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Effect of calcium and calmodulin antagonists on contractile responses of the human uterine artery

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1 The dependence on extracellular calcium of contractile responses of intramyometrial arteries (0.5–2 mm diameter), as well as the effects of various types of calcium antagonists on these responses, were studied. Contractions were induced by K-depolarization (K) and noradrenaline (NA).

2 Whereas the K response was completely abolished in a calcium-free medium containing 2 mM $LaCl_3$, the NA response was substantially maintained.

3 Nimodipine strongly inhibited the K response but had a relatively weak effect on the NA response; the IC_{50} values for the K and NA responses being 2 nM and 6 μM, respectively. Corresponding values for verapamil were about 0.7 and 10 μM.

4 Calmodulin antagonists, particularly trifluoperazine and flunarizine, caused a greater inhibition of the NA than of the K response.

5 These results indicate that besides the extracellular calcium which appears to be the major source of activator calcium, there is an intracellular pool of calcium which can be utilized to activate, albeit to a limited extent, drug-induced contractile responses.

Introduction

Vasoconstriction in the uterine vascular bed is considered to be an important factor in the development of dysmenorrhea. The control of uterine vascular dynamics and contractility is modulated by a number of factors including steroids and biogenic amines. Although the underlying mechanism of the modulatory processes is not known, the final determinant of contractile activity in smooth muscle cells appears to be the intracellular level of free calcium ions. An increase in the intracellular concentration of calcium may be achieved by an accelerated entry of extracellular calcium or increased calcium release from intracellular storage sites (Daniel & Janis, 1975; Batra, 1977).

It has been proposed that calcium enters the smooth muscle cell upon stimulation by depolarization and by agonists through separate channels, the so-called voltage-dependent channels (VDC) and receptor operated channels (ROC), respectively (Bolton, 1979). Various organic compounds with a considerable structural diversity are known to block the entry of extracellular calcium into the cell. Some of these and related compounds bind to calmodulin and thereby interfere with calmodulin participation in the activation of contractile proteins. Calmodulin forms an integral part in the calcium-dependent activation of the contractile appartus within the smooth muscle cells (Kerrick *et al.*, 1980; Hartshorne & Siemankowski, 1981).

In this work we examined the effects of both calcium entry blockers and calmodulin antagonists on contractile activity of the intramyometrial arteries. In order to investigate the possibility of different pathways of calcium entry, the smooth muscle of the uterine artery was stimulated by both K-depolarization and noradrenaline (NA). Different types of calcium antagonists were used to inhibit the contractile response. Some of the agents used, such as nimodipine and verapamil, are known to act primarily by blocking the entry of extracellular calcium, whereas drugs like prenylamine, flunarizine and trifluoperazine are considered to act intracellularly by antagonizing the calcium and calmodulin interaction

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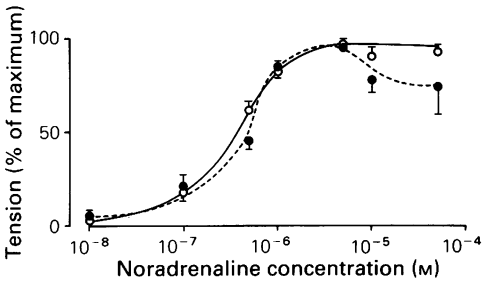

Figure 1 Concentration-response curve for noradrenaline in small (●) and large (○) uterine arteries. The diameter of small arteries was 0.5–1 mm, whereas for large arteries it was 1.3–2 mm. Each point is the mean of at least five determinations.

(Johnson & Fugman, 1983; Kreye *et al.*, 1983; Spedding, 1984; Batra, 1985).

