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In vitro effects of calcium entry blockers, chlorpromazine and fenoterol upon human pregnant myometrium contractility

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1 The inhibitory effects of nifedipine, verapamil, cinnarizine (calcium entry blockers), chlorpromazine (a putative calmodulin antagonist) and fenoterol (a β_2-adrenoceptor agonist) on contractility in human isolated pregnant myometrium were studied.

2 Spontaneous contractions (present in 93% of the preparations) were inhibited in a concentration-related manner by these compounds in the following order of potency: nifedipine > verapamil >> cinnarizine > chlorpromazine. Cinnarizine was effective only at a concentration greater than 100 μM. Fenoterol, at 10 μM, did not produce an inhibitory effect but decreased the frequency of spontaneous contractions.

3 All drugs, except fenoterol, produced a concentration-dependent relaxation of K^+-induced contractions in the following order of sensitivity: nifedipine > verapamil >> chlorpromazine. Cinnarizine produced only about 40% of relaxation. Under these conditions nifedipine and verapamil were about 80 and 5 fold more potent respectively than when tested against spontaneous contractions. The potencies of chlorpromazine and cinnarizine did not differ in the two experimental conditions.

4 Both the spontaneous and K^+-induced contractions were inhibited in a time-dependent manner in Ca^{2+}-free media and the responses were almost completely abolished in 70–100 min. Calcium addition to the medium rapidly restored both spontaneous or K^+-induced contractions.

5 To investigate further the role of intracellular calcium, K^+-depolarized preparations contracted by calcium 3 mM (40–60% of maximal contractions) were relaxed by these compounds. Nifedipine and verapamil showed a relaxation time course similar to that induced by calcium removal. Cinnarizine and fenoterol had no relaxant effect while chlorpromazine induced a slight and slow relaxation.

6 These findings suggest that calcium influx and calmodulin are involved in spontaneous contractions of pregnant human myometrium *in vitro*. Since nifedipine and verapamil were more potent against K^+-induced than spontaneous contractions, calcium channels activated by these conditions could be different. Finally, fenoterol, a β_2-adrenoceptor agonist, widely used as a tocolytic agent, blocked neither spontaneous nor K^+-induced contractions.

Introduction

Uterine contractions, as well as contractions of other smooth muscles, are dependent on the concentration of free calcium in the cell cytoplasm. An increase in cytoplasmatic free calcium concentration can be achieved by a release of the ion from intracellular store sites or by an influx of extracellular calcium through voltage- and receptor-operated channels (Cavero & Spedding, 1983).

It has been shown that several pharmacological agents that interfere with calcium influx are able to inhibit uterine contractions in rat (Fleckenstein *et al.*, 1971; Østergaard *et al.*, 1980; Forman *et al.*, 1982; Maigaard *et al.*, 1983; Csapo *et al.*, 1982; Abel & Hollingsworth, 1985) and human tissue. The potency of the different calcium antagonists in inhibiting muscle contractions depends on the stimulus employed as well as the muscle under study (Triggle & Swamy, 1983). For example, it has recently been demonstrated that cinnarizine, a very weak inhibitor of spontaneous rat uterine and portal vein contractions was more potent than nifedipine and diltiazem in inhibiting noradrenaline-induced responses in mesenteric tissue (Granger *et al.*, 1985). Since the pharmacological control of uterine contractions is a very desirable

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therapeutic goal in obstetrics and gynaecology, it is of interest to compare the effect of compounds representative of each one of the three subclasses of calcium antagonists, a calmodulin antagonist and a β-adrenoceptor agonist on the spontaneous, K^+- and Ca^{2+}-induced contractions of strips of uterine smooth muscle obtained from women at term pregnancy.

Methods

Preparation of uterine muscle

Myometrial samples were obtained from 30 women aged 24 ± 1.3 years (11 primiparous and 19 multiparous). The muscle strips were cut from the lower uterine segment during caesarian section at or near term (36–40 weeks). Tissues were immediately transferred to Krebs solution (see composition below) and transported to the laboratory, or stored at 4°C overnight and tested on the next day, as previously described (Calixto & Simas, 1984).

