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The potencies and selectivities of four calcium antagonists as inhibitors of uterine contractions in the rat *in vivo*

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1 The potencies of four calcium antagonists (nifedipine, gallopamil, verapamil and diltiazem) at inhibiting uterine contractions *in vivo* have been assessed in the conscious ovariectomized, post-partum rat. Their selectivities for this action, relative to their effects on blood pressure and heart rate, have been compared with salbutamol.

2 All compounds produced a dose-dependent inhibition of intra-uterine pressure cycles. The rank order of potency was salbutamol > nifedipine > diltiazem = gallopamil > verapamil.

3 All compounds produced a dose-dependent fall of mean blood pressure. The rank order of potency was salbutamol > nifedipine > gallopamil > verapamil > diltiazem.

4 Salbutamol and nifedipine produced a tachycardia, which was very marked with salbutamol. Gallopamil, verapamil and diltiazem induced a moderate tachycardia at low doses but temporary cessation of heart beat occurred at high doses.

5 Nifedipine and diltiazem, like salbutamol, exhibited some selectivity for inhibition of uterine contractions relative to their cardiovascular actions. Gallopamil and verapamil showed no selectivity for the uterus.

Introduction

Agonists at β-adrenoceptors such as salbutamol and ritodrine, are widely used for the treatment of preterm labour (Lippert, 1983). However, in addition to affecting uterine smooth muscle, these compounds also act at β-adrenoceptors on cardiac and vascular smooth muscle and skeletal muscle causing tachycardia, hypotension and tremor, thus limiting the effective dose which can be administered. There is, therefore, a need for compounds with a greater selectivity.

Tension development by uterine smooth muscle is dependent on extracellular calcium (Ca^{2+}) entering the cytoplasm through Ca^{2+} channels in the cell membrane (Edman & Schild, 1962; Bolton, 1979). It is predictable, therefore, that uterine contractions can be inhibited by compounds which act to limit or prevent the entry of Ca^{2+} into the myocyte. Indeed, calcium antagonists have been shown to inhibit uterine contractions in both the rat (Csapo *et al.*, 1982) and man (Forman *et al.*, 1981). Premature delivery of rats ovariectomized on day 16 of gestation was prevented by the administration of nicardipine (Csapo *et al.*, 1982) while nifedipine, verapamil and diltiazem prolonged normal parturition in this species (Hahn *et al.*, 1984).

The calcium antagonists are a structurally heterogeneous group of compounds. Based on *in vitro* and *in vivo* pharmacological profiles, three sub-classes have been recognized (Spedding 1982a,b; 1984; Granger *et al.*, 1985): a relatively hydrophilic group (e.g. diltiazem, verapamil, gallopamil), the dihydropyridines (e.g. nifedipine) and the diphenylalkylamines (e.g. cinnarizine). It has recently been demonstrated that compounds in all three groups are potent inhibitors of spontaneous and oxytocin-induced tension development by the isolated uterus of the day 22 pregnant rat (Granger *et al.*, 1985). Some selectivity for the uterus relative to cardiovascular tissues was shown by nifedipine, gallopamil and diltiazem but not by cinnarizine. The following study was carried out to extend the above observations and determine whether these compounds could inhibit uterine contractions *in vivo* at doses which did not markedly affect blood pressure and heart rate. The model used was the ovariectomized post-partum rat,

which exhibits regular, spontaneous intra-uterine pressure-cycles (Downing & Porter, 1980) and the effects of the calcium antagonists were compared with those of salbutamol, a β-adrenoceptor agonist in current clinical use.

