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Effects of calcium entry blockers on tension development and calcium influx in rat uterus

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1 Spontaneous and potassium chloride (KCl)-induced tension development of strips of whole uterus from the day-22 pregnant rat was reduced when the tissues were incubated in a calcium ion (Ca^{2+})-free medium.

2 Strips of whole uterus, in an initially Ca^{2+}-free medium, responded to the cumulative addition of Ca^{2+} with graded phasic tension development and associated rapid electrical discharges. The spasms were inhibited by gallopamil (100 nM) and diltiazem (1 μM).

3 Strips of whole uterus in a depolarizing (40 mM K^+) medium, which was initially Ca^{2+}-free, responded to the cumulative addition of Ca^{2+} with graded tonic tension development without associated electrical discharges. These spasms were inhibited by calcium entry blockers with a rank order of potency of nifedipine = gallopamil > diltiazem > cinnarizine.

4 KCl-induced tension development in endometrium-free uterine strips was antagonized by calcium entry blockers with a rank order of potency of nifedipine > gallopamil > diltiazem > cinnarizine.

5 Ca^{2+} influx into endometrium-free uterine strips was assessed by means of the 'lanthanum method'. KCl induced a concentration-dependent increase in $^{45}Ca^{2+}$ influx which was suppressed or abolished by nifedipine (2.5 nM), gallopamil (100 nM), diltiazem (500 nM) or cinnarizine (5 μM).

6 It is concluded that spontaneous and KCl-induced tension development of rat uterus involves Ca^{2+} influx from the extracellular medium into the myometrial cell. These results support the hypothesis that nifedipine, gallopamil, diltiazem and cinnarizine inhibit Ca^{2+}- and KCl-induced tension development of rat uterus by reduction of Ca^{2+} influx.

Introduction

It has long been recognized that spontaneous or KCl-induced tension development in isolated uterine smooth muscle is reduced by lowering the calcium ion (Ca^{2+}) concentration of the bathing medium (Gárcia De Jalon *et al.*, 1945; Evans *et al.*, 1958; Edman & Schild, 1962; Bengtsson *et al.*, 1984). Several agents are, nevertheless, capable of initiating tension development in uteri bathed in Ca^{2+}-free media (Mironneau *et al.*, 1984) although these mechanical responses are small relative to those obtained when physiological concentrations of Ca^{2+} are present. Thus, the uterus is generally classed as a tissue in which tension development is largely dependent on extracellular Ca^{2+} rather than on intracellular Ca^{2+} pools (Bolton, 1979).

From the above observations, tension development of uterus should be inhibited by calcium entry blockers ('calcium antagonists') since these drugs are considered to act by reduction of Ca^{2+} entry through voltage-operated Ca^{2+} channels (Bolton, 1979; Nayler & Horowitz, 1983). In support of this, gallopamil and verapamil were found to inhibit the tension development induced by KCl, acetylcholine or oxytocin in rat uterus, effects which were reversed by increasing the Ca^{2+} concentration of the bathing medium (Fleckenstein *et al.*, 1971; Varagić *et al.*, 1984). Furthermore, Granger *et al.* (1985a) have shown that several calcium entry blockers (nifedipine, gallopamil, diltiazem and cinnarizine) are potent inhibitors of spontaneous and oxytocin-induced tension development in the isolated uterus of the day-22 pregnant rat.

Compared with studies on cardiovascular tissues, there have been few investigations of the mechanisms by which the calcium entry blockers inhibit tension development in uterine smooth muscle. Therefore, the principal objective of the current study was to provide more direct evidence that these compounds can

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prevent Ca^{2+} influx into the myometrium. To achieve this, we have assessed their ability to inhibit contractions evoked by KCl and Ca^{2+} and suppress KCl-induced influx of $^{45}Ca^{2+}$. Preliminary results have been presented to the British Pharmacological Society (Granger *et al.*, 1985b).

Methods

Uteri were obtained from day-22 pregnant Sprague-Dawley rats (250–350 g) from Manchester University Animal Unit killed before 10 h 00 min by cervical dislocation. Uterine horns were freed of foetuses and placentae and placed in a physiological salt solution (PSS) at room temperature.

Strips from whole uterus

Longitudinal strips (10 × 5 mm) were mounted in tissue baths at 37.0 ± 0.5°C under a resting tension of 1 g and isometric tension development was recorded with an Ether transducer (model UFI) connected to a Grass polygraph (model 79C). Tension changes were quantified as the integral above resting tension with either a 7P10 Grass integrator preamplifier or a microcomputer system (Foster & Hollingsworth, 1985) which enabled continuous integration, data storage and analysis.