In general, 4 to 6 uterine strips from each muscle piece, approximately 2 to 3 mm wide, 1 to 2 mm thick and 15 to 20 mm long were suspended in a 10 ml organ bath containing Krebs solution at 37°C, pH 7.2–7.4, continuously gassed with 95% O_2 and 5% CO_2. The Krebs solution had the following composition (mM): NaCl 118, KCl 4.4, $CaCl_2$ 2.5, $MgSO_4$ 1.1, $NaHCO_3$ 23.9, KH_2PO_4 1.1 and glucose 5.5 prepared in distilled and deionized water.

Isotonic contractions were measured under a load of 1 g with a light lever (six fold amplification) writing on a kymograph. Tissues were equilibrated for 2 h under a rest load of 2 g before drug administration, with the solution being changed every 20 min.

Effect of calcium entry blockers, chlorpromazine and fenoterol on spontaneous contractions

After stabilization of the preparation and in the presence of spontaneous contractions, inhibitory cumulative-concentration response curves were obtained with one of the compounds studied (nifedipine, verapamil, cinnarizine, chlorpromazine and fenoterol), according to Van Rossum (1963). The maximal initial contraction obtained in each individual experiment was taken as 100%, and all contractions were calculated as a function of this value. The inhibitory potency was determined at the IC_{50} level with 95% confidence limits.

Effect of calcium entry blockers, chlorpromazine and fenoterol on potassium-induced contractions

In another set of experiments following the equilibration period, the preparations were exposed to a high-potassium solution (prepared by replacement of 80 mM NaCl with 80 mM KCl) in order to assess the effects of these compounds on the calcium influx through voltage-sensitive channels (Godfraind *et al.*, 1968). Following two control contractions for K^+, different concentration of nifedipine (0.1–300 nM), verapamil (0.01–10 μM), cinnarizine (10–300 μM), chlorpromazine (10–3000 μM) and fenoterol (0.1–10000 μM) were added to the bath solution and left in contact with the tissue for 20 min. Complete inhibitory concentration-response curves were obtained for each drug with 20 min intervals between each dose. Each agent was tested in separate strips and control experiments were performed with only KCl (80 mM) in the absence of inhibitor. The mean IC_{50} was determined as described above.

Relaxant effect of calcium entry blockers, chlorpromazine and fenoterol on calcium-induced contractions

In order to determine whether these compounds could act by blocking the effect of calcium within the cell (Spedding, 1982; Hof & Vuorela, 1983), we compared the relaxation induced by these drugs in strips previously contracted with calcium, as described previously in rat uterine smooth muscle (Calixto & Loch, 1985). After complete stabilization of the sustained tonic contractile response to calcium (3 mM, ED_{50}–ED_{80}, 60–80 min), different concentrations of nifedipine (0.03 and 0.3 μM), verapamil (0.3 and 3 μM), cinnarizine (0.3 mM), chlorpromazine (3 mM) and fenoterol (10 mM), were added to the bath. The relaxant effect was compared to that obtained when calcium was removed from the bathing solution.

Statistical analysis

The results are presented, when appropriate, as the mean ± s.e.mean. Statistical significance of differences between the means was assessed using Student's test for unpaired data. P values of less than 0.05 were considered to represent significant differences.

Drugs

The following drugs were used: nifedipine (Bayer), verapamil chloride (Knoll), cinnarizine chloride (Sigma); chlorpromazine chloride (Rhodia), fenoterol (Boehringer Ingelheim). These drugs were diluted in 0.9% w/v NaCl solution, except nifedipine (0.01 M in absolute ethanol) and cinnarizine (0.9% w/v NaCl solution containing HCl 0.1 N). The stock solutions were kept at −4°C and diluted just before use. All nifedipine experiments were protected from light.

Results

Effects of calcium withdrawal on spontaneous and K^+-induced contractions

As shown previously (Calixto & Simas, 1984), spontaneous contractions were observed in about 93% of the strips analysed. Following the equilibration period of 2 h and in the presence of spontaneous or K^+-induced contractions, the preparations were incubated in Ca^{2+}-free solution in which a progressive and time-related decrease in both spontaneous and K^+-induced contractions was observed ($t_{1/2}$ about 40 min) with abolition of activity after 70–100 min (Figure 1a and b). Addition of Ca^{2+} (2.5 mM) to the medium almost completely restored both the spontaneous and K^+-induced contractions in about 30 min.

