

Changes in the mechanisms involved in uterine contractions during pregnancy in guinea-pigs

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1. The mechanisms involved in contraction in guinea-pig myometrium were compared at mid- and late pregnancy. Tension was recorded simultaneously with either membrane potential or cytoplasmic calcium ($[Ca^{2+}]_i$) in strips exposed briefly to prostaglandin $F_{2\alpha}$ (PGF).
2. PGF-induced increases in tension were underpinned by action potentials followed by sustained depolarization and biphasic increases in $[Ca^{2+}]_i$ at mid- (peak, 879 ± 199 nM; sustained, 298 ± 35 nM, $n = 11$) and late pregnancy (peak, 989 ± 302 nM; sustained 178 ± 33 nM, $n = 8$).
3. At mid- and late pregnancy, nifedipine (10^{-6} M) reduced (a) the PGF-induced increase in tension to 84 and 35%, (b) the level attained during the depolarization by 2 and 12 mV and (c) the peak rise in $[Ca^{2+}]_i$ to 42 and 17%. The sustained rises in $[Ca^{2+}]_i$ were resistant to nifedipine.
4. In Ca^{2+} -free solution (containing 1 mM EGTA), PGF elicited an increase in tension that was 26% of that in 2.5 mM Ca^{2+} and an increase in $[Ca^{2+}]_i$ (24% of the sustained level) at mid-pregnancy but no increase in tension or $[Ca^{2+}]_i$ at term.
5. At both stages of pregnancy, PGF decreased the level of $[Ca^{2+}]_i$ required to elicit increases in tension comparable to those evoked by high K_o^+ . The slope of the tension– $[Ca^{2+}]_i$ curves were steeper in mid- than in late pregnancy.
6. In conclusion, at mid-pregnancy, the contractile response of the guinea-pig myometrium to PGF involves Ca^{2+} influx through L-type voltage-operated Ca^{2+} channels (VOCCs) and by receptor-operated mechanisms, release of Ca^{2+} from intracellular stores, and an increase in the sensitivity of the contractile apparatus to Ca^{2+} . At term the situation is different: a modest increase in the sensitivity of the contractile apparatus to Ca^{2+} persists and there is a major reliance on Ca^{2+} influx through VOCCs.

The contractile activity of the uterus changes markedly from relative quiescence during pregnancy to the generation of strong, co-ordinated contractions during labour. This transformation has been referred to as 'activation' (Challis & Lye, 1994) and is envisaged to involve changes in the identity and number of ion channels, the proteins of the contractile apparatus, second messenger systems and gap junction formation. Several classes of the K^+ channels in myometrium that are influenced by the sex steroids (in rats, Boyle *et al.* 1987; Toro *et al.* 1990) or by pregnancy (women, Khan *et al.* 1993; rats, Wang *et al.* 1998) could contribute to the changes in excitability that occur during labour. The inward Ca^{2+} current in isolated uterine smooth muscle cells in rats appears to be enhanced by progesterone (Rendt *et al.* 1992), while a Na^+ current increases during pregnancy (Inoue & Sperelakis, 1991). Coupling of G proteins to second messengers in guinea-pig myometrium (Arkininstall & Jones, 1990) and the levels of mRNA for various isoforms of phospholipase C change during pregnancy in rats (Bieber *et*

al. 1998) but the results are not always easily extrapolated to tissue contraction.

The contractile state of smooth muscle is determined predominantly by the level of phosphorylated myosin, achieved largely via myosin light chain kinase (MLCK) whose activity is regulated by Ca^{2+} –calmodulin (Somlyo & Somlyo, 1994; Walsh *et al.* 1996). The level of cytoplasmic free Ca^{2+} ($[Ca^{2+}]_i$) is influenced by the complement of ion channels, pumps and exchangers in the plasma membrane and as a result of Ca^{2+} release from the endoplasmic reticulum (see Ashida *et al.* 1988; Nelson *et al.* 1990). Dephosphorylation of myosin by myosin light chain phosphatase (MLCP) facilitates relaxation. Inactivation of MLCP, as a result of its phosphorylation by second messengers such as rho kinase or protein kinase C, can give rise to an increase in the level of phosphorylated myosin in the face of constant levels of $[Ca^{2+}]_i$ and activated MLCK. The resulting increase in the sensitivity of the contractile apparatus to Ca^{2+} accounts for the large contractile response

of many smooth muscles to excitatory agonists (Somlyo & Somlyo, 1994; Walsh *et al.* 1996), including the uterus (Izumi *et al.* 1996). The levels of tension and phosphorylated MLC are well correlated in myometrium, and the tension per phosphorylated MLC is increased during pregnancy in women (Word *et al.* 1993).

The aims of this study were to investigate (1) Ca^{2+} influx, (2) release of Ca^{2+} from intracellular stores and (3) the sensitivity of the contractile apparatus to Ca^{2+} , in order to determine the relative significance of these mechanisms in contraction of the myometrium. We also investigated whether the relative contribution of the various mechanisms to contraction might change during pregnancy. Tissues from guinea-pigs were studied because their oestrogen and progesterone profiles during pregnancy and labour resemble those of humans most closely (Bedford *et al.* 1972).

METHODS

Tissues

Guinea-pigs were killed by decapitation (Monash University Animal Experimentation Ethics Committee permit number 95077) at mid-pregnancy, days 31–38, and at term, days 62–66 of pregnancy. The day on which a plug of semen was found in the vagina was designated day 1 of pregnancy and the length of pregnancy in our colony was 67 ± 1 days ($n = 121$). Strips of outer longitudinal uterine smooth muscle, approximately 200 μm wide (one bundle), 3 mm long and full thickness, were prepared and transferred to a recording chamber (about 0.5 ml capacity), and one end was attached to an isometric force transducer (AE801, SensoNor, Horton, Norway). For simultaneous recording of membrane potential and tension, the other end was pinned to the silicone rubber base of the chamber. For simultaneous determination of cytoplasmic calcium ($[\text{Ca}^{2+}]_i$) and tension, the free end was attached to the glass bottom of the recording chamber with SupaGlue. The tissues were continuously superfused at 3 ml min^{-1} with physiological saline solution (PSS) containing (mM): NaCl, 120; KCl, 5; NaHCO_3 , 25; KH_2PO_4 , 1; MgSO_4 , 1.2; glucose, 11; CaCl_2 , 2.5, bubbled with 95% O_2 and 5% CO_2 and maintained at 35 °C. Membrane potential was recorded using intracellular glass microelectrodes filled with 1 M KCl and having resistances of 60–100 M Ω , as described previously (Parkington *et al.* 1999b).

Determination of $[\text{Ca}^{2+}]_i$

The method used to determine $[\text{Ca}^{2+}]_i$ simultaneously with tension has been described previously (Parkington *et al.* 1999b). Briefly, the tissues were alternately illuminated at 340 and 380 nm (100 Hz) and the emission at 505 nm was collected by a photomultiplier tube. Autofluorescence and background fluorescence were determined. The tissues were then incubated with solution containing (mM): NaCl, 135; KCl, 5; HEPES, 10; glucose, 11; CaCl_2 , 2.5; 5 μM fura-2 acetoxymethyl ester (fura-2 AM) and 0.01% pluronic F127 for 50–60 min at room temperature (21 °C). HEPES was used as the buffer in the loading solution because flow was suspended during this time. Following loading, solution flow was resumed using normal PSS at 35 °C. Prior to the commencement of experiments, fura-2 was removed from the extracellular environment during a 30 min wash period. During the experiments, the ratio of the fluorescence emitted upon illumination at 340 and 380 nm, $R_{340/380}$, was recorded at rest, during spontaneous contractions and during application of a maximally effective concentration of PGF

(10^{-5} M) (established in preliminary experiments). Responses were also examined in the absence and presence of various pharmacological tools (see Results). At the end of each experiment, an attempt was made to calibrate the fluorescence signal. R_{max} , the maximal $R_{340/380}$ signal, was determined using HEPES-buffered solution (see above) containing the Ca^{2+} ionophore ionomycin (2×10^{-5} M) and 5 mM $[\text{Ca}^{2+}]_o$, and R_{min} , the minimal $R_{340/380}$ signal, was determined by switching to Ca^{2+} -free solution containing EGTA (3×10^{-3} M). $R_{340/380}$, R_{max} , R_{min} and a K_d of 224 nM (Grynkiewicz *et al.* 1985) were then used to express the fluorescence signal in terms of $[\text{Ca}^{2+}]_i$. In the experiment designed to test the sensitivity of the contractile apparatus to Ca^{2+} , and in those in which the $[\text{Ca}^{2+}]_i$ profile of the response to PGF was quantified, data from some experiments were excluded from consideration in the results because of failure of the calibration. In other experiments, e.g. testing thapsigargin, cyclopiazonic acid (CPA) and caffeine, the changes in $R_{340/380}$ (e.g. in the presence of nifedipine) were small, that is, $R_{340/380}$ lay within the essentially linear range of the Ca^{2+} -ratio curve (see Hayes *et al.* 1996; Haworth & Redon, 1998), hence it was possible to use the values of $R_{340/380}$ as indicators of $[\text{Ca}^{2+}]_i$. Possible loading of intracellular organelles was tested using 5×10^{-5} M saponin for 20 min to disrupt the plasma membrane. This caused a precipitous drop in $R_{340/380}$ to near background levels. Tissues were then exposed to 1% Triton X-100 for a further 20 min to disrupt the membranes of organelles. There was no additional change in $R_{340/380}$. These results provided compelling evidence against significant loading of organelles with fura-2 in this study.