Table 1 Composition of physiological salt solutions (PSS)

	Concentration (mM)		
Component	*PSS*	*MOPS PSS*	Ca^{2+}-*free depolarizing MOPS PSS*
Na^+	143.0	135.0	102.3
K^+	5.9	5.9	40.0
Mg^{2+}	1.2	1.2	1.2
Ca^{2+}	2.55	2.55	0
$H_2PO_4^-$	1.2	0	0
SO_4^{2-}	1.2	0	0
Cl^-	128.0	148.2	144.6
HCO_3^-	25.0	0	0
MOPS	0	10.0	10.0
Glucose	11.0	11.0	11.0

PSS was bubbled with 95% O_2 and 5% CO_2; MOPS PSS with 100% O_2. MOPS PSS was prepared by bubbling with 100% O_2 at 37°C and the pH adjusted to 7.4 with 4N sodium hydroxide. Ca^{2+}-free PSS and Ca^{2+}-free MOPS PSS were prepared by omitting $CaCl_2$ from the formulations given in the Table; the Ca^{2+} concentrations of such solutions did not exceed 0.006 mM, calculated from the impurity limits of the constituents.

Experiments with non-depolarizing solutions To examine the effect of Ca^{2+} removal on spontaneous tension development, tissues were mounted in either a bicarbonate-buffered PSS or a 3-(N-morpholino)-propanesulphonic acid (MOPS)-buffered PSS (Table 1) for 45 min and integrals recorded for 5 min periods from 30 to 45 min after setting up. The bathing medium was changed to either a Ca^{2+}-free (i.e. no added calcium) PSS or to a Ca^{2+}-free MOPS PSS and the tissues washed at 15 min intervals. Integrals in subsequent 5 min periods were expressed as a percentage of the mean control integral.

The effect of the calcium entry blockers on the responses of uteri to Ca^{2+} was also measured. Tissues were initially placed in a MOPS PSS for a 45 min equilibration period. The medium was changed to a PSS containing no added calcium (0 mM Ca^{2+}) and after 1 h, $CaCl_2$ was added to the tissue bath in a cumulative manner at 10 min intervals; this produced graded, phasic tension development (Figure 1a). A second cumulative Ca^{2+}-effect curve was obtained after washing the tissue with Ca^{2+}-free MOPS PSS in the absence or presence of a calcium entry blocker for 1 h. Responses were expressed as a percentage of the maximum response to Ca^{2+} in the initial concentration-effect curve.

Experiments with depolarizing solutions The effect of KCl was studied by performing cumulative concentration-effect experiments in which KCl was added at 10 min intervals and integrals measured over that period. KCl additions were repeated after 1 h and 2 h in either PSS, Ca^{2+}-free PSS or Ca^{2+}-free PSS containing ethylene glycol-*bis*-(β-amino ethyl ether) N,N′-tetra-acetic acid (EGTA; 0.5 mM).

The ability of calcium entry blockers to inhibit responses of uteri to added Ca^{2+} was also examined in a depolarizing medium. Under the latter condition, it is considered that the voltage-dependent Ca^{2+} channels are open (Bolton, 1979) allowing rapid and constant access of Ca^{2+} to the cytoplasm from the extracellular medium. The same experimental design was adopted as above with non-depolarizing solutions but using a Ca^{2+}-free depolarizing MOPS PSS containing 0 mM Ca^{2+} and 40 mM K^+ (Table 1). The uteri responded to the cumulative addition of Ca^{2+} with graded tonic tension development (Figure 1b). Only one concentration of calcium entry blocker was tested per preparation.

Extracellular electrical recording The extracellular recording technique of Golenhofen & von Loh (1970) was used to compare the electrical changes associated with Ca^{2+} addition to uterine strips bathed in either non-depolarizing or depolarizing media. Strips of whole uterus were initially perfused with either PSS or MOPS PSS (Table 1) for 45 min. The medium was

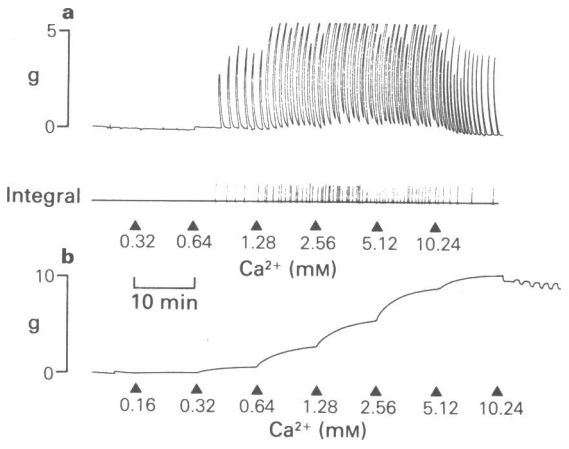

Figure 1 Whole uterus strip: (a) typical phasic tension development to Ca^{2+} added cumulatively to Ca^{2+}-free MOPS PSS; (b) typical tonic tension development to Ca^{2+} added cumulatively to Ca^{2+}-free depolarizing MOPS PSS.

changed to Ca^{2+}-free PSS or Ca^{2+}-free depolarizing MOPS PSS respectively for a further 1 h before $CaCl_2$ was added to the superfusate in a cumulative manner at 10 min intervals.