Effect of calcium entry blockers, chlorpromazine and fenoterol on the spontaneous contractions

Nifedipine (0.1–10 μM), verapamil (1–100 μM), cinnarizine (1–100 μM) and chlorpromazine (10–10000 μM), but not fenoterol (0.1–3000 μM) produced a concentration-related inhibition of the spontaneous contractions (Figure 2). In some experiments, the inhibition produced by these compounds was also associated with an increase in the tonus of the strip, especially in the experiments with cinnarizine. Fenoterol (> 10 μM) only reduced the frequency of spontaneous contractions. The mean cumulative concentration-response curves shown in Figure 3 and the results in Table 1 indicate that nifedipine was about 10, 1000 and 3350 fold more potent in inhibiting spontaneous contractions than verapamil, cinnarizine and chlorpromazine, respectively. Increasing the incubation time to 120 min did not increase the sensitivity to cinnarizine. The inhibitory effect of these compounds did not revert completely after intermittent washing of the preparations for 120 min.

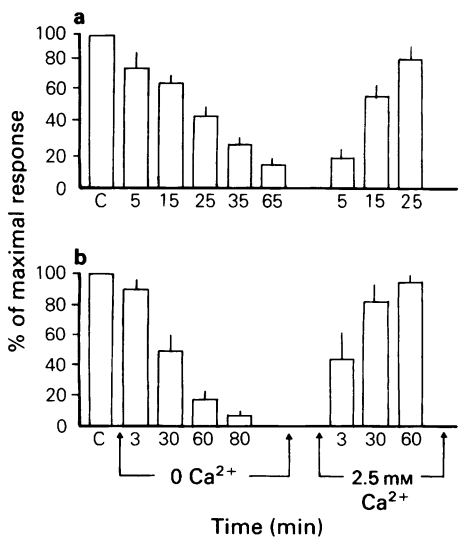

Figure 1 Time course of the relaxant effects of Ca^{2+}-free medium on spontaneous contractions (a) and K^+-induced contractions (b) of human isolated pregnant myometrium. Ca^{2+} (2.5 mM) was subsequently readded. Each column represents the mean of 7 experiments and the vertical lines the s.e.means.

Effect of calcium entry blockers, chlorpromazine and fenoterol on potassium-induced contractions

The mean-cumulative inhibitory concentration-response curves shown in Figure 4 and the data presented in Table 1 show that nifedipine (0.1–100 nM) was about 80 fold more potent in inhibiting potassium-induced contractions than spontaneous contractions, while verapamil (0.01–10 μM) was only 5.5 fold more active. In these experimental conditions, nifedipine was about 64, 10000 and 140000 fold more potent than verapamil, cinnarizine and chlorpromazine, respectively. As observed in spontaneous contractions, cinnarizine (10–300 μM) produced only a partial inhibition (about 40%), at least at the range of doses used, and the mean potency did not differ significantly between the two experimental conditions. The IC_{50} and maximal inhibition of K^+-induced contractions caused by chlorpromazine (30–3000 μM) did not differ from those observed for spontaneous contractions, while fenoterol did not interfere with K^+-induced contractions (results not shown).

Relaxant effect of calcium entry blockers, chlorpromazine and fenoterol on calcium-induced contractions

Cumulative concentration-response curves in response to calcium (0.3–88 mM) on pregnant human myometrium exposed to K^+-depolarizing calcium-free solution were reproducible at 90–120 min intervals. Figure 5 shows the mean cumulative concentration-response curves for calcium, with ED_{50} (95% confidence limits) of 6.1 (3.0–12.4 mM). Figure 6 shows the time courses of the relaxation of calcium (3 mM)-induced contractions of the depolarized uteri produced by nifedipine (30–300 nM), verapamil (0.3–3 μM), cinnarizine (300 μM) and chlorpromazine (3000 μM) or by calcium removal. All drugs with the exception of cinnarizine produced a slow partial relaxation (35–40%) that was time-, but not concentration-dependent. Nifedipine and verapamil caused relaxation with a time course similar to that produced by calcium withdrawal, while chlorpromazine produced a slow relaxation. Fenoterol had no effect.

