraction often increased after a large stretch.

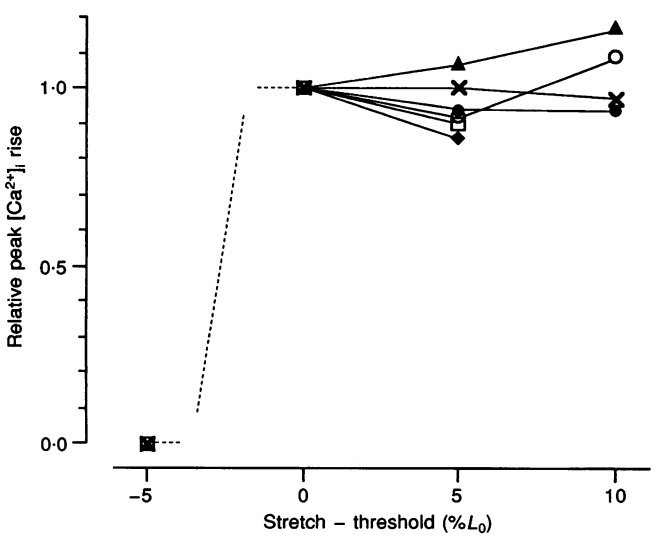

Figure 3. All-or-none property of stretch-induced $[Ca^{2+}]_i$ rise

Peak change in F_{340}/F_{380} as a function of the difference between size of stretch and threshold. Peak responses were normalized with the value at the threshold excursion in each strip. Results from 6 strips. Threshold excursion was 15% (□, ◆), 20% (●, ○, ×) and 25% (▲) of L_0.

Dependence of contraction on the velocity and magnitude of stretch

Figure 2*B* shows the responses of another muscle strip stretched by 20% of L_0 at different velocities. Stretch-induced contraction was observed when stretch was imposed in 0·5 s (●); however, when stretch was applied in 10 s (○), active contraction did not occur and only passive tension was observed. An active contraction was observed when stretch was again applied in 0·5 s to the same muscle (not shown). These results were reproduced in other muscle strips when they were stretched by either 30 or 35% of L_0. When stretched in 0·2–0·5 s, active contraction was evoked in fifty-two strips out of seventy-four, whereas slowly applied stretch (5–10 s ramp time) rarely induced contraction (1 out of 10 cases). Stretch with an intermediate velocity (1 s ramp time) induced contraction in two out of five strips. These results show that stretch-activated contraction was dependent on the velocity of stretch.

Since we changed the excursion of stretch within the constant ramp duration in the experiment shown in Fig. 2*A*, the speed of stretch was also varied. Thus, one might argue that the speed of stretch is the sole determinant factor for the initiation of stretch-induced response. However, the stretch with smaller excursion (70–80% of the minimum excursion for a successful response) but with two to four times greater speed failed to induce contraction in four out of seven strips tested. Therefore, not only the velocity but the magnitude of stretch is important for the initiation of stretch-induced contractions.

Time course of $[Ca^{2+}]_i$ and tension changes after the application of stretch

Next we studied the temporal relationship between stretch of muscle length and the resulting changes in $[Ca^{2+}]_i$ and tension. To observe clearly the time course of $[Ca^{2+}]_i$ and contraction, brief stretches were applied: 0·1 s each for rise time, plateau and fall time. A typical result is shown in Fig. 4, where the muscle strip was stretched by 40% of L_0. The $[Ca^{2+}]_i$ rise preceded the tension increase, as shown in Fig. 4*A*. To show the early time course in detail, we plotted the results on a faster time base in Fig. 4*B*. It can be seen that the rise of $[Ca^{2+}]_i$ began without a long latency, within 0·1–0·2 s after the initiation of stretch. The development of active tension, however, lagged behind the $[Ca^{2+}]_i$ change by ~1 s.