Myometrial strips

To remove the endometrium, uterine horns were opened, pinned to a wax block with the endometrial surface uppermost and superfused with cold, MOPS PSS bubbled with 100% O_2. The endometrial surface was removed by gently rubbing in a unidirectional manner with a cotton wool bud. To confirm that this procedure removed the endometrial layer, leaving circular and longitudinal muscle layers intact, samples of whole and 'stripped' preparations were fixed in Bouin's fluid, mounted in wax, sectioned, stained with 'haemotoxylin and eosin' and examined microscopically.

In initial experiments, it was found that the lanthanum-resistant $^{45}Ca^{2+}$ fraction (see below) of the whole uteri was significantly greater than that of endometrium-free uteri. Also, oxytocin increased this parameter in endometrium-free uteri but not in whole uteri (unpublished observations). Therefore, the effects of the calcium entry blockers against KCl-induced tension development were studied in endometrium-free strips prior to $^{45}Ca^{2+}$ uptake studies in this tissue. Longitudinal strips of endometrium-free uterus (7 × 2 mm) were mounted under 1 g tension in tissue baths containing MOPS PSS, maintained at 37.0 ± 0.5°C and bubbled with 100% O_2. Tension development was recorded as described previously. Tissues were allowed a 45 min equilibration period, after which concentration-effect experiments were carried out by cumulative addition of KCl (10–40 mM) to the bathing medium at 10 min intervals. The reproducibility of KCl-induced responses was improved if two preliminary concentration-effect experiments were carried out (at 30 min intervals); thus, the control curve to KCl was derived from the third exposure to KCl. Further concentration-effect experiments to KCl were repeated at 30 min intervals in the absence (time-matched controls) or presence of increasing concentrations of calcium entry blockers. The integral of tension development to KCl measured subsequently was expressed as a percentage of the initial control integral to 40 mM KCl.

Lanthanum-resistant ^{45}calcium fraction A modification of the 'lanthanum method' of Van Breemen *et al.* (1977) was used to assess the effects of the calcium entry blockers on KCl-induced $^{45}Ca^{2+}$ uptake. Strips of endometrium-free tissue (1–12 mg), or whole uteri in preliminary experiments, were impaled on syringe needles and attached to a manifold which allowed the simultaneous transfer of many tissues between racks of test tubes containing various solutions. The tissues were allowed to equilibrate in MOPS PSS at 37°C for 45 min. Tissues were then transferred to tubes containing either MOPS PSS alone or MOPS PSS with a calcium entry blocker. The antagonist concentrations chosen were the maximum concentrations used in the tissue bath studies against KCl: nifedipine (2.5 nM), gallopamil (100 nM), diltiazem (500 nM), cinnarizine (5 μM). Tissues remained in these solutions for 25 min after which they were transferred, for exactly 5 min, to solutions of MOPS PSS plus $^{45}Ca^{2+}$ (250 nCi ml^{-1}) with or without a calcium entry blocker. Tissues were then transferred to one of several treatment solutions which were: (a) MOPS PSS plus $^{45}Ca^{2+}$ with or without a calcium entry blocker or (b) MOPS PSS plus $^{45}Ca^{2+}$ plus 20 mM KCl with or without a calcium entry blocker or (c) MOPS PSS plus $^{45}Ca^{2+}$ plus 40 mM KCl with or without calcium entry blocker for a 10 min incubation period. All the above solutions were bubbled with 100% O_2 and maintained at 37.0 ± 0.5°C. The tissues were then washed in ice-cold (0°C) Ca^{2+}-free MOPS PSS containing 10 mM lanthanum chloride for a total of 120 min, with transfers between washes occurring at 1, 1, 2, 2, 4, 10, 10, 30, 30 and 30 min intervals. After the 'lanthanum chloride wash', tissues were removed from the manifold, blotted and weighed. Tissues and corresponding treatment media samples were prepared for scintillation counting and sample radioactivity was counted in a Packard Tri-carb 4640 scintillation counter linked to an Apple II computer. Tissue $^{45}Ca^{2+}$ content (the lanthanum-resistant $^{45}Ca^{2+}$ fraction) was converted to a tissue to medium ratio (nCi g^{-1} of tissue: nCi ml^{-1} of media).

Statistical analysis/Drugs

The mean $\log_{10}$ molar concentrations to produce 50% maximum response (EC_{50}) ± 95% confidence intervals were calculated according to the method of Granger *et al.* (1985a). The significance of differences between means was determined by the Mann-Whitney U-Test. For the radioactive experiments the significance of differences between means were compared by the unpaired Student's t test. As 2-tailed tests were used, significance levels are quoted as $2P$.