Responses in Ca^{2+} -free solution

The response to PGF in the absence of extracellular Ca^{2+} was also tested. Ca^{2+} was omitted from normal PSS and 10^{-3} M EGTA was added, to give 'Ca²⁺-free' PSS. The flow rate of the solution was increased to 7 ml min^{-1} for these experiments in order to facilitate more complete removal of Ca_o^{2+} . Tissues were superfused with Ca^{2+} -free PSS for 2 min prior to and during testing the response to PGF.

Sensitivity of the contractile apparatus to $[\text{Ca}^{2+}]_i$

Tissues were superfused with PSS containing 100 mM K^+ (isosmotic replacement of Na^+) to activate voltage-operated Ca^{2+} channels, and 10^{-6} M thapsigargin to prevent Ca^{2+} uptake into intracellular stores (HiK PSS). In HiK PSS, $[\text{Ca}^{2+}]_o$ was increased stepwise from 0 mM to 0.5, 1, 2.5, 5, 10 and 20 mM at 3 min intervals, thus allowing sufficient time for the $[\text{Ca}^{2+}]_i$ and tension to stabilize at each new level. The tissue was then rested in normal PSS (containing 2.5 mM Ca^{2+}) for 20 min. The procedure was repeated, this time including PGF (10^{-5} M) in the HiK PSS as $[\text{Ca}^{2+}]_o$ was increased, which was added from the final 3 min of exposure to 0 mM $[\text{Ca}^{2+}]_o$ onwards. The fluorescence signal was calibrated immediately following 10 min washout of the HiK PSS. Calibration was successful in 74% of tissues and only these data are presented in the Results (see p. 794). This method was used to investigate the effects of PGF on the sensitivity of the contractile apparatus to Ca^{2+} because high concentrations of the pore-forming agent staphylococcal α -toxin are required for guinea-pig tissues. Thus, it is possible that the toxin might fail to reach all of the smooth muscle cells within the tissue.

Drugs used

The stable form of PGF, dinoprost, stock 10^{-2} M, was used exclusively and was a gift from Upjohn (Kalamazoo, USA). Stock solutions of the following drugs were made: nifedipine (10^{-2} M in DMSO), thapsigargin (10^{-2} M in DMSO), cyclopiazonic acid (10^{-1} M in DMSO) and caffeine (dissolved directly in PSS) (all from Sigma).

Dimethyl sulfoxide (DMSO) was without a detectable effect on uterine smooth muscle activity when applied alone at 10^{-4} M.

Analysis of data

Membrane potential and tension were recorded and stored on videocassette and $[Ca^{2+}]_i$ and tension were stored on an AmLab data acquisition system (AmLab Technology, Sydney) (see Parkington *et al.* 1999*b*). The data were analysed later using the statistical software Prism (GraphPad, USA) and Origin (Microcal, USA). The normality of the data was tested using the method of Kolmogorov & Smirnov and equivalence between standard deviations was tested using Bartlett's method (both tests achieved using GraphPad Instat). The data passed these tests and subsequent analysis of variance was used to test between the two stages of pregnancy and between treatments, and Student's *t* test was used to compare means. Means \pm s.e.m. are quoted throughout. In all instances *n* designates the number of animals studied. A level of $P < 0.05$ was accepted as indicating significant difference.

To assess the sensitivity of the contractile apparatus to Ca^{2+} , tension was plotted against $[Ca^{2+}]_i$. In permeabilized blood vessels stimulated with noradrenaline, the tension- Ca^{2+} relationship is sigmoid (Nishimura & van Breemen, 1989), and thus the lower part of the sigmoid curve is essentially exponential. Hence, an exponential function was fitted to the force- $[Ca^{2+}]_i$ data obtained in control solution and in the presence of PGF for each tissue. The

curves fitted the data with $r^2 > 0.9$ for all tissues (an example is illustrated in Fig. 10*B*). From each curve, the concentrations of Ca^{2+} required to evoke contractions that were 50 and 100% of that elicited by high K^+ were calculated within each individual tissue. The values obtained for tissues from seven guinea-pigs were averaged to provide the data shown in Table 2.

RESULTS

Spontaneous contractions

Within approximately 30 min of being mounted in the chamber, spontaneous contractions occurred in all tissues obtained at mid-pregnancy (Fig. 1). The amplitudes of these contractions were $96 \pm 4\%$ ($n = 33$) of the contraction elicited by 100 mM K^+ (Fig. 2*A*). Each contraction was preceded by a complex action potential that consisted of an initial spike followed by a plateau of depolarization to -25 ± 1 mV ($n = 37$) (Fig. 3*A*). The most negative level of the membrane potential, between complex action potentials, was -56 ± 1 mV ($n = 37$).

In preparations obtained from term pregnant guinea-pigs the resting membrane potential was -58 ± 3 mV ($n = 51$).

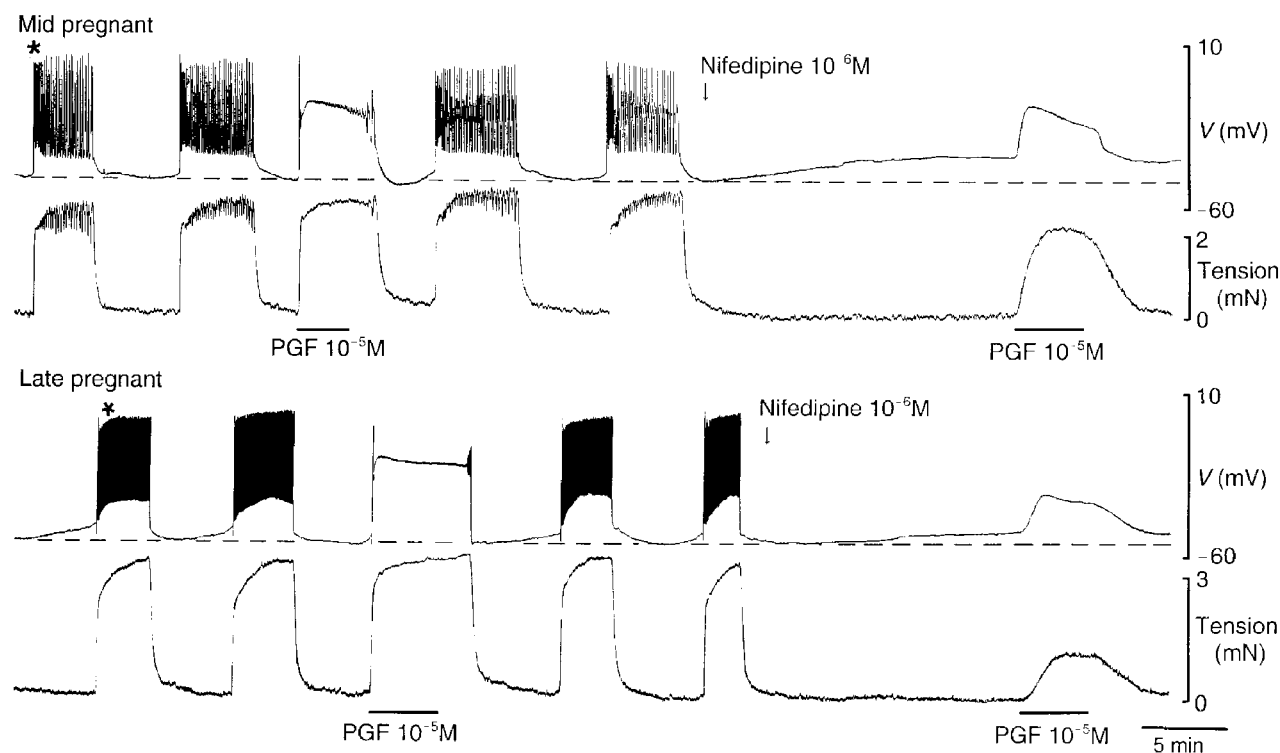


Figure 1. Spontaneous and PGF-induced electrical and contractile activity in control solution and in the presence of nifedipine in myometrium obtained from guinea-pigs at mid- and late pregnancy

Spontaneous action potentials (upper traces) were accompanied by large increases in tension (lower traces). PGF induced an initial spike followed by sustained depolarization at both stages of pregnancy. In nifedipine, commenced at the time indicated by the arrow, spontaneous activity was abolished and the membrane depolarized to a greater extent at mid- compared with at late pregnancy. The sustained membrane potential and tension responses to PGF were largely resistant to nifedipine in mid-pregnancy, while they were considerably reduced at term. Portions of the spontaneous action potentials marked * have been shown on an expanded time scale in Fig. 3.