Independence of peak $[Ca^{2+}]_i$ response on the duration of stretch

The induction of stretch-induced response was dependent on the velocity and the excursion of stretch; however, the peak response was much less dependent on the duration of the plateau of stretch. Figure 5 shows results obtained from the same muscle strip activated by stretch of various durations. In *A*, the muscle was stretched by 30% of L_0 in 0·5 s and subjected to continuous stretch of the same magnitude for 1·5 s before being released so that its length returned to the original value in the next 0·5 s. In Fig. 5*B*, the same muscle bundle as in Fig. 5*A* was stretched by the same magnitude for a longer duration (20 s). The peak rise in $[Ca^{2+}]_i$ as well as peak tension during stretch were almost the same as in *A*. Furthermore, the minimum

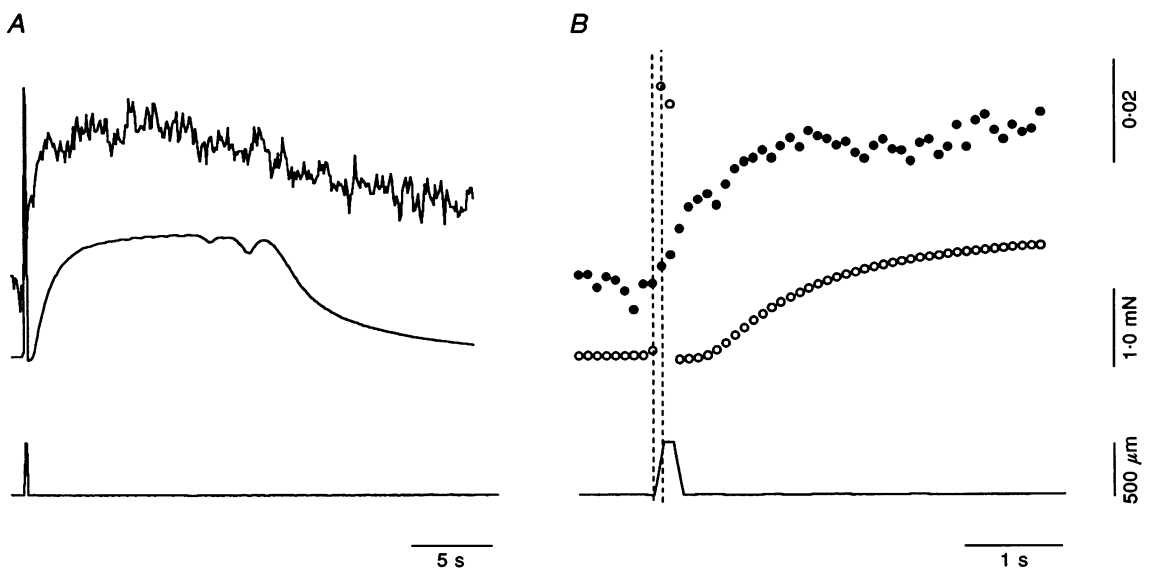

Figure 4. Time course of changes in $[Ca^{2+}]_i$ and tension after stretch

In each panel, upper, middle and lower traces show F_{340}/F_{380}, isometric tension and muscle length, respectively. Stretch of 40% of L_0 was applied to the muscle strip, 0·1 s each for the rise time, plateau and fall time. F_{340}/F_{380} (●) and isometric tension (○) were sampled every 0·1 s and were plotted on a faster time base in *B*. The rise in $[Ca^{2+}]_i$ began within 0·1–0·2 s after the start of stretch. Active contraction lagged behind $[Ca^{2+}]_i$ rise by ~1 s. $L_0 = 1·0$ mm. Representative result of 3 experiments.

excursion of stretch required for inducing contraction was almost independent of the stretch duration. The minimum excursion values of stretch for 1·5 and 20 s stretch plateaus (0·5 s for rise time) were 26·3 ± 7·5% (mean ± S.D., $n = 42$) and 27·5 ± 8·4% ($n = 38$), respectively, and there was no significant difference between them. Even when the duration of the stretch plateau was shortened to 0·1 s, the average minimum excursion of stretch required for inducing contraction was 25·8 ± 7·4% ($n = 6$). These results suggest that the peak value of stretch-induced rise in $[Ca^{2+}]_i$ and magnitude of stretch-induced contraction were almost independent of the duration of the stretch plateau above 0·1 s. Moreover, the duration of contraction was not proportionally dependent on the stretch duration, although it tended to be longer with greater duration of stretch (Fig. 5*A* *vs.* *B*). Indeed, $[Ca^{2+}]_i$ showed spontaneous decline during 20 s stretch (Fig. 5*B*), and this was observed in all muscles tested ($n = 38$).

We also examined stretch-induced contractions in the myometrium taken from pregnant rats at day 19 or 20 of pregnancy. We had to stretch the muscle to 250% of the slack length in order for L_0 to be approximately equal to the *in situ* length (see Methods). The minimum excursion of stretch with 0·5 s rise and fall times and a 1·5 s stretch plateau was 14·0 ± 6·5% ($n = 5$).