The composition of each PSS is given in Table 1; all components were of Analar grade. In experiments involving Ca^{2+} concentrations greater than 2.5 mM or La^{3+}, 3-(N-morpholino)-propanesulphonic acid (MOPS; BDH) was used as buffer (Jetley & Weston, 1980) to avoid precipitation of Ca^{2+} or La^{3+} salts.

The following drugs and chemicals were used: nifedipine (Bayer), gallopamil HCl (Knoll), (+)-*cis* diltiazem HCl (Synthelabo), cinnarizine (Janssen), lanthanum chloride (BDH) and EGTA (Sigma). Experiments involving nifedipine were performed under sodium lighting. $^{45}Ca^{2+}$ (10–40 mCi mg^{-1}) was obtained as an aqueous solution of calcium chloride from Amersham International.

Results

Strips from whole uterus

Experiments with non-depolarizing solutions Uteri exhibited spontaneous phasic tension development both in PSS and MOPS PSS. This mechanical activity declined when the medium was changed to the appropriate Ca^{2+}-free solution. After 1 h under these conditions, the tension integrals were 16.6 ± 4.7% of controls (PSS, $n = 6$) and 3.0 ± 1.1% of controls (MOPS PSS, $n = 6$).

Cumulative addition of Ca^{2+} to the tissue bath containing Ca^{2+}-free MOPS-PSS produced graded phasic tension development (Figure 1a). The EC_{50} was 2.90 ± 0.02 mM (mean ± s.e.mean, $n = 32$) and the maximum tension integral was usually observed with 2.5 mM Ca^{2+}. Phasic tension development was reduced when the Ca^{2+} concentration was raised from 5.1 mM to 10.2 mM. Incubation with gallopamil (100 nM) or diltiazem (1 μM) for 1 h produced significant antagonism of Ca^{2+}-effect curves with a rightward shift and reduction in maximum (Figure 2). There was a significant variation in response to added Ca^{2+} and time controls either were not changed from initial controls (Figure 2a) or were shifted slightly leftward with increase in maximum (Figure 2b).

In PSS there was a burst of rapid electrical discharges associated with each phasic tension development (Figure 3ai; $n = 5$). The frequency and amplitude of both phenomena declined when the tissue was superfused in Ca^{2+}-free PSS (Figure 3aii) and reappeared on the addition of Ca^{2+} (Figure 3aiii).

Experiments with depolarizing solutions KCl produced concentration-related increases in the integral of tension (Figure 4), responses which were entirely phasic at a low concentration (10 mM) and entirely tonic at a higher concentration (80 mM). These

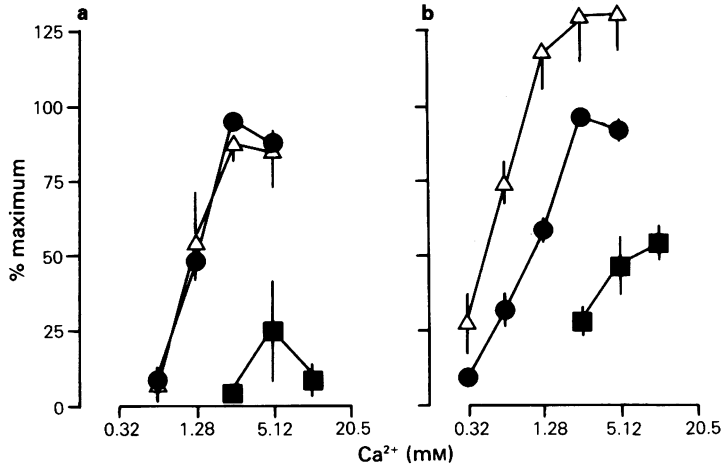

Figure 2 Whole uterus strip: concentration-effect curves to added Ca^{2+} in Ca^{2+}-free MOPS PSS. The ordinate scale is the response expressed as a percentage of the maximum response to Ca^{2+} in initial curves (●). Concentration-effect curves to Ca^{2+} were repeated after 1 h incubation in the absence (△) or the presence (■) of gallopamil (a, 100 nM;) or diltiazem (b, 1 μM;). Points are means and vertical bars the s.e.mean ($n = 4$–6).