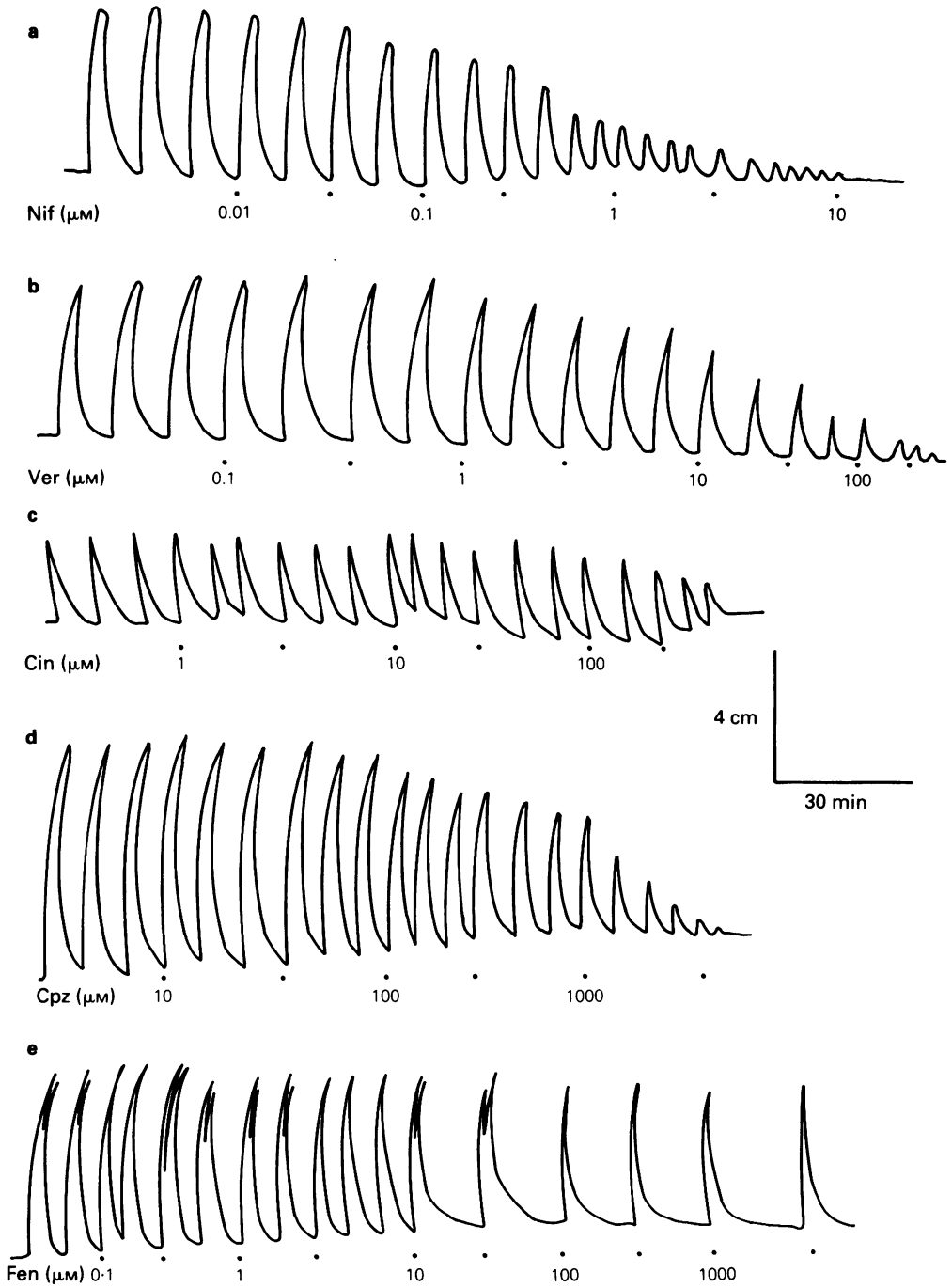

Figure 2 Typical isotonic recording of spontaneous contractions of human isolated pregnant myometrium before and after addition of cumulative concentrations of (a) nifedipine, (b) verapamil, (c) cinnarizine, (d) chlorpromazine and (e) fenoterol.