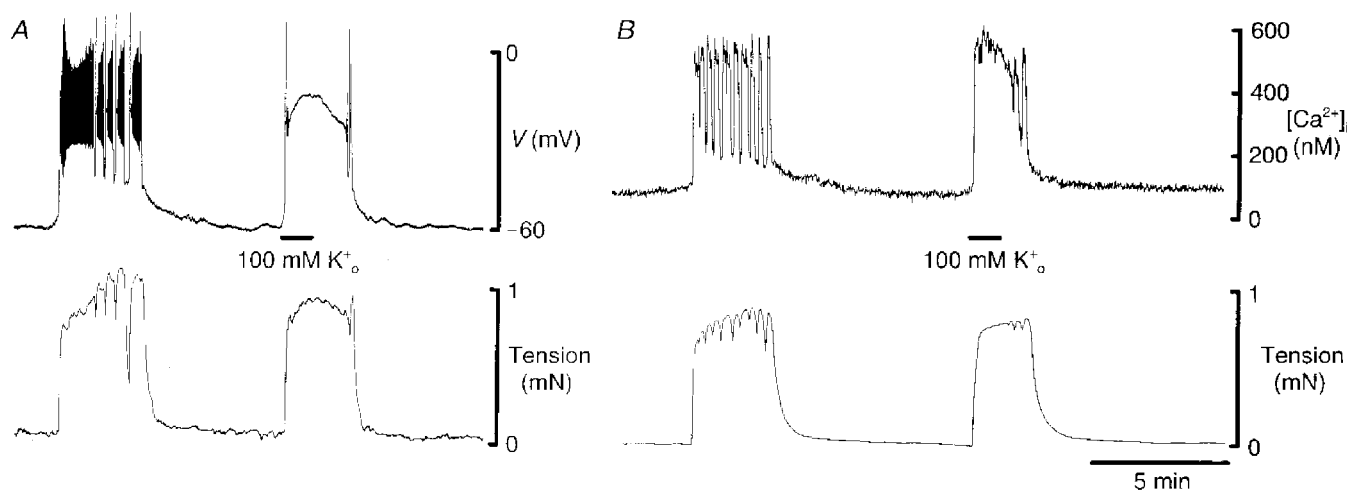


Figure 2. Contraction, membrane potential response (*A*) and $[Ca^{2+}]_i$ (*B*) evoked by 100 mM K^+_o in myometrium from a mid-pregnant guinea-pig

A, spontaneous contraction (lower trace) was underpinned by a complex action potential (upper trace). Replacement of Na^+ with 100 mM K^+ led to prompt depolarization, the initiation of spikes and contraction. *B*, spontaneous contraction (lower trace) was preceded by an increase in $[Ca^{2+}]_i$ (upper trace). Replacement of Na^+ with 100 mM K^+_o also evoked a rapid increase in $[Ca^{2+}]_i$ and contraction. Both tissues from the same mid-pregnant guinea-pig.

At this stage of pregnancy, spontaneous contractions occurred in only 31% of tissues and these contractions were $106 \pm 5\%$ of a high K^+ -induced contraction ($n = 12$, not different compared with mid-pregnancy, $P = 0.1782$). The contractions were underpinned by a burst of spikes upon a depolarization to -42 ± 5 mV ($n = 11$) in most instances (Figs 1 and 3*B*). The first spike was terminated by an undershooting hyperpolarization. Complex action potentials similar to those that occurred in mid-pregnancy were rare (plateau to -36 ± 3 mV, $n = 5$).

Resting $[Ca^{2+}]_i$ was not different in tissues obtained at mid- (88 ± 17 nM, $n = 11$) and late (91 ± 15 nM, $n = 11$) pregnancy ($P = 0.8960$). Spontaneous contractions were preceded by similar ($P = 0.4601$) increases in $[Ca^{2+}]_i$ at mid- (peak at 630 ± 28 nM, $n = 11$) and late pregnancy (peak at 669 ± 34 nM, $n = 4$) (see Fig. 4). High K^+ solution also evoked similar peak increases in $[Ca^{2+}]_i$, 623 ± 21 nM ($n = 5$) (see Fig. 2*B*) and 654 ± 23 nM ($n = 4$) at mid- and late pregnancy, respectively.

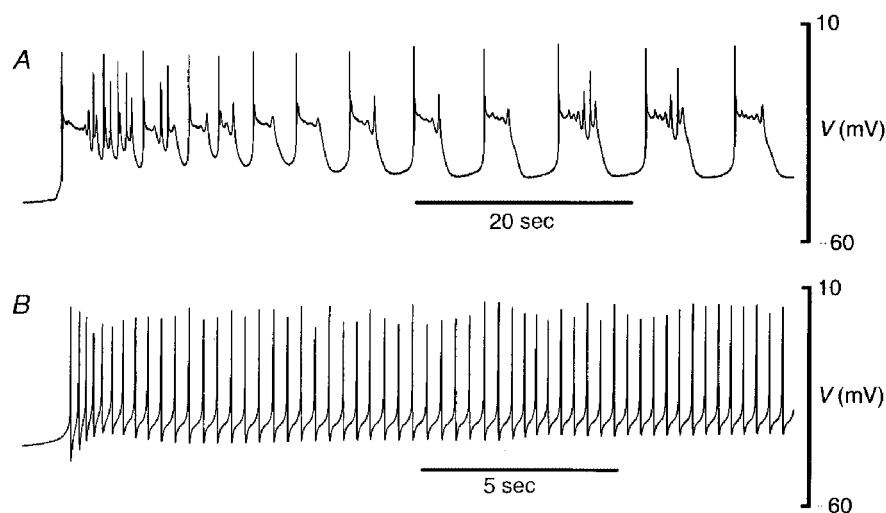


Figure 3. Spontaneous electrical activity occurring in pregnant guinea-pig myometrium

A, in a tissue obtained at mid-pregnancy, action potentials were complex in form, with spikes and plateaus of depolarization. *B*, at term, only simple spikes were recorded. Each trace is a part of the first burst of spontaneous activity from Fig. 1 (marked *), reproduced on an expanded time scale.

Prostaglandin-induced responses

The membrane potential response to a maximally effective concentration of PGF (10^{-5} M for 2–5 min) in normal PSS consisted of the initiation of a spike action potential which was followed by depolarization that peaked at -17 ± 1 mV at mid-pregnancy ($n = 21$) and -20 ± 2 mV at late pregnancy ($n = 18$) ($P = 0.1695$) (Fig. 1). The depolarization then declined to a sustained level of -20 ± 1 mV ($n = 21$) in mid-pregnancy and -22 ± 1 mV at term ($n = 18$) ($P = 0.1682$). These responses were accompanied by contraction that peaked at $157 \pm 7\%$ ($n = 21$) and $147 \pm 6\%$ ($n = 18$, $P = 0.2936$) of the peak of the contraction elicited by 100 mM K_o^+ in tissue obtained at mid- and late pregnancy, respectively.

The contraction evoked by PGF (10^{-5} M) was accompanied by a biphasic increase in $[Ca^{2+}]_i$ (Fig. 4). The initial peak, similar in amplitude at mid- and late pregnancy, decreased to a significantly lower sustained level which was larger in mid- than in late pregnancy (Table 1).

Voltage-operated Ca^{2+} channels: effect of nifedipine

Nifedipine (10^{-6} M) was used to block Ca^{2+} influx through voltage-operated Ca^{2+} channels (VOCCs). That this occurred successfully was verified by the abolition of all spikes, increases in $[Ca^{2+}]_i$, and contraction in response to 100 mM K_o^+ (not shown). Following the addition of nifedipine, spontaneous contractions did not occur. The cells no longer generated action potentials and the membrane potential depolarized to a stable level of -43 ± 1 mV ($n = 33$) at mid-pregnancy (Fig. 1). The depolarization observed in the presence of nifedipine was 13 ± 1 mV ($P < 0.0001$, paired t test).

At term, the membrane depolarized to a stable level of -52 ± 2 mV ($n = 22$) in nifedipine (Fig. 1). This was 6 ± 1 mV more depolarized than the value of the membrane potential prior to nifedipine in the same tissues. Although this depolarization was significant ($P = 0.0107$, paired t test), it was not as large as that which occurred at mid-pregnancy ($P < 0.0001$).