Methods

Animals

Post-partum Sprague-Dawley rats (Animal Unit, Manchester University) weighing between 200 and 300 g were used. Animals were housed individually after surgery in perspex cages 30 × 30 × 21 cm in a constant lighting regime (14 h light, 10 h dark) with free access to food and water. Bilateral ovariectomy was carried out within 48 h of delivery under ether anaesthesia. A small latex balloon, made from a surgical finger cot (Type B, LRC Industries, London) attached to pp50 polythene tubing (Portex Ltd, Hythe, Kent) was implanted in the right uterine horn. The balloon was expanded with 0.2 ml distilled water and the catheter sealed with a stainless steel pin. The left jugular vein and left carotid artery were cannulated using pp50 polythene tubing. All three cannulae were exteriorized at the back of the neck and were protected for the first 10 cm by a lightweight coil of stainless steel wire. The jugular cannula was filled with sterile 0.9% w/v NaCl solution (saline; Travenol Laboratories Ltd, Thetford, Norfolk) containing 200 iu of heparin (Leo Laboratories Ltd, Princes Risborough, Bucks.) per ml and sealed with a stainless steel pin. The arterial cannula was kept patent by continuous infusion of sterile saline containing 20 iu heparin per ml at a rate of 0.3 ml h^{-1}. The saline was infused through a perspex swivel device based on the design of Weeks & Collins (1964), modified according to Hardy *et al.* (1983), held vertically above the cage thus allowing unrestricted movement of the animal. The pp50 tubing did not prove strong enough to operate the swivel and was, therefore, heat-welded to pp100 tubing shortly after leaving the carotid artery. Forty-eight hours were allowed for recovery from surgery.

Assessment of drug effects

The cannula from the micro-balloon was connected to a pressure transducer (Bell and Howell, Basingstoke, Hants., or Elcomatic, Glasgow). Intra-uterine pressure was recorded continuously on a 4-channel polygraph (Grass instruments, Quincy, Mass.,

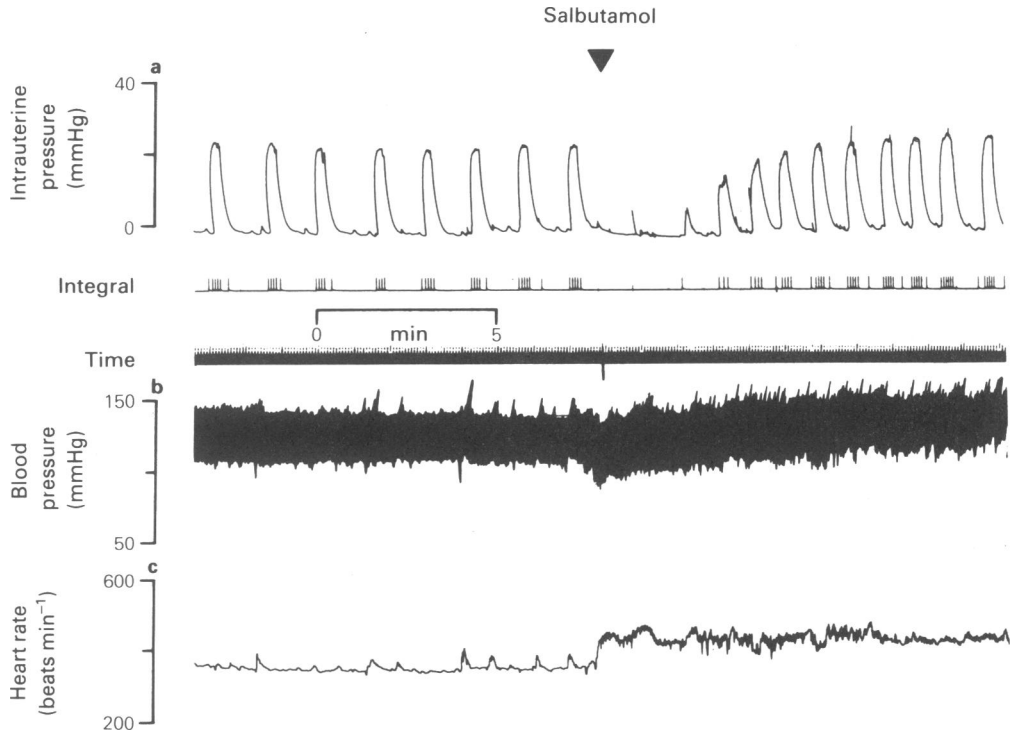

Figure 1 The effect of salbutamol (3.2 μg kg^{-1}) on (a) intra-uterine pressure and its integral, (b) blood pressure and (c) heart rate in the conscious ovariectomized post-partum rat.

