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Influence of extracellular calcium and calcium antagonists on contractions induced by potassium and prostaglandin $F_{2\alpha}$ in isolated cerebral and mesenteric arteries of the cat

K.-E. Andersson, L. Edvinsson, E.T. MacKenzie*, T. Skärby & A.R. Young*

Department of Clinical Pharmacology, University Hospital of Lund, S-221 85 Lund, Sweden and Cerebral Circulation and Metabolism Group*, LERS-Synthélabo, 31, ave P.V. Couturier, 92220-Bagneux, France

1 The effects of a number of calcium antagonists (diltiazem, nifedipine, nimodipine and verapamil) have been studied on feline isolated pial arteries contracted by potassium (127 mM) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 2.5 μM) and mesenteric arteries contracted by potassium (127 mM).

2 Withdrawal of Ca^{2+} from the extracellular medium for 30 min reduced the contractile response to potassium in cerebral vessels by 92% and in mesenteric vessels by 96%. Subsequent addition of Ca^{2+} caused reproducible contractions which were inhibited by both nifedipine and nimodipine.

3 The four calcium antagonists relaxed the isolated middle cerebral artery contracted either by potassium or $PGF_{2\alpha}$, and mesenteric arteries contracted by potassium, in the following order of potency: nimodipine > nifedipine > verapamil > diltiazem.

4 Nimodipine was more potent than nifedipine in cerebral arteries, and more potent in cerebral than in mesenteric arteries. Otherwise, the potassium-contracted cerebral and mesenteric vessels showed no major differences in sensitivity to calcium antagonists.

Introduction

Cerebrovascular smooth muscle tone is regulated by a complex system of input mechanisms by vasomotor nerves, hormones and local physical and chemical factors, all of which have been thoroughly described for the peripheral circulation (Johansson, 1978). These factors implicate a final common step in the process linking membrane excitation and contraction; namely, the concentration of calcium ions in the vicinity of the contractile proteins. This calcium ion concentration determines the degree of activation of the vascular smooth muscle cells. Many factors may trigger the release of calcium ions from intracellular stores (such as mitochondria, sarcoplasmic reticulum) and/or increase the calcium ion influx from the extracellular medium through specific membrane channels (Bolton, 1979).

Several approaches have been used to examine these fundamental processes and to estimate the relative importance of different calcium pools for the mechanism of activation. Among the tools used are different antagonists of the calcium involved in the excitation-contraction coupling (Fleckenstein, 1977). Although some reports have demonstrated the necessity of extracellular calcium for cerebrovascular muscle contraction (Betz & Csornai, 1978; Toda, 1976), knowledge of the role of extracellular calcium for activation and relaxation in the vascular bed is still limited.

In the present study, we have examined the influence of variations in extracellular calcium concentration on the contractions induced by either potassium or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in feline pial or mesenteric vessels. Furthermore, the action of four different calcium antagonists was examined and compared.

Methods

Preparation and mounting

Cats of either sex were anaesthetized with sodium pentobarbitone (30 mg/kg, i.p.) and killed by exsanguination and decapitation. The skull was opened, the brain removed, placed in a Petri dish and soaked in a cold Krebs buffer solution. The middle cerebral arteries were subsequently removed and placed in a glass beaker containing the same buffer. Immediately after, a part of the mesentery, containing arteries of

similar diameter to the cerebral vessels, was removed. With the aid of an operating microscope, arterial segments were dissected free and placed in a glass beaker containing the cold buffer solution. Part of the material was used immediately in the experiments, while the rest was stored in a refrigerator (+4°C) for up to 24 h. There was no difference in response to potassium or $PGF_{2\alpha}$ between fresh and day-old arteries.

Vessel segments (300–400 μm in diameter, 2–4 mm long) were mounted in 5 ml temperature-controlled tissue baths (one segment in each bath) containing a system of L-shaped metal holders for recording isometric circular contractions (Högestätt, Andersson & Edvinsson, 1983). Contractions were measured by means of Grass FT-03 transducers, amplified and recorded on a Grass polygraph.

The baths contained a Krebs buffer solution of the following composition (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, NaH_2PO_4 1.2 and glucose 11.0. A solution with a high potassium content (127 mM) was obtained by replacing NaCl with equivalent amounts of KCl. In experiments involving a calcium-free solution, $CaCl_2$ was omitted from the normal Krebs solution and EGTA 10 μM added. The bath and stock solutions were kept at 37°C and aerated continuously with a mixture of 95% O_2 and 5% CO_2 to maintain a pH of 7.4. Chemicals used were of analytical grade.