Source of Ca^{2+} for stretch-induced $[Ca^{2+}]_i$ rise

After removal of extracellular Ca^{2+} with addition of 5 mM EGTA for 2–5 min the stretch-induced transient $[Ca^{2+}]_i$ rise and accompanying contraction were not observed ($n = 9$), although a stretch of the same magnitude and velocity induced contractions in the same muscle in the normal PSS. Furthermore, stretch-induced responses were suppressed completely in the presence of 10 nM nicardipine ($n = 5$).

To examine whether Ca^{2+} release from internal stores is involved in stretch-induced responses, we have studied the effect of CPA (10 nM), which inhibits the Ca^{2+}-ATPase of intracellular Ca^{2+} stores and thereby suppresses the Ca^{2+} release from the stores. With 10 nM CPA, a concentration sufficient to inhibit oxytocin-induced Ca^{2+} release in rat uterus (Kasai, Iino, Tsutsumi, Taketani & Endo, 1994*b*), we found no inhibitory effect on stretch-induced responses. The peak magnitude of the transient contraction was rather augmented to 1·20 ± 0·1 (mean ± S.D., $n = 5$) of that of control. Moreover, the half-width of both the transient $[Ca^{2+}]_i$ rise and contraction became greater than those of the control.

Absence of neural influence in stretch-induced responses

We then examined the possibility of neural involvement in stretch-induced responses. Neither 1 μM TTX, a voltage-dependent Na^+-channel blocker, nor 1 μM atropine, a muscarinic receptor antagonist used to block parasympathetic influence, had any apparent inhibitory effect on stretch-induced increase in $[Ca^{2+}]_i$ or contraction. The peak magnitude of the transient contraction in the presence of TTX and atropine were 0·99 ± 0·18 (mean ± S.D., $n = 5$) and 0·96 ± 0·04 ($n = 4$) of those of control, respectively.

Effects of stretch on oxytocin-induced rhythmic contractions

Oxytocin induces rhythmic contractions in uterus. We therefore studied the effects of stretch on the rhythmic contractions. The frequency of rhythm in the presence of 10 nM oxytocin was 0·75 ± 0·16 min^{-1} in our muscle preparations (mean ± S.D., $n = 21$).

If stretch was applied in 0·5 s during the relaxation phase of the rhythmic contraction (0·75T_0 or 0·5T_0 after the onset of the previous contraction, where T_0 represents the average period of rhythmic contractions), the first contraction after the stretch occurred earlier than that expected from the rhythm before the stretch, as shown in Fig. 6*A* and *B*. Therefore, stretch has a phase-advancing effect on the oxytocin-induced rhythm. In Fig. 6*A* the muscle was stretched by 15% of L_0 at 0·75T_0 during the relaxation phase. The first contraction occurred ~4 s after

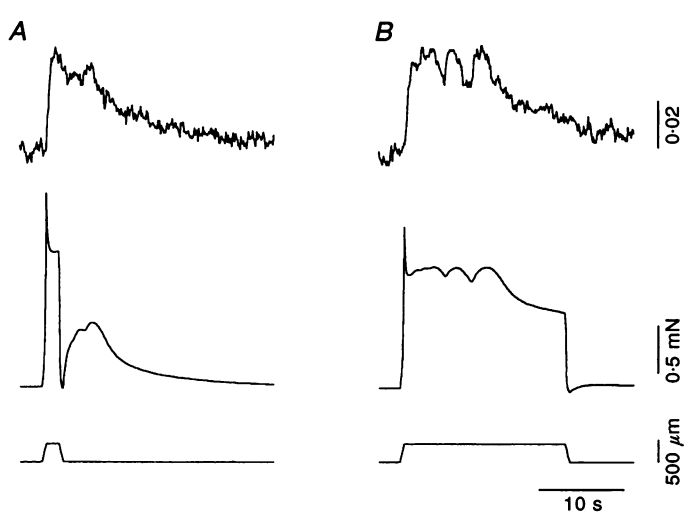

Figure 5. Independence of peak $[Ca^{2+}]_i$ rise on the duration of stretch

In each panel, upper, middle and lower traces show F_{340}/F_{380}, isometric tension and muscle length, respectively. *A*, the muscle strip in PSS was stretched by 30% of L_0 in 0·5 s and was subjected to continuous stretch of the same magnitude for 1·5 s before being released so that its length returned to the original value in the next 0·5 s. *B*, the same muscle bundle was stretched by the same magnitude for longer duration (20 s). $L_0 = 1·25$ mm.