U.S.A.) and quantified by the integral of intra-uterine pressure above basal pressure using a 7P10B integrator pre-amplifier. The arterial cannula was connected to a pressure transducer and blood pressure continuously recorded. The signal was taken from the driver amplifier of the channel recording blood pressure and fed into a tachograph pre-amplifier to obtain a continuous record of heart rate.

Drugs were administered by bolus i.v. injection, in ascending dose order at not less than 40 min intervals, one drug per rat. Drug volume was 100 μl, followed by 200 μl of sterile saline. The volume of the venous cannula was approximately 130 μl. Integrals of intra-uterine pressure were counted for the 10 min period immediately after drug injection and expressed as a percentage of the integral for the 10 min before injection. This time period was selected as encompassing the peak effect of all the drugs. Changes in blood pressure were expressed as the maximum fall (in mmHg) and alterations in heart rate as the maximum change (beats min^{-1}) immediately after injection.

Drugs

The following drugs were used: nifedipine (Bayer) verapamil HCl (Knoll), gallopamil HCl (Knoll), (+)-*cis* diltiazem HCl (Synthelabo) and salbutamol sulphate (Glaxo). Stock solutions of verapamil, diltiazem and salbutamol were prepared in sterile saline, kept at 4°C and dilutions made in sterile saline. Stock solutions of gallopamil were prepared in absolute ethanol (Analar, BDH) and subsequent dilutions made in sterile saline. The highest dose administered was in 10% ethanol in saline. Nifedipine solutions were prepared fresh each day in polyethylene glycol: ethanol: water (15:15:50; v:v:v), and dilutions made in the same mixture. Solutions were protected from direct light and all experiments with this compound were carried out under sodium light. Doses are expressed as the base.

Analysis of results

Linear regression, using the least squares method (Armitage, 1971), was performed on % inhibition of intra-uterine pressure and fall in blood pressure against log dose for the linear part of the relationship for each drug. These were used to calculate the dose to produce 60% of control intra-uterine pressure and 30 mmHg fall in mean blood pressure. The dose to produce half the maximum increase in heart rate was also determined. These measurements were used for comparison as they were approximately in the middle of the linear portions of the dose-response curves. The results are expressed as means ± 95% confidence limits.