Experimental procedure

Shortly after the arterial preparations had been mounted in the organ baths, the vessels were subjected to a load of 5 mN. Test drugs were administered when a stable base line was reached, usually after 60 to 90 min. Contractions were induced by potassium (127 mM), resulting in a response which was stable for more than 30 min.

In some experiments the vessels were contracted by the addition of $PGF_{2\alpha}$ (2.5 μM) which produced a response that was stable for at least 30 min. The calcium antagonists were added to the organ baths in a cumulative manner in concentrations ranging from 0.1 nM to 100 μM. Subsequently, log concentration-response curves were constructed from the data obtained. Calculations were made of the IC_{50} value (the concentration of drug producing half maximum inhibition) and the I_{max} value (the maximum inhibitory response produced, expressed as a percentage of the stable contraction induced by either $PGF_{2\alpha}$ or a high concentration of potassium). Vessels which did not respond to potassium or $PGF_{2\alpha}$ with reproducible contractions were rejected.

Drugs

The following drugs were used: diltiazem (Tanabe); nifedipine (Bayer); nimodipine (Bayer); verapamil (Knoll); and $PGF_{2\alpha}$ (Amoglandin, Astra). These substances were dissolved in 0.9% w/v NaCl solution (saline). All concentrations are expressed as the final molar concentrations in the bath. Nifedipine and nimodipine were kept in a dark environment due to the possibility of light-induced decomposition.

Results

Influence of extracellular calcium

The contractile responses to potassium of both pial and mesenteric vessels were concentration-dependent and reproducible in the standard Krebs solution. The threshold concentration for contraction was approximately 15–20 mM in cerebral vessels and 5–10 mM higher in mesenteric arteries; contraction was almost maximal at 60 mM for both types of vessel. Exposure of cerebral and mesenteric arteries to a calcium-free Krebs solution for 30 min reduced the contractile effect of potassium (127 mM) to 8±2% and 4±1% of the controls respectively. The vessel segments contracted promptly upon re-administration of calcium; this response again being concentration-related with the maximum effect at 4 mM calcium (Figure 1a and 1b).

In the absence of extracellular calcium, nifedipine (0.3 μM) and nimodipine (0.21 μM) respectively caused a further decrease in the response induced by potassium to 3±1% and 2±1% in cerebral arteries ($P<0.001$; Student's t test), and that of mesenteric arteries to 0%. These two calcium antagonists markedly inhibited the contraction produced by re-introducing calcium into the extracellular medium (Figures 1a and b).

Effects of calcium antagonists

Cerebral arteries: The four calcium antagonists relaxed pial arteries, contracted by either 2.5 μM $PGF_{2\alpha}$ or 127 mM potassium, in the following order of potency: nimodipine > nifedipine > verapamil > diltiazem (Table 1). Vessels contracted either by $PGF_{2\alpha}$ or potassium were almost completely relaxed (Figures 2a and b). There was a slight, but statistically significant difference ($P<0.05$) in potency between nifedipine and nimodipine in relaxing potassium-contracted arteries, the latter being approximately four times more potent (Table 1). However, the IC_{50} value for relaxation of $PGF_{2\alpha}$-contracted vessels by nimodipine was about 10 times lower ($P<0.001$) than that of nifedipine (Table 1). The IC_{50} values for the calcium antagonists to relax the arteries were otherwise similar whether the vessels had been contracted by potassium or by $PGF_{2\alpha}$ (Table 1).