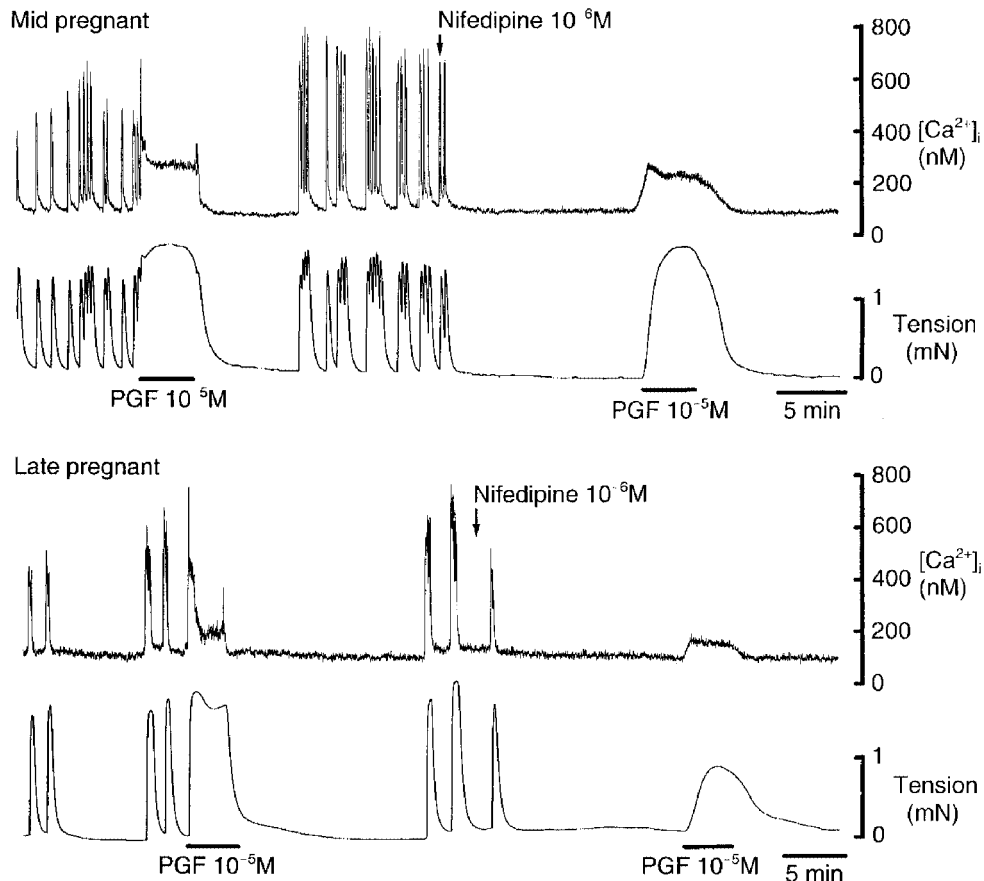


Figure 4. Spontaneous and PGF-induced increases in $[Ca^{2+}]_i$ and contractile activity in control solution and in the presence of nifedipine in myometrium obtained from guinea-pigs at mid- and late pregnancy

Spontaneous increases in tension (lower traces) were preceded by increases in $[Ca^{2+}]_i$ (upper traces). PGF induced a biphasic increase in $[Ca^{2+}]_i$, with a transient peak followed by a lower sustained increase. Nifedipine, introduced as indicated by the arrow, markedly reduced the PGF-induced peak in $[Ca^{2+}]_i$ and the tension response at term. The sustained rise in $[Ca^{2+}]_i$ persisted to a greater extent, as did the tension response at mid-pregnancy.

Nifedipine had no detectable effect on resting $[Ca^{2+}]_i$ at either stage of pregnancy (90 ± 18 nM, $n = 11$ at mid-pregnancy and 89 ± 14 nM, $n = 11$ at term) (Fig. 4).

Effect of nifedipine on the responses to PGF

It has been suggested that the dihydropyridine VOCC blocking drugs, especially at higher concentrations, may be capable of interfering with agonist-induced contractile mechanisms in smooth muscle (Kobayashi *et al.* 1991). In tissues obtained at mid-pregnancy, increasing the concentration of nifedipine decreased the maximum rate of rise of the initial spike on the upstroke of the membrane potential response to PGF, from 11 ± 2 V s⁻¹ at 10^{-9} M nifedipine to 1 ± 1 V s⁻¹ ($n = 6$) at 10^{-7} M. Spikes were abolished at higher concentrations of the blocker (Fig. 5). However, the sustained depolarization phase of the response persisted (only a 2 mV reduction) and was not affected when the concentration of nifedipine was increased to 10^{-5} M. In nifedipine, the PGF-induced increase in tension was $84 \pm 3\%$ ($n = 19$) of the control response to PGF (Fig. 1 and Table 1).

Treatment with nifedipine had a dramatic effect on the responses evoked by PGF at term. Again spikes were abolished but, in addition, the depolarization phase of the membrane potential response reached only -30 ± 2 mV ($n = 9$) (a 12 mV reduction) and tension was reduced to $35 \pm 2\%$ ($n = 9$) of control (Fig. 1 and Table 1).

At both stages of pregnancy the initial peak in $[Ca^{2+}]_i$ evoked by PGF was significantly reduced in the presence of

nifedipine (Fig. 4 and Table 1). The reduction was to 42% at mid- and to 17% at late pregnancy. In contrast, the sustained increases in $[Ca^{2+}]_i$ were not significantly different in nifedipine compared with control (paired *t* tests) at either stage of pregnancy (Fig. 4 and Table 1).

Ca²⁺ influx during depolarization and contraction

Ca²⁺ can enter smooth muscle cells via pathways other than through VOCCs, e.g. Ca²⁺ 'leak' or store-depleted Ca²⁺ influx, and the involvement of such mechanisms can be tested by varying $[Ca^{2+}]_o$ and by using other divalent cations, such as Cd²⁺ (Somlyo & Somlyo, 1994; Pacaud *et al.* 1996). In the presence of nifedipine, $[Ca^{2+}]_o$ was decreased for 2 or 5 min prior to and during exposure to PGF and tension was expressed as a percentage of the response to PGF in PSS containing normal 2.5 mM Ca_o²⁺, also in the presence of nifedipine. The level of depolarization and the amplitude of the tension response evoked by 10^{-5} M PGF in tissues obtained at both stages of pregnancy progressively decreased as $[Ca^{2+}]_o$ was lowered from 2.5 mM to 1, 0.3 and 0 mM (Fig. 6A). The level of membrane potential attained during the sustained depolarization decreased by 12.1 ± 0.7 mV ($n = 6$) and tension declined by $43 \pm 3\%$ per 10-fold decrease in $[Ca^{2+}]_o$ (Fig. 6A). The rate of the decrease was less steep at term (4.4 ± 0.7 mV and $31 \pm 4\%$ per 10-fold decrease in $[Ca^{2+}]_o$, respectively, $n = 6$, Fig. 6A).

Increasing the concentration of Cd²⁺ in the perfusate between 10^{-5} and 10^{-3} M progressively reduced the level of the depolarization and the contraction in response to PGF at

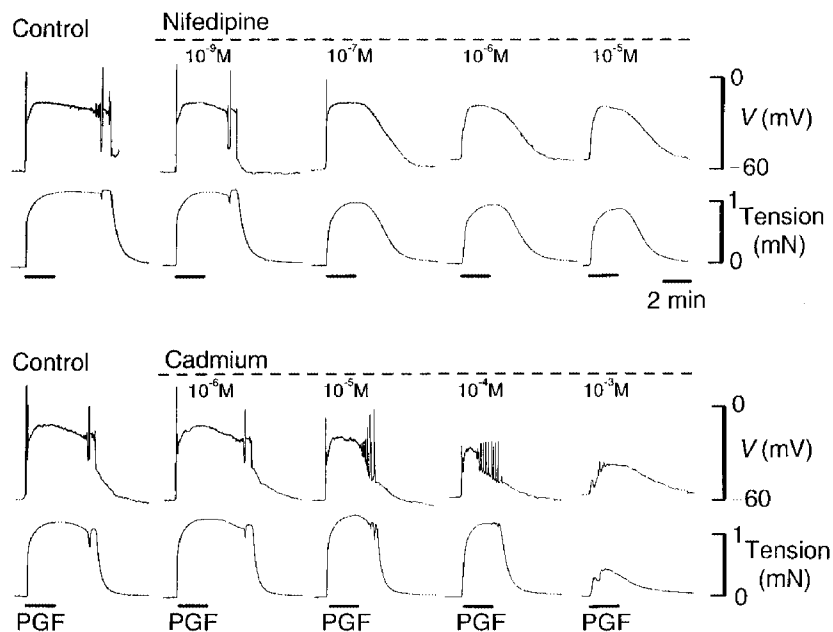


Figure 5. Effect of nifedipine and Cd²⁺ on the membrane potential and tension responses to PGF in myometrium of guinea-pig at mid-pregnancy

Increasing concentrations of nifedipine reduced and then abolished the spike component of the membrane potential responses (upper traces) evoked by PGF (10^{-5} M) while leaving intact the sustained depolarization and the increase in tension (lower traces). Increasing concentrations of Cd²⁺ progressively suppressed both the spikes and the depolarization but only decreased the amplitude of the tension response at the highest concentration.