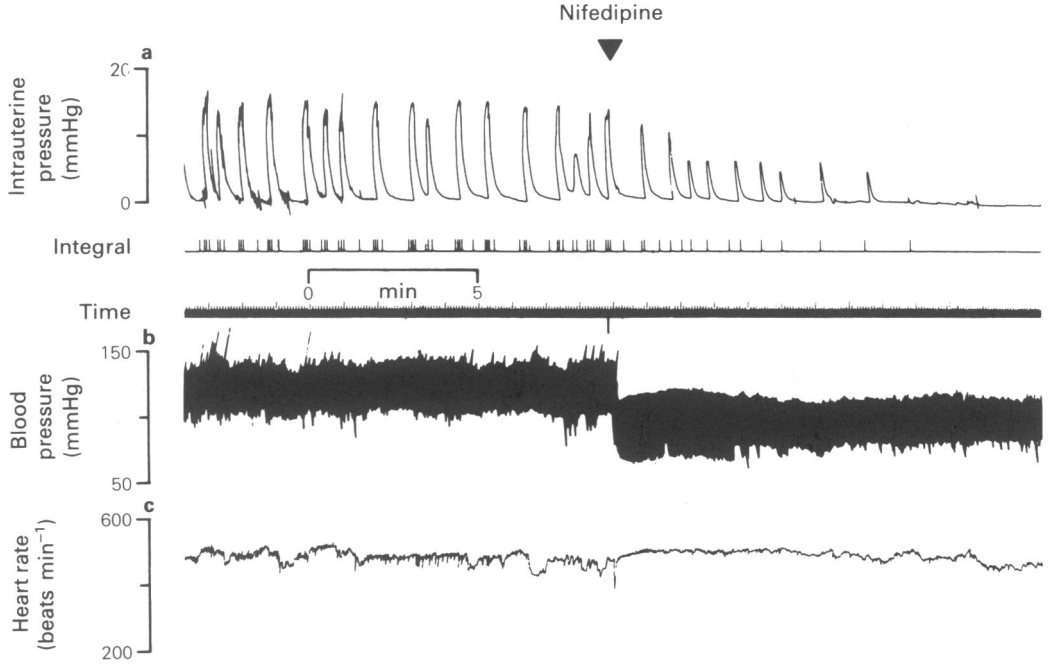

Figure 2 The effect of nifedipine (200 μg kg^{-1}) on (a) intra-uterine pressure and its integral, (b) blood pressure and (c) heart rate in the conscious ovariectomized post-partum rat.

Results

The mean blood pressure and heart rate before drug administration were 117.3 ± 1.5 mmHg and 385.3 ± 5.2 beats min^{-1} (mean ± s.e.mean, $n = 49$) respectively. Mean amplitude of intra-uterine pressure was 21.6 ± 1.3 mmHg ($n = 46$) in these animals. In five rats which received seven consecutive injections of saline at 40 min intervals there was no significant change in blood pressure, heart rate or intra-uterine pressure. In five rats given bolus injections of increasing concentrations of ethanol in saline, no significant change in the above parameters was seen until 0.1 ml of 50% ethanol in saline was reached. This induced a bradycardia, associated with a fall in blood pressure at higher doses.

The effects of salbutamol and two of the calcium antagonists on intra-uterine pressure, blood pressure and heart rate are shown in Figures 1, 2 and 3 and the dose-response curves for salbutamol and the four calcium antagonists in Figures 4, 5 and 6. Salbutamol produced a dose-dependent inhibition of uterine contractions with 60% inhibition occurring at a dose of 12 $\mu g\ kg^{-1}$. The onset of the effect of salbutamol on the uterus was rapid and the offset was often associated with an increased frequency and amplitude of intra-uterine pressure cycles (Figure 1). Salbutamol induced a moderate fall in blood pressure (Figure 5) but a marked tachycardia with an increase of 84 ± 21 beats-min^{-1} at a dose of 25 $\mu g\ kg^{-1}$ (Figure 6). The four calcium antagonists also produced a dose-dependent inhibition of uterine contractions. The rank order of potency was nifedipine > diltiazem = gallopamil > verapamil (Figure 4, Table 1). Their onset of action on the uterus was slower than that of salbutamol and there was less rebound associated with the offset of their action. These drugs also induced a fall in blood pressure but here the rank order was nifedipine > gallopamil > verapamil > diltiazem (Figure 5, Table 1). The slope of the curve relating fall in mean blood pressure to dose was shallower for salbutamol than for the calcium antagonists (Figure 5). Nifedipine resembled salbutamol in producing a dose-dependent tachycardia but the maximum increase (in beats min^{-1}) was significantly less ($2P < 0.05$) than that seen with salbutamol at doses which produced a similar inhibition of uterine contractions. Gallopamil, verapamil and diltiazem also produced a small tachycardia at low doses but in contrast to nifedipine a bradycardia and a temporary cessation of the recording of heart rate occurred for up to 20 s at high doses. This was most evident with gallopamil, occurring in 1/6 animals at a dose of 400 $\mu g\ kg^{-1}$ and 4/5 animals at a dose of 800 $\mu g\ kg^{-1}$. Salbutamol was the most potent chronotropic agent; the calcium antagonists were less potent and of a similar potency to each other (Table 1).