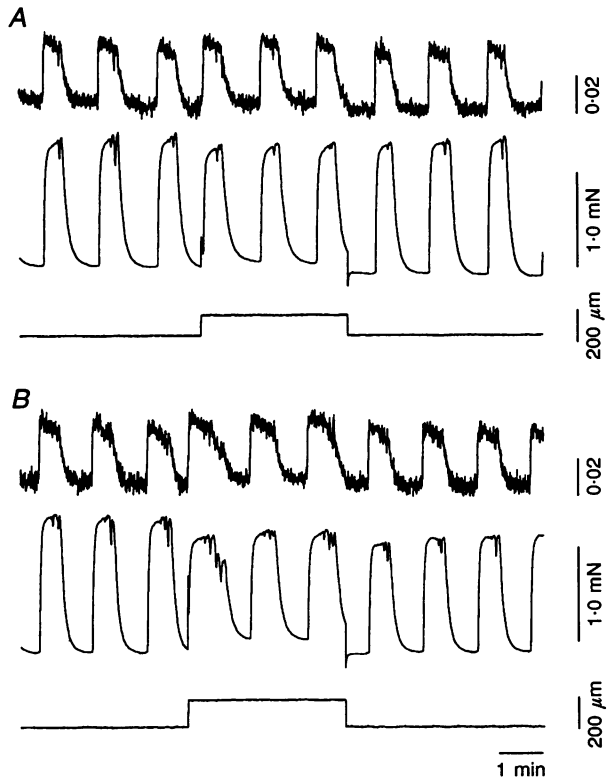

Figure 6. Effect of stretch on oxytocin-induced rhythmic contractions

In each panel, upper, middle and lower traces show F_{340}/F_{380}, isometric tension and muscle length, respectively. Rhythmic contractions were induced by 10 nM oxytocin. A stretch was applied in 0·5 s during the relaxation phase of the rhythmic contraction at $0{\cdot}75T_0$ (T_0: average period of rhythmic contractions of this muscle) and the muscle was subjected to continuous stretch of constant magnitude for three contractions before being released so that its length returned to the original value. *A*, the muscle was stretched by 15% of L_0. *B*, the same muscle strip was stretched by 25% of L_0. $L_0 = 1{\cdot}0$ mm. Representative result of 7 muscle strips.

the stretch, although it was expected to take place after ~21 s based on the control rhythm. When the same muscle strip was stretched by 25% of L_0 at $0{\cdot}75T_0$ (Fig. 6*B*), the contraction took place without a significant lag period. Furthermore, the half-width of both rise in $[Ca^{2+}]_i$ and tension became greater during the stretch. The frequency of the rhythm during continued application of stretch usually returned to the control level, as shown in Fig. 6*A*. However, in some cases with large increases in the half-width of contraction, the frequency was reduced during stretch, as seen in Fig. 6*B*. When the muscle strips were released to the original length, the frequency of the rhythm and the half-width of contraction returned to the control values.

When a brief stretch lasting ~2 s was imposed during the relaxation phase, the next rhythmic contraction took place earlier than expected from the control rhythm, as was the case with continued stretch ($n = 5$). However, there was no effect of brief stretch on the half-width of the subsequent rhythmic contractions.

Slowly applied stretch was also effective in inducing changes in oxytocin-evoked rhythmic contractions. In Fig. 7*A* a slow ramp stretch (30% L_0) was applied during the relaxation phase of the rhythmic contractions induced by 10 nM oxytocin. The stretch was initiated at $0{\cdot}75T_0$ and the ramp duration was 10 s. The muscle was subjected to continuous stretch of constant magnitude for two contractions before being released so that its length