Methods

Pieces of uterine artery as well as ascending branches of uterine artery were obtained from non-pregnant premenopausal women undergoing hysterectomy. The excised tissue was immediately placed in oxygenated Tyrode solution at 2°C and transported to the laboratory. Under a dissecting microscope, vessels were cut first helically and strips (6 mm long and 1 mm wide) were obtained. The strips were then mounted in an organ bath containing 20 ml of physiological Na-HEPES solution (composition mM: NaCl 135, KCl 4.6, $MgCl_2$ 1.2, $CaCl_2$ 1.5, glucose 11, HEPES 10) maintained at 37°C and bubbled with 100% O_2. The pH of the Na-HEPES solution was adjusted to 7.4 by titration with NaOH. A preincubation period of 45–60 min was used for accommodation of the tissue. Isometric contractions were recorded at a resting tension of 3 mN as described in detail previously (Laudanski *et al.*, 1984).

Contractile responses induced by K-depolarization (K) and noradrenaline (NA) were studied in strips from the same vessel.

Depolarization was induced by elevating the KCl concentration to 80 mM while removing an equimolar amount of NaCl in Na-HEPES solution. The concentration of NA was 5×10^{-5} M, which was found to give a maximum response. The contact time with the agonists was 10–15 min. After recording a control response, the strips were washed 3 times with Na-HEPES solution. The strips were then exposed to antagonists for 10 min and responses recorded again. This process was repeated with successively increasing concentrations of the antagonist in the bathing medium.

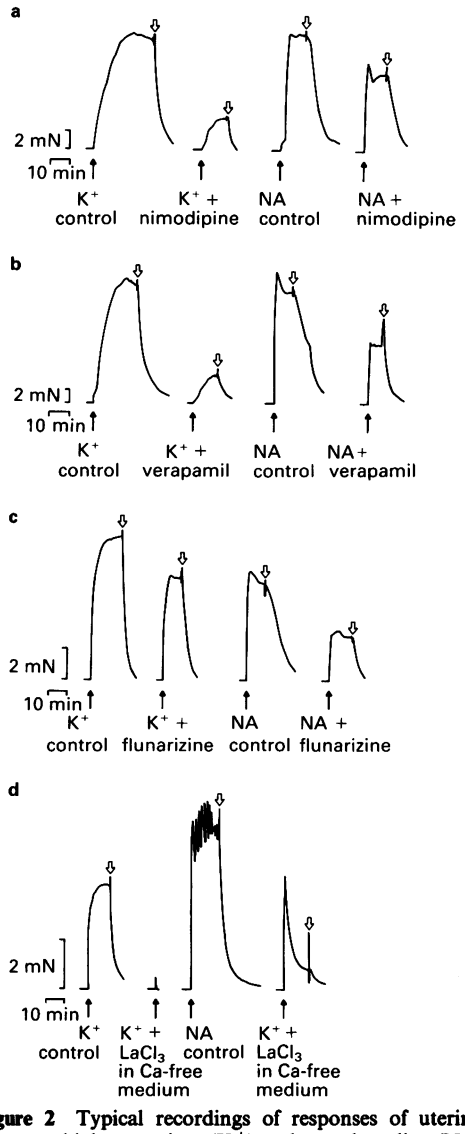

Figure 2 Typical recordings of responses of uterine artery to high potassium (K^+) and noradrenaline (NA) and their inhibition following exposure to various antagonists: (a) nimodipine (10^{-7} M), (b) verapamil (10^{-5} M), (c) flunarizine (2×10^{-6} M) and (d) 2 mM $LaCl_3$ in Ca-free medium. An exposure period of 10 min was allowed with each antagonist. Arrows on top represent washout with Na-HEPES solution.

The effect of test substances was evaluated by comparing control responses (set as 100%) with the experimental preparation. The mean values ± s.e. mean were calculated for each test group. The significance level for any differences (P-value) was determined by use of the Mann-Whitney U-test.