Table 1. The membrane potential, $[Ca^{2+}]_i$ and tension responses to PGF (10^{-5} M) and the effect of nifedipine (10^{-6} M) on these responses in myometrium from guinea-pigs at mid- and late pregnancy

	Initial component of response		Sustained component of response	
	Mid	Late	Mid	Late
Depolarization level (mV)				
Control	-17 ± 1 (21)	-20 ± 2 (18)	-20 ± 1 (21)	-22 ± 1 (18)
Nifedipine	-20 ± 2 (21)	$-31 \pm 2^{*\dagger}$ (18)	-22 ± 1 (21)	$-30 \pm 2^{*\dagger}$ (18)
Cytoplasmic calcium (nM)				
Control	879 ± 199 (11)	989 ± 302 (8)	298 ± 35 (11)	$178 \pm 33^*$ (8)
Nifedipine	$367 \pm 141^\dagger$ (11)	$173 \pm 28^\dagger$ (8)	230 ± 32 (11)	163 ± 36 (8)
Tension (%)				
Control	—	—	100	100
Nifedipine	—	—	84 ± 3 (19)	$35 \pm 2^*$ (9)

PGF evoked membrane depolarization, an increase in $[Ca^{2+}]_i$ and an increase in tension. Nifedipine decreased the initial transient increase in $[Ca^{2+}]_i$ at both stages of pregnancy. The blocker had little effect on the depolarization, the sustained rise in $[Ca^{2+}]_i$ or on tension at mid-pregnancy but markedly suppressed these parameters at late pregnancy. The number of tissues examined is given in parentheses. * Significantly different from mid-pregnancy. † Significantly different from control.

both stages of pregnancy in the absence of nifedipine (illustrated for mid-pregnancy in Fig. 5). In the presence of nifedipine, Cd^{2+} (10^{-3} M) reduced the level of the PGF-induced depolarization to -40 ± 1 mV and the contraction to $35 \pm 3\%$ ($n = 5$) of control at mid-pregnancy. At term, these parameters were reduced to -38 ± 3 mV and $12 \pm 2\%$ ($n = 5$), respectively (Fig. 6B). Ni^{2+} was without effect on PGF-evoked depolarization or increase in tension, in the presence of nifedipine, until the concentration was increased above 2×10^{-3} M ($n = 5$, data not shown).

The endoplasmic reticulum

Release of Ca^{2+} from the endoplasmic reticulum (ER) contributes to the initial rise in $[Ca^{2+}]_i$ and to the contraction evoked by many spasmogens of smooth muscle and a role for this system in the response of the guinea-pig myometrium to PGF was investigated. Cyclopiazonic acid (CPA, 1 or 2×10^{-5} M), which prevents refilling of the ER with Ca^{2+} , evoked action potential discharge and contraction in three quiescent tissues obtained at late pregnancy and these were abolished by nifedipine (data not shown). At mid-pregnancy, CPA increased activity by either increasing the

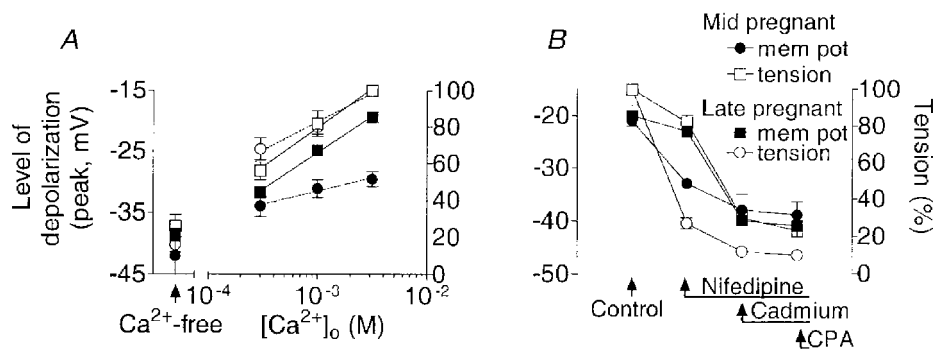


Figure 6. Effect of low Ca^{2+}_o , nifedipine, Cd^{2+} and CPA on the depolarization and tension responses evoked by PGF in myometrium of pregnant guinea-pigs

A, the level of membrane depolarization evoked by PGF (10^{-5} M), applied following 2–5 min in low Ca^{2+}_o and nifedipine (10^{-6} M), was plotted against $[Ca^{2+}]_o$. The increase in tension was expressed as a percentage of the responses in normal 2.5 mM Ca^{2+}_o at mid- ($n = 6$) and late ($n = 6$) pregnancy. The responses in nominally Ca^{2+} -free solution (no EGTA) are shown on the left. B, Cd^{2+} (10^{-3} M) caused the greatest reduction in the depolarization and tension responses to PGF (10^{-5} M) at mid-pregnancy ($n = 5$) while nifedipine (10^{-6} M) had the greatest effect at term ($n = 5$). Cyclopiazonic acid (CPA, 2×10^{-5} M) had no further effect on the responses at either stage of pregnancy.

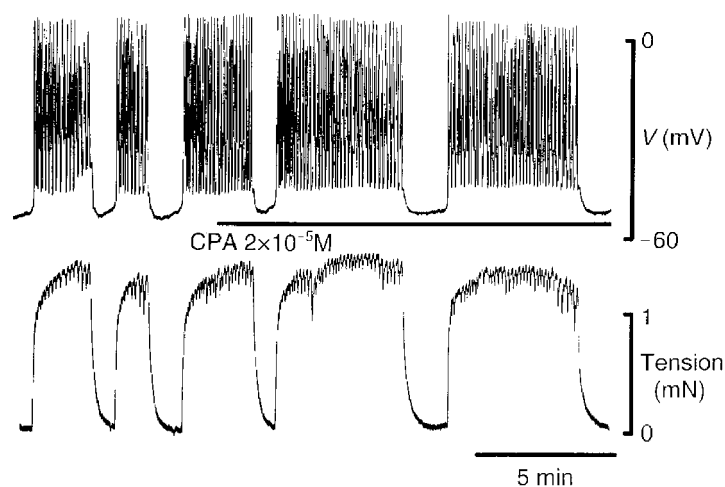


Figure 7. Effect of CPA on membrane potential and tension in normal solution

Cyclopiazonic acid (CPA, 2×10^{-5} M) increased the period of firing of spikes (upper trace) and prolonged the duration of contraction (lower trace).

frequency (with a decrease in the duration) or an increase in the duration (with a decrease in frequency) (Fig. 7) of bursts of action potentials/contractions, with no effect on the amplitude of contraction ($89 \pm 5\%$ of prior values, $n = 5$, $P = 0.0858$). The variability in the response was reflected in large standard errors and consequent lack of statistical significance in the changes in frequency ($104 \pm 12\%$, $n = 5$, $P = 0.7174$) and duration ($111 \pm 20\%$, $n = 5$, $P = 0.617$). The underlying depolarization evoked by CPA was clearly revealed in nifedipine (effect at mid-pregnancy shown in Fig. 8). The amplitude of this depolarization was greater at mid-pregnancy (12 ± 2 mV, $n = 6$) than at late pregnancy (7 ± 1 mV, $n = 5$, $P = 0.0457$). In the absence of nifedipine, CPA was without effect on the amplitude of the contractions evoked by 100 mM K_0^+ ($98 \pm 2\%$, $n = 10$) or PGF ($98 \pm 3\%$, $n = 10$) at mid-pregnancy. In the presence of nifedipine, the

level of depolarization and tension attained by PGF were unaltered by CPA (Fig. 8).

Thapsigargin (10^{-6} M), a structurally unrelated inhibitor of the ER Ca^{2+} pump, was without effect on spontaneous action potentials or on the frequency ($97 \pm 2\%$ of prior values, $n = 5$), duration ($103 \pm 5\%$, $n = 5$) or amplitude ($100 \pm 3\%$, $n = 5$) of the attendant contractions at mid-pregnancy. Thapsigargin was also without effect on membrane potential in nifedipine (Fig. 8).

The effects of thapsigargin (5×10^{-7} or 10^{-6} M) and CPA (1 or 2×10^{-5} M) (for 10–20 min) on $R_{340/380}$, an indication of $[Ca^{2+}]_i$, and on tension were also examined in the presence of nifedipine. Thapsigargin was without effect on resting $R_{340/380}$ or on tension at mid- ($n = 5$) or late ($n = 5$) pregnancy. In the same tissues and in the presence or absence

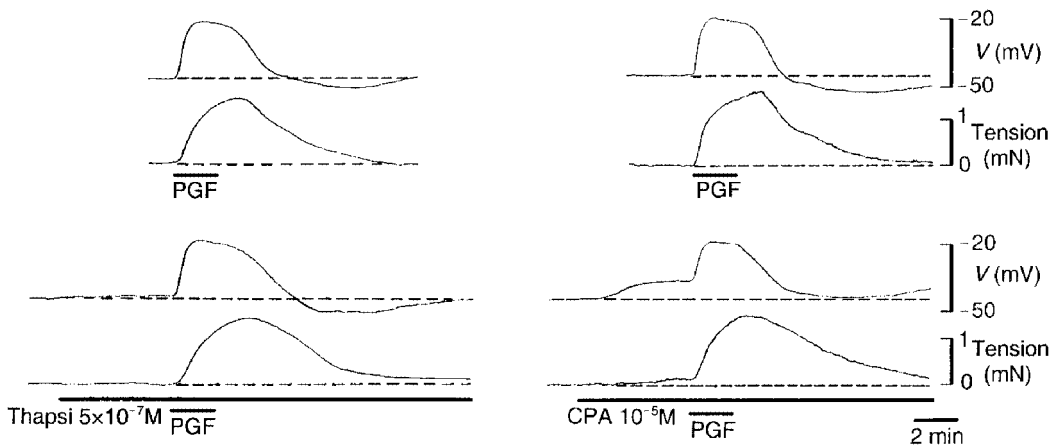


Figure 8. Effect of thapsigargin and CPA on the membrane potential and tension responses in the presence of nifedipine in myometrium of guinea-pig at mid-pregnancy