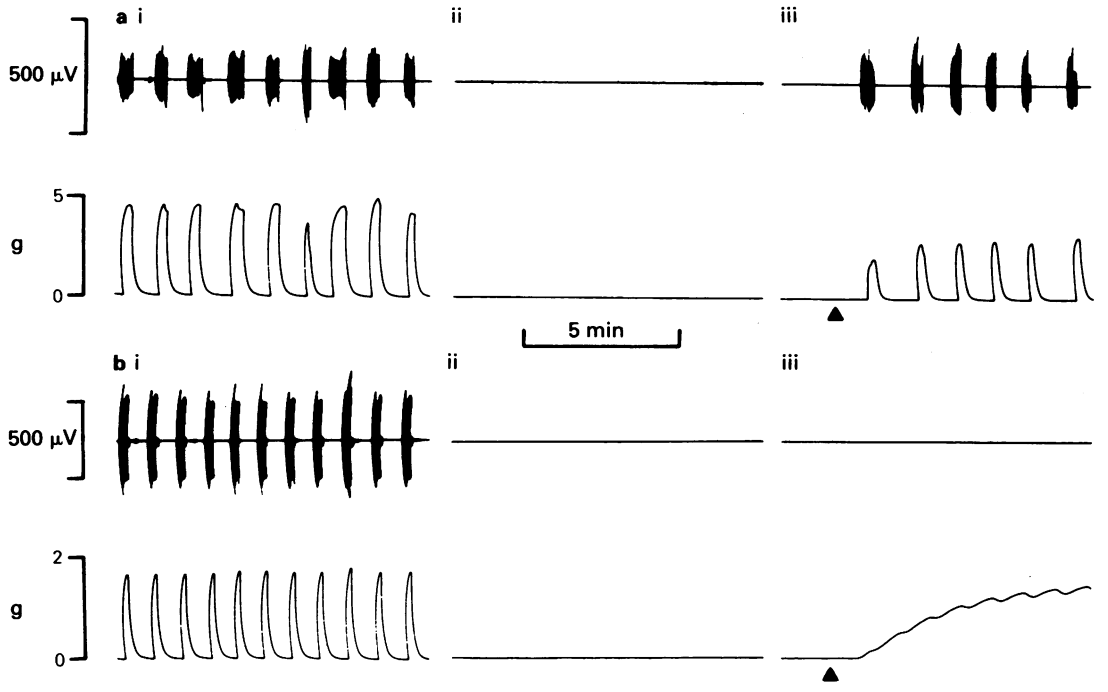

Figure 3 Whole uterus strip: extracellular electrical recording (upper traces) and mechanical changes (lower traces) in (a) PSS and (b) MOPS PSS. (a) (i) PSS; (a) (ii) from 55 min in Ca^{2+}-free PSS; (a) (iii) with addition of 0.64 mM Ca^{2+} (▲). (b) (i) MOPS PSS; (b) (ii) from 55 min in Ca^{2+}-free depolarizing MOPS PSS; (b) (iii) with addition of 0.64 mM Ca^{2+} (▲).

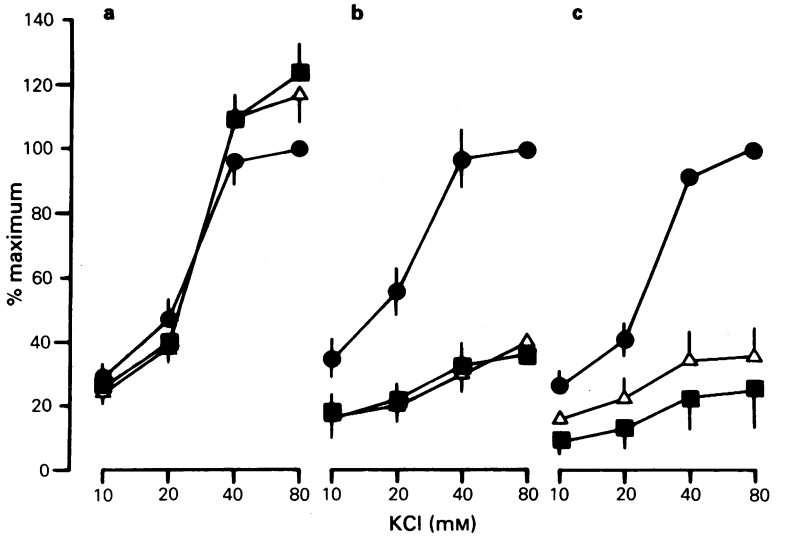

Figure 4 Whole uterus strip: KCl concentration-effect curves in PSS (●) and after incubation for 1 h (△) or 2 h (■) in (a) PSS; (b) Ca^{2+}-free PSS; (c) Ca^{2+}-free PSS + EGTA (0.5 mM). Ordinate scale is response expressed as a percentage of maximum response in initial curves. Points are means and vertical bars the s.e.mean (n = 5–7).

effects were markedly reduced after 1 h or 2 h incubation in a Ca^{2+}-free PSS over which time there was little change in control responses to KCl (Figures 4a and b). The presence of EGTA (0.5 mM) in the Ca^{2+}-free PSS had little further inhibitory effect on responses to KCl (Figure 4c).