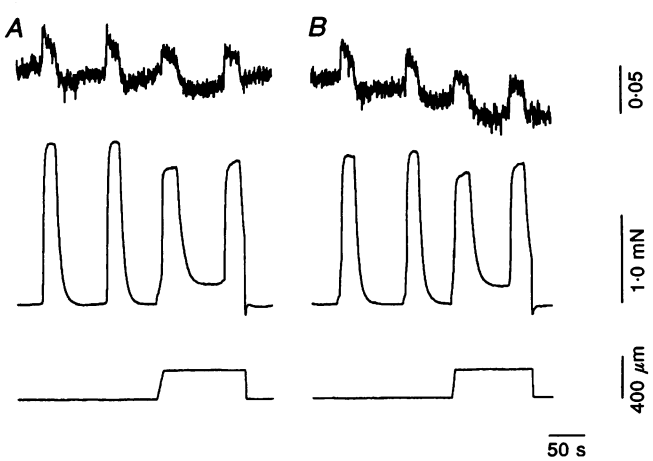

Figure 7. Effect of slow stretch on oxytocin-induced rhythmic contractions

In each panel, upper, middle and lower traces show F_{340}/F_{380}, isometric tension and muscle length, respectively. A slow ramp stretch (30% L_0) was applied during the relaxation phase of the rhythmic contraction induced by 10 nM oxytocin. The stretch was initiated at $0{\cdot}75T_0$ and the muscle was subjected to continued stretch of constant magnitude for two contractions before being released so that its length returned to the original value in 0·5 s. The ramp durations were 10 s (*A*) and 5 s (*B*). $L_0 = 1{\cdot}0$ mm. Representative result of 5 muscle strips.

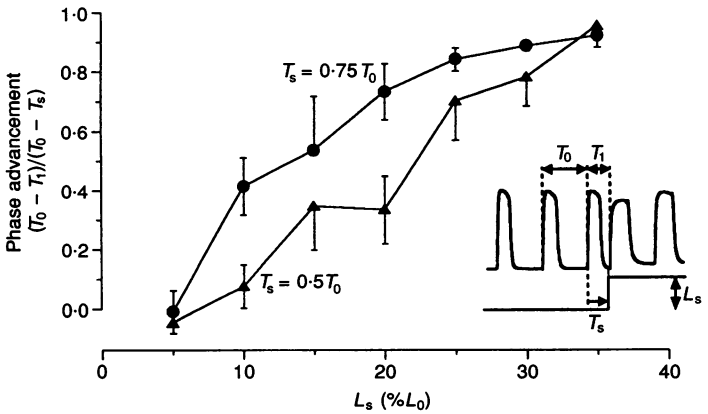

Figure 8. Phase-advancing effect of stretch on oxytocin-induced rhythmic contractions
The magnitude of stretch (L_s) is shown on the abscissa. The phase-advancing effect was evaluated from $(T_0 - T_1)/(T_0 - T_s)$ (see text for explanation). The stretch was applied at both $0{\cdot}75T_0$ (●) and at $0{\cdot}5T_0$ (▲). Means ± S.E.M., $n = 4$–5 for $L_s = 5\%$, $n = 6$–8 for $L_s = 10$–30%, $n = 2$–3 for $L_s = 35\%$.

returned to the original value in 0·5 s. The rhythmic contraction immediately after the stretch occurred earlier than expected from the control rhythm and its half-width was greater than that of the control. With faster ramp stretch (5 s ramp, Fig. 7*B*), the phase-advancing effect became greater.

Phase-advancing effect of stretch

Figure 8 summarizes the phase-advancing effect of stretch (0·5 s ramp) on the rhythmic contractions induced by 10 nM oxytocin. To evaluate the phase-advancing effect, $(T_0 - T_1)/(T_0 - T_s)$ was calculated (T_1: period from the initiation of previous rhythmic contraction to that of the next contraction immediately after stretch; T_s: time at which application of stretch is initiated, measured from the onset of previous contraction; see inset of Fig. 8). $(T_0 - T_1)/(T_0 - T_s)$ is zero when there is no effect of stretch on the rhythm so that $T_1 = T_0$, while it becomes greater with greater phase-advancing effect and approaches a limiting value of unity when stretch immediately induces the next contraction so that $T_1 = T_s$. (Since tension rise lags behind $[Ca^{2+}]_i$ increase by a few seconds, the maximum value of phase advancement is slightly below 1·0.) With the increase in the magnitude of stretch (L_s), the phase advancement value approached 1·0, which means that the latent time from stretch to contraction was diminished.

The phase advancing effect of stretch was greater when stretch was applied towards the end of the relaxation phase. When the stretch was applied at $0{\cdot}75T_0$, significant phase advancement was observed with L_s of 10% of L_0 or greater (●), whereas when stretch was applied at $0{\cdot}5T_0$, L_s of 15% of L_0 was required for significant phase advancement (▲). With L_s of 35% of L_0, the lag time between stretch and initiation of contraction ($T_1 - T_s$) became less than 2 s when stretch was applied at either $0{\cdot}75T_0$ or $0{\cdot}5T_0$.