In the presence of nifedipine (10^{-6} M), thapsigargin (Thapsi) had little effect on resting membrane potential or tension or on the responses to PGF (10^{-5} M). Cyclopiazonic acid (CPA) alone evoked depolarization and an increase in tension but had no effect on the responses to PGF. Upper traces, membrane potential; lower traces, tension.

of thapsigargin, CPA increased $R_{340/380}$ by $20 \pm 3\%$ of that induced by high K_o^+ (obtained prior to application of nifedipine) at mid-pregnancy and by $9 \pm 3\%$ at term. The associated increases in tension were 11 ± 4 and $1 \pm 0.3\%$, respectively, of the peak tension induced by $100 \text{ mM } K_o^+$. Neither of the pump inhibitors had a measurable effect on the increase in $R_{340/380}$ or on tension in response to a first or a second application of PGF ($n = 4$) in the presence of nifedipine (data not shown).

We examined the possibility that Ca^{2+} influx through VOCCs might induce Ca^{2+} release from the ER (Ca^{2+} -induced Ca^{2+} release, CICR). Ryanodine ($2 \times 10^{-5} \text{ M}$), which releases this store (Taggart & Wray, 1998), had no effect on spontaneous activity or on the responses to PGF (not shown). Caffeine (10^{-2} M), which also releases Ca^{2+} from the CICR store (Somlyo & Somlyo, 1994), did not increase $R_{340/380}$ in tissues obtained at mid-pregnancy ($n = 7$; see example in Ca^{2+} -free PSS, Fig. 9C) or late pregnancy ($n = 5$). In tissues in which calibration was successful, caffeine reduced $[Ca^{2+}]_i$ to $17 \pm 9 \text{ nM}$ ($n = 4$).

In view of the fact that an initial peak in $[Ca^{2+}]_i$ in response to PGF persisted in the presence of nifedipine and/or

thapsigargin, we tested the effect of PGF on $[Ca^{2+}]_i$ in PSS from which Ca^{2+} had been omitted and which contained 1 mM EGTA. Superfusion with this solution caused a prompt decline in $[Ca^{2+}]_i$ (Fig. 9). After 2 min, and still in Ca^{2+} -free solution, PGF elicited a brief increase in $[Ca^{2+}]_i$ that was $24 \pm 7\%$ of the sustained, nifedipine-resistant component of the PGF-induced increase in $[Ca^{2+}]_i$ in the seven tissues tested at mid-pregnancy (Fig. 9B). This increase in $[Ca^{2+}]_i$ was accompanied by an increase in tension that was $26 \pm 9\%$ ($n = 7$) of that elicited in normal PSS. In four of these tissues the increase in $[Ca^{2+}]_i$ consisted of two distinct peaks (the response in one of these tissues is illustrated in Fig. 9), and in one of the remaining three an inflection on the rise in $[Ca^{2+}]_i$ suggested the possibility of more than one component. Inflections were apparent on the associated increases in tension (Fig. 9B). The earlier of the two peaks in $[Ca^{2+}]_i$ was abolished when thapsigargin was included in the Ca^{2+} -free PSS, leaving the second peak intact (Fig. 9D). All increases in $[Ca^{2+}]_i$ were abolished in the combined presence of thapsigargin and caffeine (Fig. 9E). In three tissues examined at term, PGF did not evoke an increase in $[Ca^{2+}]_i$ or contraction in Ca^{2+} -free PSS (data not shown).

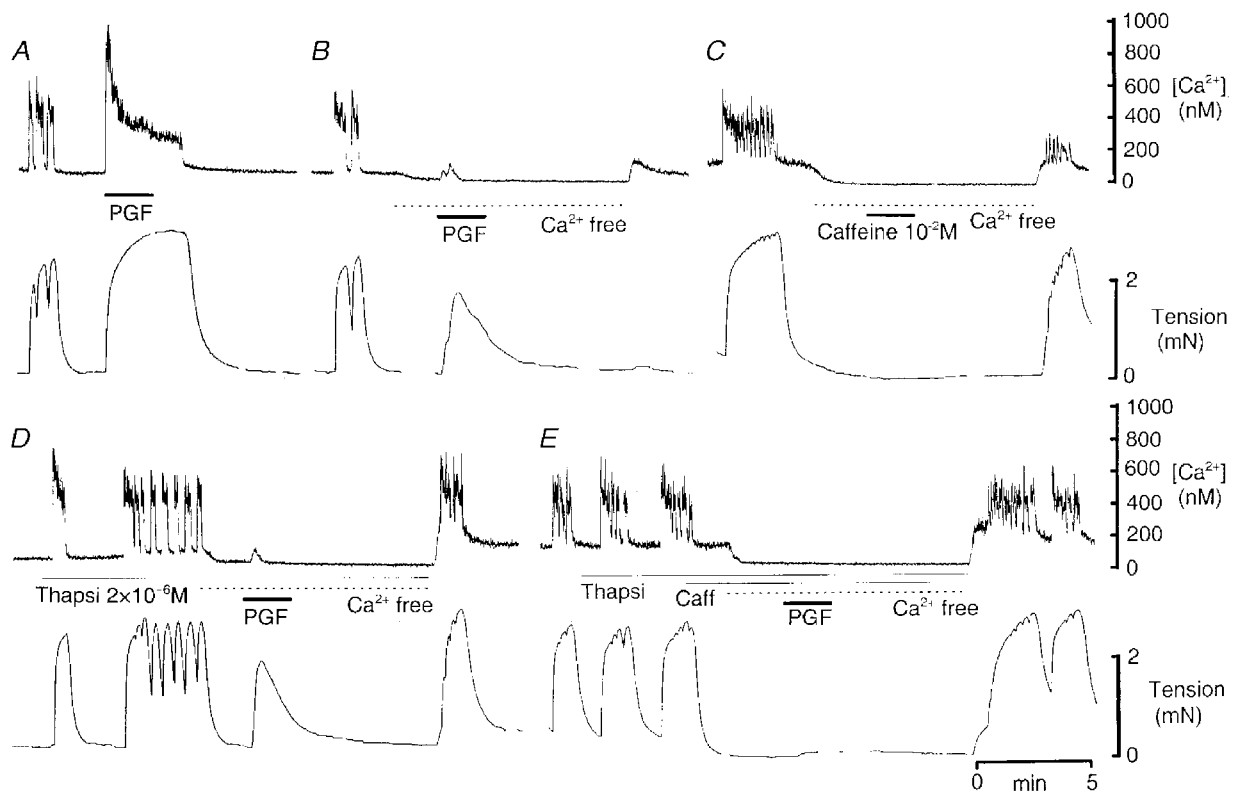


Figure 9. Effect of thapsigargin and caffeine on the increase in $[Ca^{2+}]_i$ and tension evoked by PGF in Ca^{2+} -free solution, containing 1 mM EGTA, in myometrium obtained from a guinea-pig at mid-pregnancy

A, PGF (10^{-5} M) evoked the usual biphasic increase in $[Ca^{2+}]_i$ and a large increase in tension in control solution. B, these responses were markedly reduced in Ca^{2+} -free solution containing 1 mM EGTA. C, caffeine failed to evoke a response in Ca^{2+} -free solution. D, thapsigargin (Thapsi) abolished the earlier component of the rise in $[Ca^{2+}]_i$ evoked by PGF in Ca^{2+} -free solution. E, thapsigargin plus caffeine (Caff) abolished all responses to PGF in Ca^{2+} -free solution. Upper traces, $[Ca^{2+}]_i$; lower traces, tension.

Sensitivity of the contractile apparatus to Ca^{2+}

When Ca^{2+} in the PSS superfusing strips of myometrium was increased from 0 to 20 mM, in the presence of 100 mM K_o^+ and 10^{-6} M thapsigargin, there was a concentration-dependent increase in $[\text{Ca}^{2+}]_i$ (Fig. 10A) and an increase in tension (Fig. 10B). The relationship between tension and $[\text{Ca}^{2+}]_i$ in control solution, that is, in the absence of PGF, was not different at mid- (Fig. 10C) and late (Fig. 10D) pregnancy. When $[\text{Ca}^{2+}]_o$ was raised in the presence of PGF, $[\text{Ca}^{2+}]_i$ increased as in control solution (Fig. 10A) but the tension was dramatically increased (Fig. 10B) at both stages of pregnancy. This increase in the sensitivity of the contractile apparatus to Ca^{2+} in mid-pregnant tissues was unaffected by staurosporine (10^{-7} M) ($n = 4$, data not shown).