When uteri were bathed in Ca^{2+}-free depolarizing MOPS PSS, cumulative addition of Ca^{2+} produced a graded tonic tension development (Figure 1b). The EC_{50} was 3.13 ± 0.02 mM (mean ± s.e.mean, $n = 66$) and the maximum tension development was usually observed with 5.1 mM Ca^{2+}. Control concentration-effect curves to Ca^{2+} repeated 1 h later were not significantly different from the initial control curves. Incubation with nifedipine (10 and 100 nM), gallopamil (1, 10 and 100 nM), diltiazem (100 nM and 1 μM) or cinnarizine (100 nM, 1 and 10 μM) for 1 h produced significant inhibition of these Ca^{2+}-effect curves (Figure 5). Typically, there were significant rightward shifts of the Ca^{2+}-effect curves without a reduction in maximum response with the lower antagonist concentrations and a further rightward shift with a reduction in maximum at the higher antagonist concentrations. With control tissues, increasing the Ca^{2+} concentration to 10.2 or 20.4 mM resulted in a reduction in tension, compared to that seen with 5.1 mM Ca^{2+}, sometimes with superimposed phasic tension development. The latter was also seen with high Ca^{2+} concentrations in the presence of the calcium entry blockers. These features prevented the construction of full concentration-effect curves in the presence of the calcium entry blockers. The antagonist concentrations required to produce 50% inhibition of the maximum response to Ca^{2+} were: nifedipine, 7.63 ± 0.21; gallopamil, 7.52 ± 0.44; diltiazem, 6.33 ± 0.28; cinnarizine, 5.75 ± 0.13 (−log M,

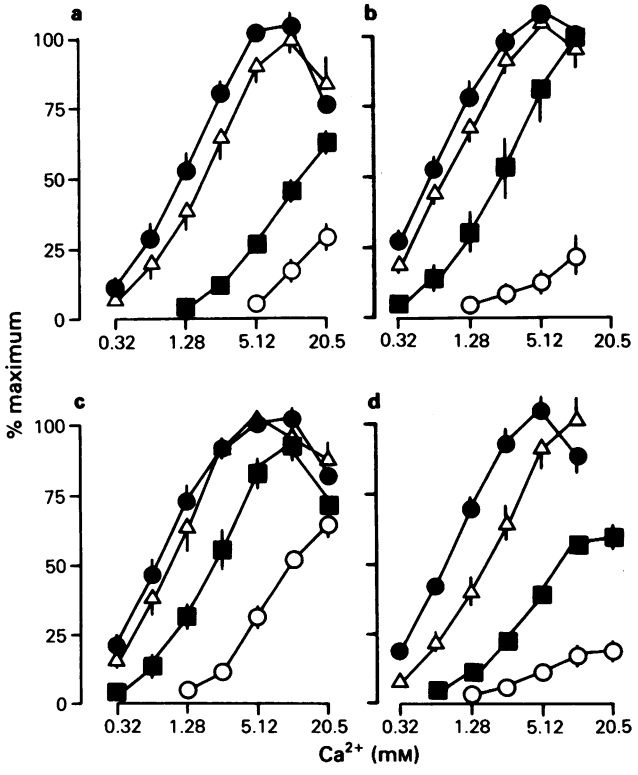

Figure 5 Whole uterus strip: Ca^{2+} concentration-effect curves in Ca^{2+}-free depolarizing MOPS PSS. Ordinates are responses expressed as a percentage of the maximum response to Ca^{2+} in initial curves. Control curves repeated after 1 h (●) were not significantly different from initial curves, latter not shown for clarity. Other curves are after 1 h incubation with (a) nifedipine (△ 1 nM, ■ 10 nM, ○ 100 nM); (b) gallopamil (△ 1 nM, ■ 10 nM, ○ 100 nM); (c) diltiazem (△ 10 nM, ■ 100 nM, ○ 1 μM); (d) cinnarizine (△ 100 nM, ■ 1 μM, ○ 10 μM). Points are means and vertical bars the s.e.mean ($n = 5-6$).