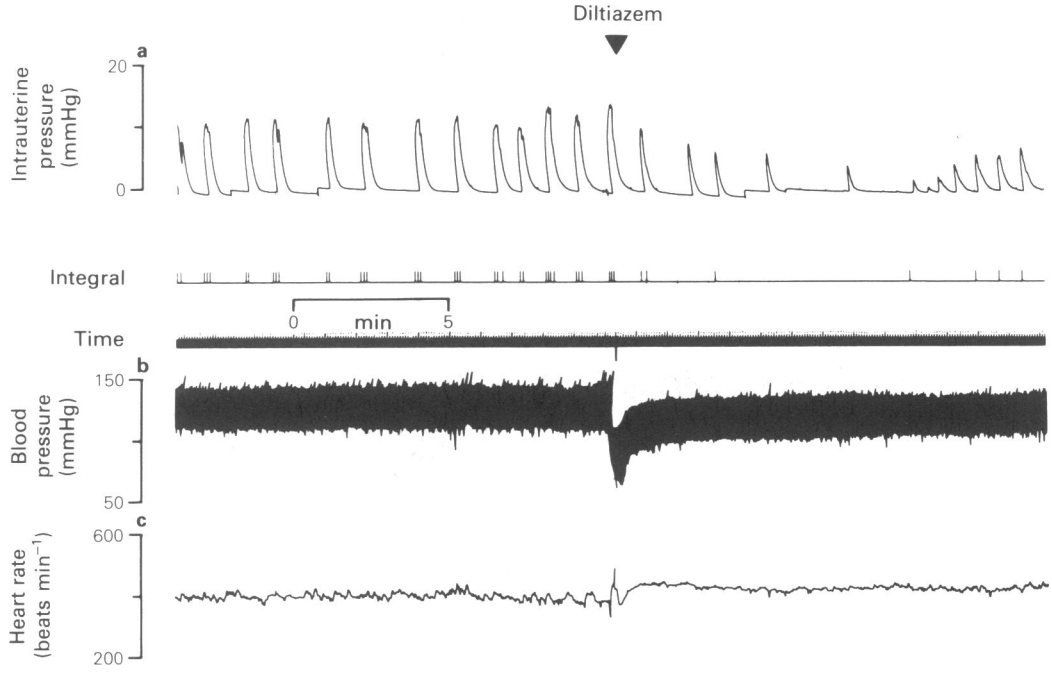

Figure 3 The effect of diltiazem (2 mg kg^{-1}) on (a) intra-uterine pressure and its integral, (b) blood pressure and (c) heart rate in the conscious ovariectomized post-partum rat.