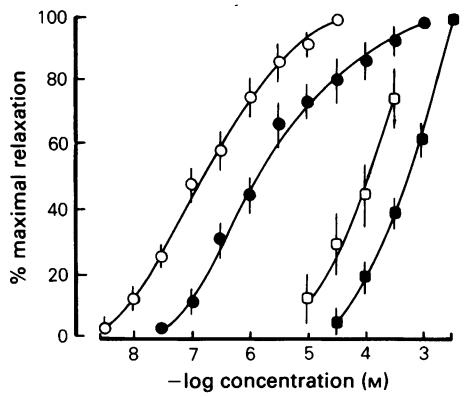

Figure 3 Mean inhibitory concentration-response curves to nifedipine, (○), verapamil (●), cinnarizine (□) and chlorpromazine (■) on the spontaneous contractions of the human isolated pregnant myometrium. Each point represents the mean of 6 to 9 experiments and the vertical lines the s.e.mean.

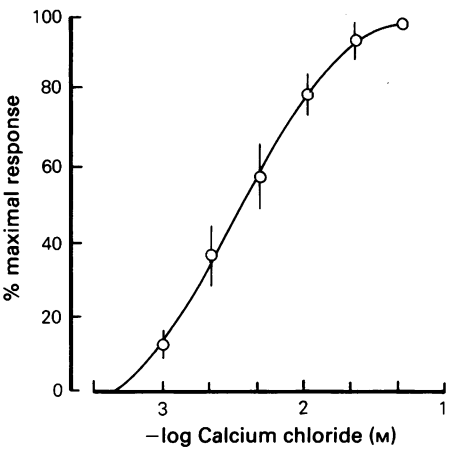

Figure 5 Mean concentration-response curves to $CaCl_2$ obtained in human isolated, K^+-depolarized, pregnant myometrium. Each point represents the mean of 8 experiments and the vertical lines the s.e.means.

Discussion

The present study demonstrates that nifedipine, verapamil, cinnarizine and chlorpromazine inhibit in a concentration-related way both spontaneous and K^+-induced contractions of human myometrial strips obtained at or near term. The inhibitory effect produced by calcium entry blockers is well correlated with a reduced calcium influx across the cell membrane (for review see Cavero & Spedding, 1983; Godfraind & Miller, 1985; Loutzenhiser *et al.*, 1985). In both experimental conditions, the rank order of potency was nifedipine > verapamil ➤ cinnarizine > chlorpromazine. Nifedipine and verapamil were 80 and 5.5 fold, respectively, more potent in inhibiting

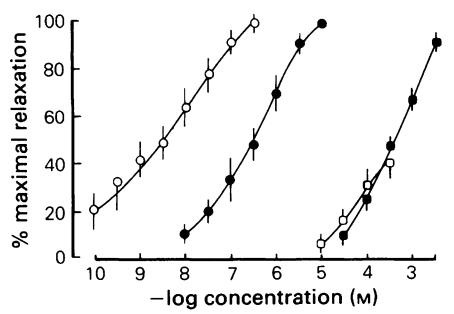

Figure 4 Mean inhibitory concentration-response curves to nifedipine (○), verapamil (●), cinnarizine (□) and chlorpromazine (■) on the K^+-induced contraction of the human isolated pregnant myometrium. Each point represents the mean of 8 experiments and the vertical lines the s.e.mean.

K^+-induced than spontaneous contractions. These potency differences indicate that the human pregnant myometrium contractile response evoked by high K^+ relies like other smooth muscles (Forman *et al.*, 1978; Pedersen *et al.*, 1979; van Breemen & Siegel, 1980; Fanta *et al.*, 1982; Godfraind & Miller, 1985; Granger *et al.*, 1986) on Ca^{2+} influx, possibly through voltage-sensitive channels. On the other hand, the reduced inhibitory effect of these drugs on spontaneous contractions together with the presence of contractions in Ca^{2+}-free solution suggest that these contractions depend on calcium from both extracellular and intracellular sources.

The mechanism for the relaxant effect of nifedipine and verapamil is still controversial and appears to differ according to the tissue studied and the animal species (Triggle, 1984; Godfraind & Miller, 1985). The hypothesis of voltage-operated and receptor-operated calcium channels relies largely on functional rather than biochemical and electrophysiological evidence (Hagiwara & Byerley, 1983). In general, it has been accepted that the concentration of calcium channel blockers required to block receptor-operated channels (Braunwald, 1982; Cauvin *et al.*, 1983; Snyder & Reynolds, 1985) are much higher than those necessary to block voltage-operated channels, but in some tissues the action of nifedipine on voltage- and receptor-operated channels is observed in the same concentration range (Braunwald, 1982). The order of potency of nifedipine and verapamil against K^+-induced contractions found in this study is consistent with other reports on voltage-operated channels (Cauvin *et al.*, 1983). Studies with isolated arteries indicate that nifedipine is approximately a hundred fold more