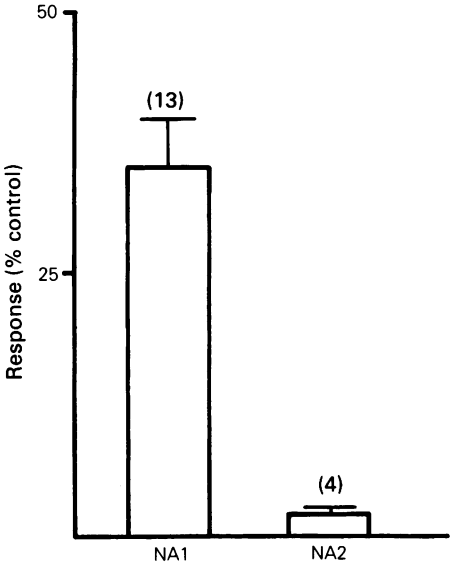

Figure 3 Reduction of the contractile response to noradrenaline (NA) in Ca-free lanthanum-containing medium. The first response was obtained after a 10 min exposure and the second after an additional 10 min in the above medium. Columns represent mean values and vertical lines s.e.mean of the number of determinations indicated within parentheses.

Drugs

Nimodipine, verapamil, fendiline, trifluoperazine, prenylamine and flunarizine, were obtained from Bayer (Sweden), Knoll (W. Germany), Hoechst (W. Germany), Thiemann (W. Germany), Leo Rhodia (Sweden) and Jansson (Belgium), respectively, Diethylstilboestrol was purchased from Sigma Chemical Co.

Results

Potassium depolarization induced a contraction characterized by an initial rapid rise in tension followed by a decrease in tension to a certain plateau which was generally sustained until the agonist was washed out. Phentolamine (10^{-6} M) occasionally caused 5–10% inhibition of the K response. A similar pattern of contraction was observed with NA except that the initial rise in tension was faster than that observed with the K response and the maximum response was somewhat higher than that of the K response. A comparison between the responses obtained by NA in small (diameter 0.5–1 mm) and large (diameter 1.3–2 mm) arteries is shown in Figure 1. The dose-response curves were similar and the concentration of NA giving maximum response was around 10^{-5} M in both cases. Data from both small and large arteries were therefore pooled.

Figure 2 shows some typical responses to K and NA, and inhibition thereof by nimodipine, verapamil, flunarizine and lanthanum. Whereas nimodipine caused considerably greater inhibition of the K response than the NA response, flunarizine had the opposite effect.

Lanthanum in Ca-free solution almost completely inhibited the K response, but caused only 60–70% inhibition of the NA-induced contraction. When NA was applied after an additional 10 min exposure to lanthanum-containing Ca-free solution, practically no response could be obtained with NA (Figure 3).

The dose-response curves for the inhibition by various compounds of both the K and NA responses are shown in Figure 4. Increasing concentrations of nimodipine caused a successive inhibition of the K response, reducing it to less than 10% at a concentration of 10^{-7} M. The NA response on the other hand was inhibited by about 50% with a concentration as high as 10^{-5} M (Figure 4a). Inhibition by verapamil reached close to 100% for both the K and NA responses, but the NA response was less sensitive to the blocking action of verapamil (Figure 4b). The calmodulin antagonists, trifluoperazine and flunarizine caused a greater inhibition of the NA response than of the K response. The effect of diethylstilboestrol was also tested. Although it caused a greater inhibition of the K response than of the NA response, the difference was not statistically significant. The calculated IC_{50} values from the curves are presented in Table 1. Nimodipine was the most potent inhibitor as far as the response to K was concerned, the IC_{50} being about 2 nM. The corresponding value for the NA response was 6 μM. Verapamil also had considerably lower IC_{50} value (about 15 fold) for the K responses than for the NA responses. The mean IC_{50} for the effect of fendiline on the K-induced response was one half of that for the NA-induced response and the difference was statistically significant (Table 1). The difference in the IC_{50} values for prenylamine for the two responses was not significant. Although both trifluoperazine and flunarizine caused a greater inhibition of the NA response than of the K response, the IC_{50} value for trifluoperazine only was significantly higher for the K response than for the NA response ($P < 0.01$).