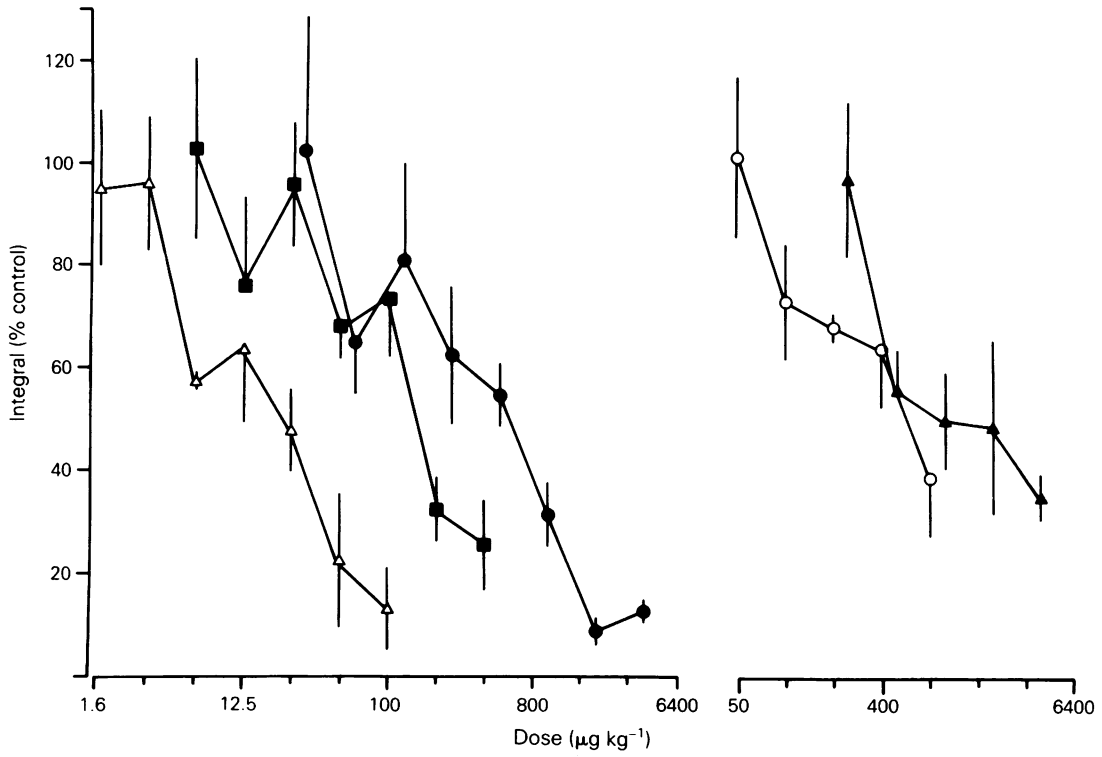

Figure 4 Inhibition of intra-uterine pressure by salbutamol (△), nifedipine (■), diltiazem (●), gallopamil (○) and verapamil (▲). The ordinate scale is the integral of intra-uterine pressure in the 10 min period after drug injections as a % of that in the 10 min before injection. For salbutamol, nifedipine, gallopamil and verapamil, each point is the mean of observations from 4–7 animals and for diltiazem 7–10 animals. The vertical lines denote s.e.mean.

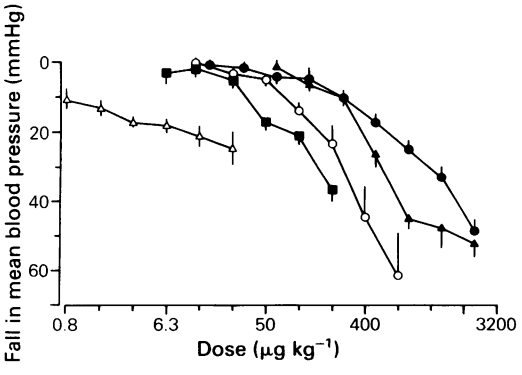

Figure 5 Fall in mean blood pressure produced by salbutamol (△), nifedipine (■), gallopamil (○), verapamil (▲) and diltiazem (●). For salbutamol, nifedipine, gallopamil and verapamil, each point is the mean of observations from 4–7 animals and for diltiazem, 5–11 animals. The vertical lines denote s.e.mean.

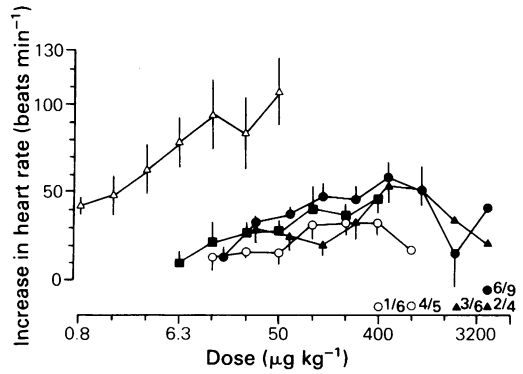

Figure 6 Change in heart rate produced by salbutamol (△), nifedipine (■), gallopamil (○), verapamil (▲) and diltiazem (●). For salbutamol, nifedipine, gallopamil and verapamil, each point is the mean of observations from 3–7 animals and for diltiazem, 5–11 animals. The vertical lines denote s.e.mean. The symbols on the abscissa scale indicates the number of animals from the group in which there was temporary cessation of recording of heart rate at the dose shown.