Effect of stretch on the period of oxytocin-induced rhythmic contractions

Figure 9 shows the effect of stretch on the relative period of rhythmic contractions. The relative period was measured by T_i/T_0, where T_i represents the period between the $(i-1)$th to ith oscillations, $i = 1$ for the first contraction after stretch (see Fig. 9 inset). T_s was fixed at $0{\cdot}5T_0$ and three rhythmic contractions were observed during stretch. It can be seen that T_1 was decreased but that average values of both T_2 and T_3 during stretch were similar to the control values before the stretch. When muscle strips were stretched by 25–35% of L_0 (●), T_1 was smaller than that in the case of stretch by 5–20% of L_0 (▲). When muscle strips returned to the original length, there was no significant change in the relative period.

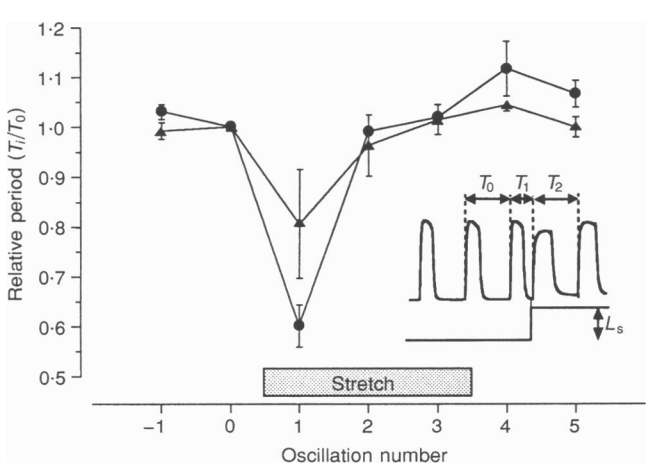

Figure 9. Changes in the relative period of rhythmic contractions in response to stretch
Relative period of rhythmic contractions (T_i/T_0) plotted as a function of oscillation number. T_i represents the period between the $(i-1)$th contraction and the ith contraction. ●, stretch of 25–35% of L_0 ($n = 10$) and ▲, stretch of 5–20% of L_0 ($n = 5$). Means ± S.E.M.

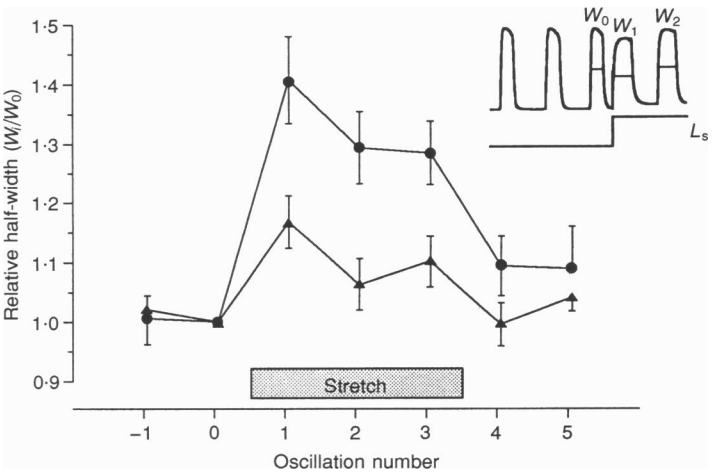

Figure 10. Effect of stretch on the half-width of the rhythmic contraction
Relative half-width of rhythmic contractions (W_i/W_0) plotted against the oscillation number. ●, stretch by 25–35 % of L_0 ($n = 8$) and ▲, stretch by 5–20 % of L_0 ($n = 10$)). Means ± S.E.M.

Stretch prolongs the duration of rhythmic contractions

Figure 10 shows the effect of stretch on the half-width (W_i) of the ith rhythmic contraction. Relative half-width, expressed as W_i/W_0, was plotted as a function of the oscillation number. It was increased during stretch compared with those before and after the stretch. W_1/W_0 was larger than W_2/W_0 or W_3/W_0, but the relative half-width of contractions remained increased during stretch, unlike the phase-advancing effect (Fig. 9). The increase in contraction duration was greater with greater magnitude of stretch.