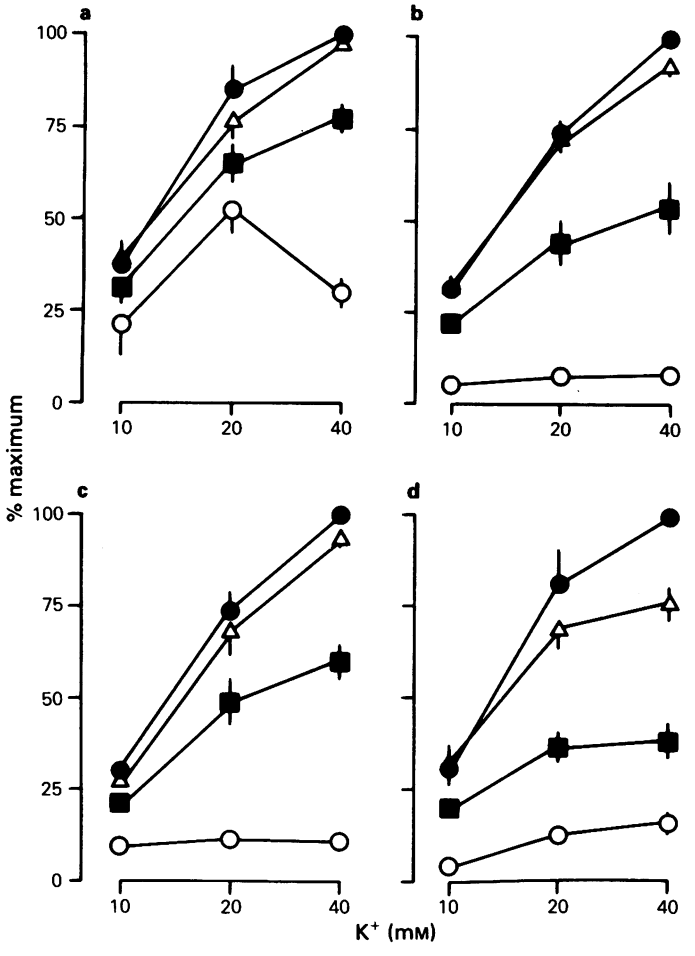

Figure 6 Myometrial strip: KCl concentration-effect curves in PSS (●) and after 30 min incubation with (a) nifedipine (Δ 100 pM, ■ 500 pM, ○ 2.5 nM); (b) gallopamil (Δ 1 nM, ■ 10 nM, ○ 100 nM); (c) diltiazem (Δ 5 nM, ■ 50 nM, ○ 500 nM); (d) cinnarizine (Δ 50 nM, ■ 500 nM, ○ 5 μM). Ordinates are responses expressed as percentage of the maximum responses to KCl in initial curves. Points are means and vertical bars the s.e.mean ($n = 4-6$).

means ± 95% confidence intervals, $n = 6-8$). The rank order of potency was, therefore, nifedipine = gallopamil > diltiazem > cinnarizine.

The patterns of rapid electrical discharge and of phasic tension development in MOPS PSS (Figure 3bi, $n = 5$) were similar to those observed in PSS (Figure 3ai). After 1 h of incubation in Ca^{2+}-free depolarizing MOPS PSS both phenomena had ceased. Re-addition of Ca^{2+} produced tonic tension development without electrical discharges.

Myometrial strips

In initial experiments it was observed that the lanthanum-resistant $^{45}Ca^{2+}$ fraction of whole uterus was significantly greater ($2P < 0.01$) than that of endometrium-free myometrial strips (0.39 ± 0.02 and 0.14 ± 0.01 ml g^{-1} respectively, $n = 8$) suggesting very significant lanthanum-resistant $^{45}Ca^{2+}$ binding to the endometrium. Histological studies demonstrated that rubbing of the endometrial surface removed the majority of the endometrium but left the smooth muscle layers intact.

In tissue bath studies, KCl produced concentration-related tension changes similar to those seen with whole uterus. The calcium entry blockers each produced a concentration-dependent reduction of both slopes and maxima of concentration-effect curves to KCl (Figure 6). Control curves, repeated at 30 min intervals, were reproducible over the course of

an experiment. The antagonist concentrations to produce 50% inhibition of the response induced by 40 mM KCl were: nifedipine, 8.89 ± 0.20; gallopamil, 7.98 ± 0.29; diltiazem, 7.18 ± 0.21; cinnarizine, 6.54 ± 0.36 (−log M, means ± 95% confidence intervals, $n = 4-6$). The rank order of potency was, therefore, nifedipine>gallopamil>diltiazem>cinnarizine.

The non-stimulated lanthanum-resistant $^{45}Ca^{2+}$ fraction was unaffected by nifedipine (2.5 nM), gallopamil (100 nM) or diltiazem (500 nM) but significantly ($2P < 0.05$) reduced by cinnarizine (5 μM) (Table 2). Exposure to 20 mM KCl for 10 min produced a significant increase in the lanthanum-resistant $^{45}Ca^{2+}$ fraction of uterine muscle. This increase was completely inhibited by diltiazem (500 nM) and cinnarizine (5 μM) but not significantly ($2P > 0.05$) affected by nifedipine (2.5 nM). Exposure to 40 mM KCl for 10 min produced a further increase in the lanthanum-resistant $^{45}Ca^{2+}$ fraction which was completely inhibited by gallopamil (100 nM) and significantly reduced by the other three calcium entry blockers.

Discussion

The present study has shown that several calcium entry blockers are able to inhibit a variety of calcium-dependent processes in whole uterine muscle. The rank order of potency of these drugs (nifedipine>gallopamil>diltiazem>cinnarizine) was the same against the various stimuli and identical to that found previously against spontaneous and oxytocin-induced tension development (Granger *et al.*, 1985a). The conclusion from such observations is that the mechanism and site of action of the calcium entry blockers is the same against these stimuli and is likely to be located at the voltage-operated calcium channel as described for other smooth muscles (Bolton, 1979).