Table 1 Potencies of calcium entry blockers, chlorpromazine and fenoterol on human pregnant myometrium *in vitro*

	IC_{50}(M)		
Drugs	*Spontaneous contractions*	K^+ (80 mM)	IC_{50} *ratio*
Nifedipine	2.0×10^{-7} (0.6– 6.5)[a]	2.5×10^{-9} (0.2–10.3)	80*
Verapamil	1.6×10^{-6} (0.4– 7.2)	$2.9 - 10^{-7}$ (1.4–10.9)	5.5*
Cinnarizine	$>10^{-4}$	$>10^{-4}$	–
Chlorpromazine	6.7×10^{-4} (3.6–12.5)	3.5×10^{-4} (2.8– 4.3)	1.9
Fenoterol	b	b	–

[a]95% confidence intervals; [b]without effect up to 10^{-2} M.
*$P < 0.05$
Each group represents the mean of 6 to 8 experiments.

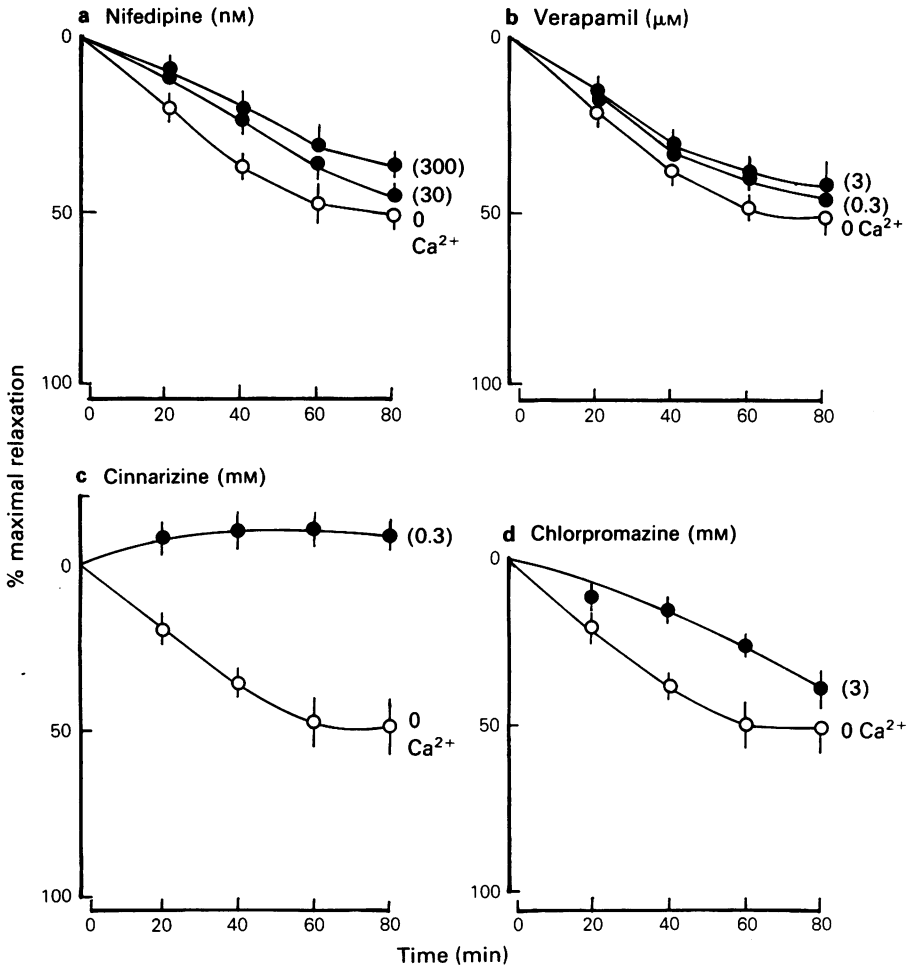

Figure 6 Time-course of the relaxant effects of different concentrations of (a) nifedipine (3 and 30×10^{-8} M), (b) verapamil (3 and 30×10^{-7} M), (c) cinnarizine (3×10^{-4} M) and (d) chlorpromazine (3×10^{-3} M) compared with those produced by ommission of Ca^{2+} from the bathing solution (O) on $CaCl_2$ (3×10^{-3}M)-induced contractions of the human isolated pregnant myometrium in K^+-depolarizing medium. The concentration of these compounds in the bath are indicated by the numbers in parentheses. Each point represents the mean of 4 to 7 experiments and the vertical lines the s.e.means.