DISCUSSION

Using a method to impose controlled length changes on muscle strips, we studied quantitative relationships between stretch and contraction in uterine smooth muscle. We found that a stretch with sufficient magnitude and velocity applied to quiescent muscle strips induced a rise in $[Ca^{2+}]_i$ accompanied by contraction, which had an all-or-none property. It was also found that stretch had profound effects on oxytocin-induced rhythmic contractions. Stretch shifts the phase of subsequent rhythmic contractions without changing the frequency of rhythm, and increases the duration of rhythmic contractions. Although we have carried out a systematic study only in non-pregnant uterus, qualitatively similar results were obtained in pregnant uterus. These results suggest that stretch is one of the important regulating factors of the contractile responses of uterine smooth muscle.

Comparison with previous studies

There have been only a few studies on the stretch-dependent contractions of uterine smooth muscle. Kleinhaus & Kao (1969) demonstrated the effects of stretch on action potentials in uterine smooth muscle. When a quiescent rabbit myometrial preparation was subjected to a quick and short stretch, it became electrically active. Their experiments were qualitative, and they did not vary the magnitude of applied stretch.

It has been proposed that stretch applied to cerebral and coronary arteries produces myogenic contraction by mobilization of Ca^{2+} from both extracellular spaces and intracellular storage sites (Nakayama & Tanaka, 1993). A similar effect of stretch on intracellular Ca^{2+} stores has been suggested in rat uterine strips. Csabina, Bárány & Bárány (1986) observed active force development and myosin light chain phosphorylation subsequent to transient stretch 1 min after removal of extracellular Ca^{2+} with 1 mM EGTA. They did not observe stretch-induced contraction after EGTA treatment for 50 min, when Ca^{2+} stores were presumably depleted. From these observations, the authors concluded that Ca^{2+} mobilized from intracellular stores was involved in stretch-induced contraction of rat uterus. However, since they used rather thick preparations (presumably cross-sectional area of ~10 mm^2), it seems likely that Ca^{2+} in the extracellular space was not completely removed within 1 min. On the other hand, we used much thinner preparations (~0·04 mm^2 cross-sectional area) to facilitate diffusion. We observed no stretch-induced contractions either in the absence of extracellular Ca^{2+} or in the presence of a Ca^{2+} channel blocker, nicardipine. Furthermore, we found no inhibitory effect of CPA, which is a potent inhibitor of Ca^{2+}-ATPase in Ca^{2+} stores. Our results suggest that stretch-induced contractions in uterine smooth muscle are dependent on Ca^{2+} influx from the extracellular space.

Stretch-induced contractions in quiescent muscle

Stretch-induced transient contraction in uterine smooth muscle was not influenced by TTX or atropine. This result suggests that neural stimulation is not involved in this type of contraction and might be explained by the existence of stretch-sensitive channels in the uterine smooth muscle cells. SA channels were first demonstrated in cultured skeletal muscle cells (Guharay & Sachs, 1984) and have

since been characterized in a number of different cell types including smooth muscle cells. The SA channel is a non-selective cation channel which permits the passage of Na^+, K^+ and Ca^{2+}. This flux of cations results in membrane depolarization, leading to activation of the voltage-gated Ca^{2+} channels (Kirber *et al.* 1988; Meininger & Davis, 1992).

As shown in Fig. 2, the magnitude and the velocity of stretch are important for the contraction. However, the peak magnitudes of stretch-evoked responses were almost independent of the strength of stretch and the responses showed an all-or-none property (Fig. 3). These results suggest that stretch induced depolarization by activation of SA channels, and that if the depolarization reached the threshold level then all-or-none Ca^{2+} spikes were generated as has been shown in urinary bladder myocytes by Wellner & Isenberg (1994).

Dependence of stretch-induced responses on the velocity of stretch suggests the presence of an accommodation process. Accommodation may be due to a viscoelastic property of muscle strips, or to stress–relaxation. The tension in the plasma membrane may decline rapidly after stretch, and slowly applied stretch would fail to induce sufficient passive tension for the activation of SA channels. It could also be due to adaptation of SA channels. It has been shown that the SA channel activity in the patch membrane of *Xenopus* oocytes declined rapidly after the application of constant pressure to the membrane (Hamill & MacBride, 1992), although such adaptation seems to be absent in vascular and urinary bladder myocytes (Davis, Donovitz & Hood, 1992; Wellner & Isenberg, 1993).