Table 1 Potencies of salbutamol and four calcium antagonists on intra-uterine pressure, blood pressure and heart rate of the ovariectomized, post-partum rat

Drug	n	*60% of control intra-uterine pressure*	*30 mmHg fall in blood pressure*	*50% of maximum increase in heart rate*
Salbutamol	7	12 (7–18)	74 (30–589)	2
Nifedipine	7	98 (66–145)	178 (138–251)	15
Diltiazem	13	302 (166–467)	1230 (1000–1549)	28
Gallopamil	6	324 (182–933)	204 (145–282)	22
Verapamil	7	832 (372–1622)	661 (457–851)	49

Results show mean doses ($\mu g\,kg^{-1}$) needed to produce each effect; 95% confidence limits are given in parentheses. For conversion of doses to $\mu mol\,kg^{-1}$ divide by the following molecular weights of the base – salbutamol 239 daltons, nifedipine 347 daltons, diltiazem 414.5 daltons, gallopamil 484.6 daltons, verapamil 454.4 daltons.

Discussion

All four calcium antagonists produced a dose-dependent inhibition of intra-uterine pressure in the conscious ovariectomized post-partum rat. The regular and sustained pressure cycles which develop in this animal after removal of the ovaries make it a useful model for testing such compounds. Nifedipine was the most potent of the calcium antagonists, confirming the findings *in vitro* in which uterine strips from day 22 pregnant rats were used (Granger *et al.*, 1985). Diltiazem resembled nifedipine in being able to inhibit totally uterine contractions at high doses whereas the dose of verapamil, and to a greater extent gallopamil, that could be given was limited by their effects on the cardiovascular system. The present studies provide no evidence for any specific mechanism of action by which the calcium antagonists inhibit uterine contractions *in vivo*. However, in view of their high potency it is likely that their action involves inhibition of Ca^{2+} entry into the myometrium, as has been described for other smooth muscles (Nayler & Horowitz, 1983).

Nifedipine was again the most potent of the calcium antagonists as a vasodepressor, and the rank order of potency of the compounds was found to be in line with the observations of Spedding (1982b) in the angiotensin-infused pithed rat. All four calcium antagonists produced a tachycardia but this was less than that seen with the β-adrenoceptor agonist, salbutamol. The tachycardia with salbutamol presumably results from a direct action on the sino-atrial node together with a reflex increase in sympathetic drive resulting from the fall in blood pressure, whereas with the calcium antagonists only the latter mechanism will be operative as they have either no action or a negative chronotropic effect on the heart *in vitro* (Nayler & Horowitz, 1983). The temporary cessation of recording of heart rate at high doses of gallopamil, verapamil and diltiazem probably reflects their ability to inhibit conduction in the atrio-ventricular node (Narimatsu & Taira, 1976; Spedding, 1982b) and could contribute to the observed vasodepression.

Salbutamol is a potent inhibitor of uterine contractions *in vivo* but at the dosage required produces a marked tachycardia and a small fall in blood pressure (Hollingsworth & Schnieden, 1973; Liggins & Vaughan, 1973; current studies) and so exhibits only a moderate selectivity for the uterus. Verapamil and gallopamil produced heart block and a marked vasodepression at the doses necessary to inhibit uterine contractions and therefore exhibited no selectivity for the uterus. Nifedipine and diltiazem at doses which produced inhibition of uterine contractions comparable to that seen with salbutamol induced a smaller tachycardia but a greater fall in blood pressure than that seen with the β-adrenoceptor agonist. There is, therefore, the promise of some selectivity for the uterus within the calcium antagonists. Differences between the calcium antagonists in their tissue selectivities could reflect differences in their disposition or their sites of action within the myometrium. The high potency of nifedipine on the uterus parallels its high potency on other smooth muscles and reflects its specificity for these tissues relative to the myocardium (Narimatsu & Taira, 1976). Although diltiazem is at present grouped with the hydrophilic calcium antagonists verapamil and gallopamil (Spedding, 1982a,b), in contrast to these drugs it appears to show some selectivity for the uterus.

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