potent in inhibiting calcium cell influx via voltage-operated calcium channels than verapamil (Fleckenstein & Fleckenstein-Grun, 1980; Fanta & Drazen, 1983; Andersson *et al.*, 1983). Our data, however, do not permit us to determine whether these drugs shared a common mechanism of action in pregnant human myometrium, but do indicate that calcium influx is necessary for the development of K^+-induced contractions and to a lesser extent for spontaneous contractions. It is possible that in the case of spontaneous contractions the calcium entry blockers are acting by a different mechanism, since it has been shown that these drugs may also act at intracellular sites in relation either to an increase of calcium efflux or to stimulation of calcium uptake (Saida & Van Breemen, 1983; Spedding, 1983; Cohen *et al.*, 1984). An intracellular effect may also have explained why nifedipine and verapamil were also able to inhibit partially the sustained contraction induced by calcium in K^+-depolarized tissues. This effect was not concentration-dependent, but increased with time. The period of time necessary for complete relaxation to occur after verapamil or nifedipine was longer (about 80 min) than that reported in similar studies performed in guinea-pig isolated taenia and nonpregnant rat myometrium (about 5–30 min) (Spedding, 1982; Calixto & Loch, 1985) but was related to the time course of Ca^{2+} washout. The response of the myometrium may, however, depend on animal species since the sensitivity to calcium in human and nonpregnant dog (Calixto & Antonio, 1986) was not different, whereas in myometrium from nonpregnant rats the potency of $CaCl_2$ to induce contractions in high K^+-Ca^{2+}-free medium was about 30–40 fold higher (Calixto & Loch, 1985) than in the present study.

Cinnarizine, a postulated Ca^{2+} antagonist with vascular selectivity (Van Neuten & Vanhoutte, 1981), showed a very low inhibitory potency against both spontaneous and K^+-induced uterine contractions. This observation is consistent with some results obtained in the uterus and portal vein of rats (Granger *et al.*, 1985) and in the guinea-pig taenia (Spedding, 1982). Since cinnarizine belongs to a subgroup of Ca^{2+} antagonists that is different from nifedipine or verapamil it is reasonable to postulate that if the effect of cinnarizine is consequent to blockade of calcium entry, its site of action must be different from that of nifedipine or verapamil.

Chlorpromazine-induced relaxation in the human isolated pregnant myometrium was compatible with the potency observed in some other smooth muscle preparations (Karaki *et al.*, 1982; Burka, 1984). However, its IC_{50} was about 10 fold higher than the concentration needed to inhibit calmodulin (ID_{50} = 40 μM) (Hidaka *et al.*, 1979; Kanamori *et al.*, 1981). It is possible that additional sites of action for chlorpromazine may be involved in its relaxant effect in pregnant human myometrium *in vitro* (Karaki *et al.*, 1982).

Fenoterol did not modify either spontaneous, K^+- or Ca^{2+}-induced contractions on human isolated pregnant myometrium. In addition, in the same preparation other putative selective β_2-adrenoceptor agonists such as orciprenaline, terbutaline and isoxsuprine, inhibited spontaneous, but not K^+-induced contractions at high concentrations, effects which were not blocked by propranolol (at 100 μM) (Calixto & Simas, 1984). If spontaneous and K^+-induced contractions of human uterine strips *in vitro* obtained from the lower segment are representative of the contractions that occur in preterm labour then these findings indicate that the tocolytic effect of fenoterol may not be related to its β_2-adrenoceptor agonist properties as has been suggested for other drugs of this group (Caritis, 1983; Calixto *et al.*, 1984).

In summary, the present findings furnish additional evidence indicating that calcium entry blockers and a calmodulin inhibitor have a direct relaxant effect on spontaneous, K^+- and calcium-induced contractions of pregnant human myometrium *in vitro*. In addition, these results show that there are marked differences in the potencies and mechanism of the relaxant effects produced by the different types of Ca^{2+} antagonist in the human isolated pregnant myometrium.

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