Both gadolinium (Gd^{3+}) and amiloride have been shown to inhibit SA channels (Yang & Sachs, 1989; Berrier, Coulombe, Szabo, Zoratti & Ghazi, 1989; Hamill, Lane & MacBride, 1992). Although 100 μM Gd^{3+} inhibited the stretch-induced responses in rat uterus, it also inhibited 80 mM K^+-induced contractions (Y. Kasai & M. Iino, unpublished observation) in accordance with the report that Gd^{3+} is a potent L-type Ca^{2+} channel blocker (Lacampagne, Gannier, Argibay, Garnier & Le Guennec, 1994). Thus we could not distinguish the effects of Gd^{3+} on the SA channels from those on the voltage-dependent Ca^{2+} channels. Amiloride (up to 3 mM) failed to inhibit stretch-induced responses in our preparations (Y. Kasai & M. Iino, unpublished observation), suggesting that SA channels in rat uterus are less sensitive to amiloride.

In pregnant rat uteri, the threshold excursion value of stretch applied in 0·5 s was ~14% which was smaller than that of uterine muscle from the non-pregnant rat (~26%). As described in Methods, we had to stretch the preparation from pregnant rats to 250% of the slack length to bring it to the *in situ* length. Therefore, a further study is required to clarify whether the apparent increase in the sensitivity was due to real change in the smooth muscle cells or due simply to altered initial muscle length.

Effect of stretch on oxytocin-induced rhythmic contractions

Oxytocin induces rhythmic contractions in uterus. During each contraction a burst of action potentials is observed subsequent to slow depolarization, which is thought to be the pacemaker potential (Parkington & Coleman, 1990). Uterine smooth muscle cells have internal Ca^{2+} stores, and oxytocin at a sufficiently high concentration induces release of Ca^{2+} from the stores. However, intracellular Ca^{2+} stores are unlikely to be involved in oxytocin-induced rhythmic contractions because depletion of Ca^{2+} stores by CPA had no effect on the contractions (Kasai *et al.* 1994*b*). Therefore, Ca^{2+} influx during action potentials seems to be the major source of $[Ca^{2+}]_i$ rise associated with the rhythmic contractions. However, the mechanism of generation of the pacemaker potential has not yet been elucidated. Since stretch had a profound effect on the rhythmic contractions, the results may provide some clues to this important mechanism.

Stretch of sufficient magnitude shifted the subsequent contraction to a time point earlier than that predicted by the control rhythm. The effect was more pronounced when the stretch was applied towards the end of the relaxation phase (Fig. 8). However, the effect of stretch on the period of rhythm was transient and the period returned to the control value during sustained stretch (Fig. 9). In other words, stretch reset the phase of rhythm without changing the frequency. One of the possible mechanisms behind this is that stretch facilitated pacemaker potential and that this effect was transient due, for example, to rapid accommodation. It will therefore be important to measure membrane potential under these conditions, although such measurement was not possible in our current experimental set-up.

The mechanism involved in maintenance of the burst of action potentials during each rhythmic contraction is not yet known either. Stretch prolongs the duration of contraction and this effect remains during sustained stretch, unlike the transient effect on the period of rhythm, although the effect is greater in the contraction that immediately follows the application of stretch (Fig. 10). The differential effects of stretch on the two aspects of the rhythmic contractions may suggest that the mechanism involved in the generation of the pacemaker potential is different from that involved in the maintenance of the burst of action potentials. It seems possible that stretch may have a direct effect on the voltage-gated Ca^{2+} channels, which then results in the prolongation of the burst of action potentials. It is interesting to note that stretch of cell membrane by either application of direct positive pressure through a pipette or osmotic swelling increased the voltage-gated inward Ca^{2+} current in the rat basilar arterial myocyte under conventional and perforated patch whole-cell clamp conditions (Langton, 1993). However, there are

conflicting results on the direct effect on the Ca^{2+} currents. Stretch failed to modify the current–voltage relationship of voltage-gated Ca^{2+} currents in single cells of porcine coronary artery under whole-cell voltage clamp configuration (Davis *et al.* 1992). Furthermore, stretch reduced the Ca^{2+} current in urinary bladder myocytes (Wellner & Isenberg, 1994). This possibility, therefore, requires further study.

The effects of stretch reported here imply that stretch has a profound influence on the function of the uterus. Steady increase in distending force is expected to enhance the duration of rhythmic contractions with little change in the frequency of rhythm. Intermittent stretch, for example due to the movement of a fetus, may advance the phase of rhythm. In either case, stretch of uterine muscle is expected to have a strong influence on the contractile force of the organ.

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