Discussion

Since the contraction-relaxation cycle of smooth muscle is regulated by the level of free myoplasmic Ca and since Ca entry blockers reduce the amount

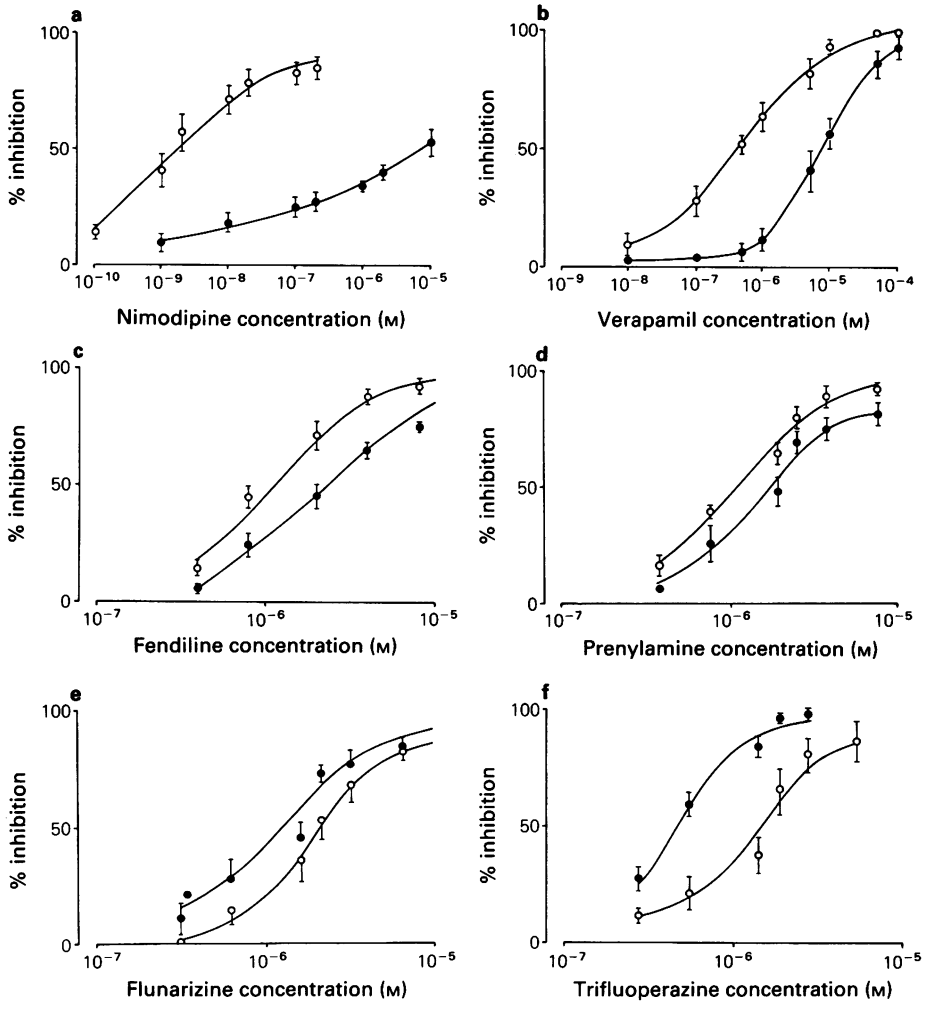

Figure 4 Dose-response curves for the inhibition by (a) nimodipine, (b) verapamil, (c) fendiline, (d) prenylamine, (e) flunarizine and (f) trifluoperazine of the contractile responses to K^+ (○) and noradrenaline (NA; ●) in human uterine arteries. Values are means of from 4–10 experiments; vertical lines indicate s.e.mean.

of Ca reaching the interior of the cells, they have been found to be highly effective inhibitors of contractile activity. Calmodulin antagonists on the other hand cause relaxation of the smooth muscle primarily by inhibiting the interaction of Ca and calmodulin. The inhibition of contracile activity of smooth muscle can therefore, in principle be brought about by both types of agents (Johnson & Fugman, 1983; Spedding, 1983).