At both stages of pregnancy, equivalent tension development required a lower level of $[\text{Ca}^{2+}]_i$ in the presence of PGF than in its absence (Fig. 10C and D). At lower $[\text{Ca}^{2+}]_i$, the increase in the sensitivity of the contractile apparatus to Ca^{2+} brought about by PGF appeared to be similar at mid- (73 ± 7 nM, $n = 7$) and late (117 ± 21 nM, $n = 7$) pregnancy (increases in tension that were 50% of those evoked by high K^+ , Table 2). However, as $[\text{Ca}^{2+}]_i$ increased, the sensitivity

to Ca^{2+} was significantly greater in tissues obtained at mid-pregnancy (only 114 ± 8 nM Ca^{2+} required to achieve a level of tension that was 100% of that in response to high K_o^+) compared with late pregnancy (203 ± 24 nM Ca^{2+} required to achieve a 100% increase in tension, Table 2).

It has been suggested that arachidonic acid may contribute to the increase in the sensitivity of the contractile apparatus to Ca^{2+} evoked by spasmogens in some smooth muscles (Somlyo & Somlyo, 1994). Since PGF induced a greater sensitivity to Ca^{2+} at mid-pregnancy, arachidonic acid was tested in these tissues. The level of $[\text{Ca}^{2+}]_i$ required to achieve tensions that were 50% and 100% of that evoked by high K_o^+ was decreased in the presence of 10^{-5} M arachidonic acid, but the effect was not as great as that which was achieved by PGF (Fig. 10C and Table 2).

DISCUSSION

The results of this study demonstrate that the relative importance of the mechanisms underpinning PGF-induced contraction of uterine smooth muscle changes dramatically between mid- and late pregnancy in guinea-pigs. At mid-pregnancy, PGF stimulated Ca^{2+} entry into the smooth

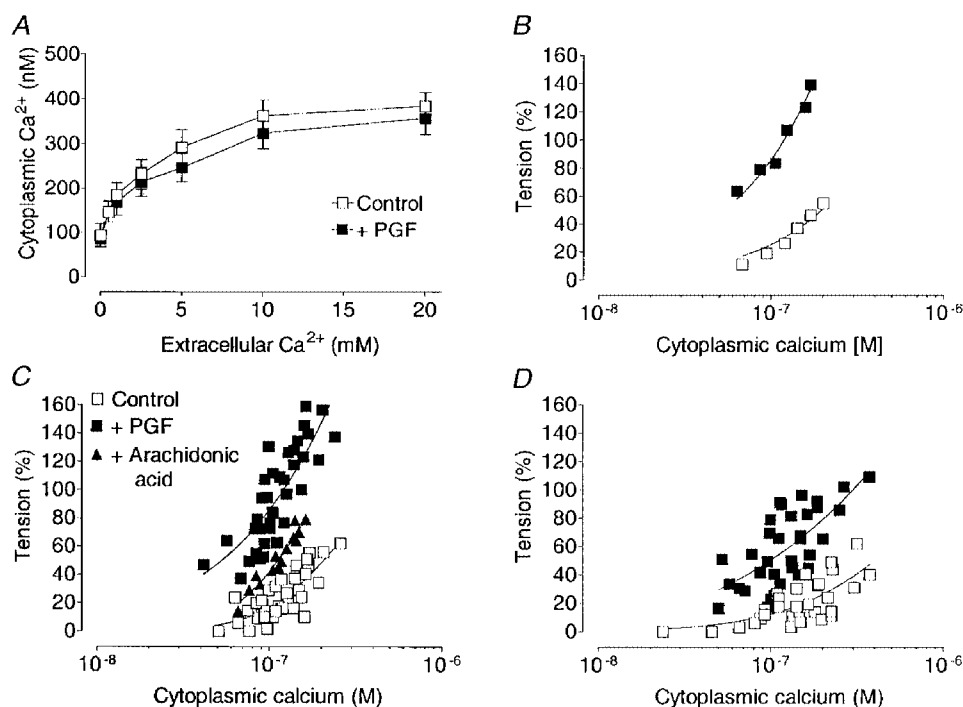


Figure 10. Effect of increasing extracellular Ca^{2+} on $[\text{Ca}^{2+}]_i$ and tension and the influence of PGF on the relationship between $[\text{Ca}^{2+}]_i$ and tension in guinea-pig myometrium during pregnancy

A, increasing Ca_o^{2+} induced similar increases in $[\text{Ca}^{2+}]_i$ in PSS containing high K^+ (100 mM, control) and in PSS containing both high K^+ and PGF (10^{-5} M). B, an example of the effect of increasing levels of $[\text{Ca}^{2+}]_i$ on tension (expressed as a percentage of the initial peak contraction evoked by high K^+ in normal 2.5 mM Ca^{2+}) in an individual strip illustrates that the relationship was well fitted by an exponential function (continuous lines). The relationship between $[\text{Ca}^{2+}]_i$ and tension in control solution, was not different in mid- (C) and late (D) pregnancy. PGF (10^{-5} M) increased the tension response for any given value of $[\text{Ca}^{2+}]_i$ at both mid- (C, $n = 7$) and late (D, $n = 7$) pregnancy but was more effective at mid-pregnancy. At mid-pregnancy, arachidonic acid (10^{-5} M) ($n = 4$) also increased the sensitivity of the contractile apparatus to $[\text{Ca}^{2+}]_i$ but to a lesser extent than PGF (C).

Table 2. Effect of PGF on the $[Ca^{2+}]_i$ at which tissues produced tension that was 50% and 100% of the tension evoked by 100 mM K_o^+

	$[Ca^{2+}]_i$ producing 50% tension (nM)		$[Ca^{2+}]_i$ producing 100% tension (nM)	
	Mid	Late	Mid	Late
Control	191 ± 15	236 ± 30	550 ± 21 *	999 ± 26 *†
+ PGF	73 ± 7 ‡	117 ± 21 ‡	114 ± 8 ‡	203 ± 24 ‡†
+ Arachidonic acid	116 ± 12 ‡	—	249 ± 9 *‡	—

In strips from 7 mid-pregnant guinea-pigs, and from 7 guinea-pigs at late pregnancy, PGF (10^{-5} M) decreased by approximately half, the concentration of $[Ca^{2+}]_i$ required to evoke contraction that was 50% of that elicited by 100 mM K_o^+ in the same strip. In 4 of the mid-pregnant tissues the effects of arachidonic acid (10^{-5} M) were also tested and it was found to decrease the Ca^{2+} requirement for contraction. In order to elicit tension that was equal to 100% of that evoked by 100 mM K_o^+ only one-fifth the concentration of $[Ca^{2+}]_i$ was required in the presence of PGF. Data from individual animals were used to obtain these means and s.e.m. * Data were obtained by extrapolation. † Significantly different from mid-pregnant. ‡ Significantly different from control.

muscle cells through VOCCs and through a receptor-operated mechanism, Ca^{2+} was released from intracellular stores, and there was an increase in the sensitivity of the contractile apparatus to Ca^{2+} . At term, Ca^{2+} influx was predominantly through VOCCs, with direct receptor-mediated Ca^{2+} influx markedly reduced, little if any release of Ca^{2+} from stores, and a blunted ability of PGF to increase the sensitivity of the contractile apparatus to Ca^{2+} .

Effects of pregnancy

It is likely that the ion channel profile in the myometrium of guinea-pigs changes between mid- and late pregnancy as indicated by the following observations. (1) The action potential in mid-pregnant tissues was complex in form, consisting of simple spikes followed by a sustained depolarization to around -25 mV, while only simple spikes occurred at term. It has been suggested previously that the plateau potential in the circular myometrium of pregnant rats reflects a weaker K^+ and a stronger Ca^{2+} conductance (Osa & Kawarabayashi, 1977), and in that tissue there is also a switch from complex action potential to simple spikes at term (Bengtsson *et al.* 1984; Osa & Ogasawara, 1984). (2) A pronounced after-hyperpolarization terminated the first spike action potential at term but was never recorded in tissues obtained at mid-pregnancy. The outward K^+ current density in rat myometrial cells is almost doubled in late pregnancy (Wang *et al.* 1998), an additional persistent K^+ current is recorded during labour in human myometrial cells (Khan *et al.* 1993), and oestrogen, elevated in late pregnancy and labour, stimulates the appearance of a K^+ current in rat (Mironneau & Savineau, 1980; Boyle *et al.* 1987; Toro *et al.* 1990) and guinea-pig (Vassort, 1975) myometrium. These currents resulted from voltage-dependent K^+ (K_V) channels, rather than Ca^{2+} -activated K^+ (K_{Ca}) channels. (3) Nifedipine was associated with a significantly greater membrane depolarization, by 7 mV, at

mid- versus late pregnancy. Suppression of activity of K_V and K_{Ca} channels has been reported for this drug, including one study involving K_{Ca} channels in rat myometrium (Miyoshi *et al.* 1991). (4) Significantly fewer tissues displayed spontaneous contractile activity at term, which could reflect a decline in pacemaker activity. However, it could be that the membrane potential recorded in the presence of nifedipine reflects the 'true' resting level and that the value recorded between action potentials at mid-pregnancy reflects the activation of K_{Ca} channels resulting from Ca^{2+} influx through VOCCs. In that case, a decrease in the importance of K_{Ca} with a greater reliance on K_V at term (rat, Wang *et al.* 1998; human, Khan *et al.* 1993) could explain the reduced incidence of spontaneous activity, the after-hyperpolarization which terminates the action potential, the more negative membrane potential in nifedipine, and perhaps the absence of the plateau component that determines the shape of the complex action potential at this time.