An interesting observation was that incubation of whole uterus strips for 1 h or 2 h in a nominally Ca^{2+}-free medium greatly reduced, but failed to abolish completely, the responses to added KCl, even when EGTA was present in the PSS. It should be noted that the Ca^{2+} content of these solutions is negligible compared to that required to induce contractions. Since the responses to KCl were completely abolished by calcium entry blockers this suggests that significant amounts of extracellularly-bound calcium are still available for tension development under these circumstances, supporting the observations of van Breemen *et al.* (1966).

Extracellular recording was used as a simple monitor of electrical changes in uterine strips. The rapid electrical discharges observed in normal, calcium-containing PSS were the equivalent of the spike potentials observed in uterine muscle using intracellular microelectrodes (Marshall, 1968). Addition of calcium to tissues bathed in Ca^{2+}-free PSS produced a return of rapid electrical discharges and of normal phasic tension development, the latter was shown to be inhibited by diltiazem and gallopamil. When the tissue was in Ca^{2+}-free depolarizing PSS, addition of Ca^{2+} did not evoke detectable spike discharges but led to an increase in tonic tension development, which was also inhibited by diltiazem and gallopamil. It is therefore concluded that in both these conditions Ca^{2+} influx was occurring by similar mechanisms since the sensitivities of uterine strips to added Ca^{2+} and to calcium entry blockers were similar (Figures 1, 2, 3 and 5).

In order to provide more direct evidence of an action of the calcium entry blockers on Ca^{2+} influx itself, experiments were carried out using the lanthanum method. The use of a MOPS PSS for these

Table 2 The lanthanum-resistant $^{45}Ca^{2+}$ fraction of endometrium-free rat uterus

Treatment	*Nifedipine* (2.5 nM)	*Gallopamil* (100 nM)	*Diltiazem* (500 nM)	*Cinnarizine* (5 μM)
Control	0.16 ± 0.01	0.19 ± 0.01	0.15 ± 0.01	0.18 ± 0.02
Calcium entry blocker alone	0.15 ± 0.01	0.18 ± 0.01	0.13 ± 0.01	0.14 ± 0.01[a]
KCl 20 mM	0.21 ± 0.01[b]	ND	0.22 ± 0.01[b]	0.21 ± 0.01[a]
KCl 20 mM + calcium entry blocker	0.19 ± 0.01	ND	0.15 ± 0.01[d]	0.18 ± 0.01[d]
KCl 40 mM	0.26 ± 0.02[b]	0.28 ± 0.01[b]	0.26 ± 0.01[b]	0.29 ± 0.02[b]
KCl 40 mM + calcium entry blocker	0.20 ± 0.02[ac]	0.19 ± 0.01[d]	0.17 ± 0.01[ad]	0.21 ± 0.01[ad]

Data are presented as tissue: medium ratio (ml g^{-1}) for $^{45}Ca^{2+}$ as means ± s.e.mean ($n = 8$). Significantly different from respective control group (a, $2P < 0.05$; b, $2P < 0.01$) or respective KCl group (c, $2P < 0.05$; d, $2P < 0.01$). ND = not determined.

experiments had little effect on the electrical and mechanical responses developed by the uterus and suggests that the use of this buffer did not markedly affect uterine responses, findings in agreement with similar observations on other tissues (e.g. Foster *et al.*, 1983).

Early attempts to use the lanthanum method in uterine muscle were unsuccessful (see Krejci & Daniel, 1970) but recent modifications (Batra, 1982; Ichida *et al.*, 1984; present study) have enabled a more accurate and meaningful assessment of $^{45}Ca^{2+}$ influx into this tissue. These modifications have included the use of endometrium-free myometrial strips to eliminate the large $^{45}Ca^{2+}$ binding to endometrium, the use of a prolonged lanthanum chloride wash to maximize the displacement of extracellularly-bound $^{45}Ca^{2+}$, an action that is optimized with a Ca^{2+}-free lanthanum chloride wash, and performing the lanthanum chloride wash at 0°C to minimize lanthanum-resistant, energy-dependent loss of $^{45}Ca^{2+}$ from the tissue.

KCl induced a concentration-dependent increase in $^{45}Ca^{2+}$ influx in myometrial strips and this was well-correlated with the associated tension development in this tissue. These increases in $^{45}Ca^{2+}$ influx were prevented by the calcium entry blockers at concentrations that were equi-effective in suppressing KCl-induced tension development. Batra (1985) has also recently shown that nitrendipine and gallopamil inhibited KCl-stimulated $^{45}Ca^{2+}$ influx in the myometrium of the non-pregnant rat. Our observations on the inhibition of $^{45}Ca^{2+}$ influxes, together with those concerning the inhibition of KCl- and Ca^{2+}-induced contractions, strongly support the hypothesis that the inhibitory actions of calcium entry blockers in the uterus can be explained by an inhibition of Ca^{2+} influx, as in other smooth and cardiac muscles (Nayler & Horowitz, 1983). The present findings provide a strong base for their use as pharmacological tools in this tissue.

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