Our data showed that nimodipine was highly potent in inhibiting contractile activity induced by K-depolarization (IC_{50} = 2.3 nM) but much less effective, even in high concentrations, in inhibiting the NA response (IC_{50} = 5.7 μM). These findings are in agreement with the recent data of Maigaard *et al.* (1985) showing an insignificant inhibition of the NA response in myometrial arteries with as high as 10^{-7} M nifedipine. A similar concentration of nimodipine in the present study had no effect on the NA response, whereas the K response was completely inhibited. A similar but less dramatic difference was also observed for verapamil in the inhibition of the K and NA responses.

It has been previously shown that drugs inducing a contraction in vascular smooth muscle may, in addition to opening receptor-operated channels release Ca from intracellular stores (Deth & van Breemen, 1977; Deth & Lynch, 1981; Godfraind &

Table 1 Inhibition by various drugs of the contractile response induced by noradrenaline (NA) and high potassium (K)

Drug	(n)	IC_{50} values (M) NA	(n)	K
Nimodipine	(4)	$5.7 \pm 1.7 \times 10^{-6}$	(6)	$2.3 \pm 1.1 \times 10^{-9}$**
Verapamil	(7)	$9.7 \pm 2.5 \times 10^{-6}$	(6)	$7.2 \pm 3.2 \times 10^{-7}$**
Trifluoperazine	(10)	$5.6 \pm 1.0 \times 10^{-7}$	(7)	$1.7 \pm 0.3 \times 10^{-6}$**
Fendiline	(6)	$2.2 \pm 0.4 \times 10^{-6}$	(6)	$1.1 \pm 0.2 \times 10^{-6}$**
Prenylamine	(7)	$1.8 \pm 0.2 \times 10^{-6}$	(6)	$1.2 \pm 0.2 \times 10^{-6}$
Flunarizine	(8)	$1.6 \pm 0.3 \times 10^{-6}$	(9)	$2.4 \pm 0.5 \times 10^{-6}$
Diethylstilboestrol	(7)	$1.4 \pm 0.5 \times 10^{-5}$	(6)	$6.0 \pm 2.3 \times 10^{-6}$

The IC_{50} is the concentration causing 50% inhibition of the respective response. Values are means ± s.e.mean of the number of experiments indicated within parentheses. Significance of difference between NA and K is indicated by $*P < 0.05$, $**P < 0.01$.

Dieu, 1981; Godfraind *et al.*, 1983). The extent of the release of intracellular Ca probably varies greatly between different types of smooth muscle (Godfraind & Dieu, 1981; Cauvin *et al.*, 1983). In the present study a Ca-free medium containing lanthanum was used to see whether intracellular Ca participates in the drug-induced response. Lanthanum had previously been shown to block all influx of Ca. The data showed that the response to K but not that to NA was completely inhibited in a medium lacking Ca but containing lanthanum. This observation strongly argues for an intracellular Ca pool which can be liberated by NA, as has previously been shown for arterial smooth muscle (Deth & van Breemen, 1977; Deth & Lynch, 1981; Godfraind & Dieu, 1981; Godfraind *et al.*, 1983). Recent data of Maigaard *et al.* (1985) on myometrial arteries appear to support this view.

Our data on the inhibition by calmodulin antagonists indicate that flunarizine and trifluoperazine most probably act by inhibiting the formation of the Ca and calmodulin complex rather than blocking Ca entry, because they caused significantly greater inhibition of the NA response than of the K response. Furthermore, the apparent dissociation constant of binding of these drugs to calmodulin (Johnson & Fugman, 1983) and the IC_{50} value of flunarizine for inhibition of the calmodulin-dependent phosphodiesterase complex (Lugnier *et al.*, 1984) were very similar to the IC_{50} value for the inhibition of contraction found here. A recent study by Spedding (1983) showed that trifluoperazine caused a significant inhibition of a skinned taenia preparation but not of an intact preparation indicating an intracellular site of action. Prenylamine may have a mixed action, since in the present study we found no significant difference in the IC_{50} values for the inhibition the K and NA responses.

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