Frequent spontaneous contractions have been observed universally in myometrium isolated from rats (Osa & Ogasawara, 1984), mice (Osa, 1974), rabbits (Kleinhaus & Kao, 1969), cats (Bülbring *et al.* 1968), sheep (Parkington, 1985), women (Word *et al.* 1992; Parkington *et al.* 1999a,b), and now guinea-pigs, during mid-pregnancy, a time when uterine smooth muscle is relatively quiescent *in vivo*. This suggests that endogenous inhibitory influences act to maintain a normal pregnancy but the myometrium is capable of contracting if necessary, for instance, in the event of fetal death. Myometrium isolated from guinea-pigs, sheep and women at term is more quiescent. These observations suggest that a large proportion of the VOCCs are in the closed, available state and hence can facilitate maximal Ca^{2+} influx upon depolarization by stimulatory hormones during labour, thus allowing greater control by these hormones.

Voltage-operated Ca^{2+} channels and contraction

During mid-pregnancy, PGF is capable of eliciting a substantial increase in tension, underpinned by depolarization and a sustained increase in $[\text{Ca}^{2+}]_i$ that is independent of Ca^{2+} influx through VOCCs. A similar depolarization is evoked by oxytocin in rat myometrium as a result of activation of a cation channel with significant Ca^{2+} permeation. Ca^{2+} released from intracellular stores has been implicated in the activation of these channels (Arnaudeau *et al.* 1994). A biphasic increase in $[\text{Ca}^{2+}]_i$, also with a plateau to around 270 nM, is evoked by bradykinin and histamine in human airway smooth muscle (Murray & Kotlikoff, 1991). The PGF-induced depolarization and the sustained component of $[\text{Ca}^{2+}]_i$ reported here in myometrium decreased significantly at term (Table 1). Of note is the greater effectiveness of Cd^{2+} in reducing the nifedipine-resistant depolarization in mid-pregnant tissues than at term (Fig. 6B), and this strengthens the notion of greater Ca^{2+} permeation in response to PGF in mid-pregnancy. The increased effectiveness of nifedipine at term revealed the importance of Ca^{2+} influx through VOCCs at this time. Again, this is very similar to observations in term human myometrium (Parkington *et al.* 1999a).

The endoplasmic reticulum

In the present study, the existence of a Ca^{2+} store that is released by PGF has been demonstrated in mid-pregnant tissues, confirming previous observations in our laboratory in which contractility alone was recorded (Coleman *et al.* 1988). However, thapsigargin or CPA, which prevent filling of intracellular stores, were without effect on the response to PGF, despite the ability of these agents to suppress the transient increase in $[\text{Ca}^{2+}]_i$ by PGF in Ca^{2+} -free PSS. CPA caused depolarization and an increase in $[\text{Ca}^{2+}]_i$. Thapsigargin did not induce depolarization, but observations in vascular smooth muscle raise the possibility that thapsigargin may inhibit the Ca^{2+} pump in only a component of the endoplasmic reticulum, unlike CPA which appears to deplete the entire store (Shima & Blaustein, 1992). Another possibility is that thapsigargin may be less effective in blocking the pump than CPA (see Taylor & Broad, 1998). It has been suggested that when the endoplasmic reticulum is replete with Ca^{2+} , some of that Ca^{2+} is released into the space between the endoplasmic reticulum and the plasma membrane, where it activates K_{Ca} channels before being extruded from the cell by pumps or exchangers. Depolarization by CPA has been explained in terms of removal of this mechanism (Suzuki *et al.* 1992). Similar activation of Ca^{2+} -dependent Cl^- channels is also a possibility.

Ca^{2+} influx through VOCCs can release Ca^{2+} from stores in some smooth muscles, presumably via CICR (Imaizumi *et al.* 1998). On the one hand, caffeine, which releases this store in many tissues, abolished the transient rise in $[\text{Ca}^{2+}]_i$ evoked by PGF in Ca^{2+} -free solution. On the other hand, even prolonged exposure to ryanodine, which putatively releases the store involved in CICR, was entirely without

effect on spontaneous or PGF-induced contraction or $[\text{Ca}^{2+}]_i$, and caffeine itself was ineffective in releasing Ca^{2+} . A similar lack of effect of caffeine in rat myometrium has been interpreted in terms of the absence of CICR in this tissue (Savineau & Mironneau, 1990; Taggart & Wray, 1998). Other putative actions of caffeine, e.g. inhibition of phosphodiesterases, or inhibition of the $\text{Ins}(1,4,5)\text{P}_3$ receptor, could account for these anomalies (Ehrlich *et al.* 1994). The decrease in basal tone by caffeine was preceded by a lowering of $[\text{Ca}^{2+}]_i$ and this may reflect inhibition of phosphodiesterases.

Sensitivity of the contractile apparatus to Ca^{2+}

A tetanic contractile response was maintained for the entire 2–5 min exposure to PGF, despite a significant decline in $[\text{Ca}^{2+}]_i$ within approximately 1 min. An increase in the sensitivity of the contractile apparatus to Ca^{2+} could account for this, and a similar increase in sensitivity by stimulatory agonists has been described previously for rat uterine smooth muscle (Izumi *et al.* 1996; Taggart & Wray, 1998). The present work probed these effects further and extended observations to encompass a wider range of $[\text{Ca}^{2+}]_i$ and to investigate changes in the extent of sensitization with pregnancy. The fura-2 method of estimating $[\text{Ca}^{2+}]_i$ used in the present study is not without its pitfalls (see Hayes *et al.* 1996; Haworth & Redon, 1998). However, it has the advantage that (1) the tissue is studied under conditions identical to those used for electrophysiology, (2) it circumvents the possibility that permeabilizing agents might fail to reach all of the smooth muscle cells within the tissue, and (3) a not dissimilar approach has previously been used to study myometrium (Taggart & Wray, 1998). Special care was taken with these experiments, and only tissues with successful calibrations were included in this aspect of the study. Thus, while errors will be consistent within tissues and are likely to be consistent between tissues, the values of $[\text{Ca}^{2+}]_i$ given may not represent absolute levels. Nonetheless, upon inclusion of PGF in Ca^{2+} -free, high K^+ solution there was an immediate increase in tension without any change in the 340/380 nm ratio, clearly demonstrating an increase in the sensitivity of the contractile apparatus to Ca^{2+} induced by PGF. A G protein appears to be involved in the sensitization of the contractile apparatus to Ca^{2+} in smooth muscle (Nishimura & van Breemen, 1989; Somlyo & Somlyo, 1994), including the myometrium (Izumi *et al.* 1996). Resistance to staurosporine in the present study suggests the involvement of a small monomeric rather than a large trimeric G protein in guinea-pig myometrium, analogous to the implication of Rho in sensitization in vascular (Hirata *et al.* 1992) and visceral (ileum, Itagaki *et al.* 1995) smooth muscles. A small monomeric G protein Rnd1, related to Rho but not possessing GTPase activity, has recently been found to suppress the sensitization evoked by carbachol in rat ileum (Loirand *et al.* 1999). Of interest to the present argument is the finding that the expression of mRNA for Rnd1 was increased by oestrogen and progesterone (Loirand *et al.* 1999). In guinea-pigs, as in

women, the circulating levels of both of these steroids increase up to and during labour (Bedford *et al.* 1972) and hence this could account for the depressed ability of PGF to increase the sensitivity of the contractile apparatus to Ca^{2+} at term.

In conclusion, the results described here demonstrate that uterine contractions in response to PGF during mid-pregnancy in guinea-pigs are underpinned by a broad suite of mechanisms including Ca^{2+} influx through VOCCs and via receptor-operated mechanisms, release of Ca^{2+} from intracellular stores, and an increase in the sensitivity of the contractile apparatus to Ca^{2+} . The receptor-operated Ca^{2+} mechanisms and store release decline to insignificance at term, leaving a marked reliance on Ca^{2+} influx through VOCCs, supported by a persistent, though blunted sensitization to Ca^{2+} . It is suggested that this reliance on VOCCs, together with the absence of spontaneous action potential activity at term in guinea-pigs (present study) and in women (Parkington *et al.* 1999a) would be conducive to greater control by local or circulating hormones. Mechanisms similar to those described here in guinea-pigs are also observed in the uterus of women at term.

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