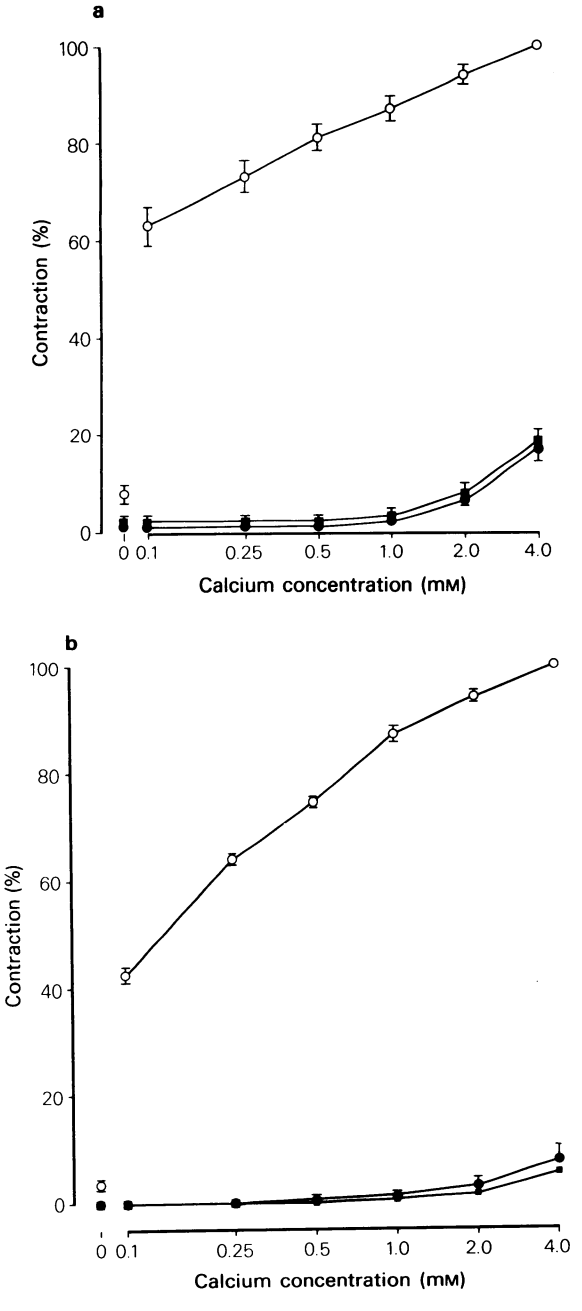

Figure 1 Responses of feline isolated (a) middle cerebral and (b) mesenteric arteries to increasing concentrations of calcium after exposure to a Krebs solution containing zero calcium. Data are given as percentage of maximum. Potassium (127 mM) was added in the absence of drug (○), and in the presence of nifedipine (0.3 μM,●), or nimodipine (0.21 μM, ■). Each point represents the mean value of 6 determinations; vertical lines show s.e.mean.

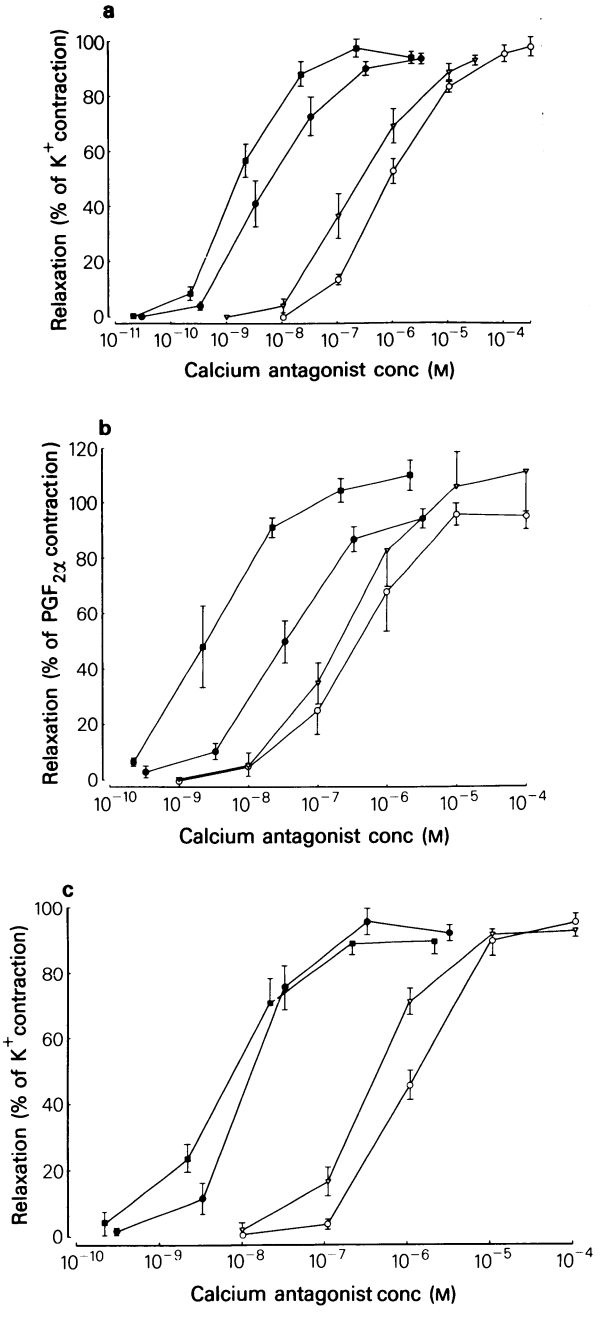

Figure 2 Inhibitory effect of nimodipine (■), nifedipine (●), verapamil (△) and diltiazem (○) each added cumulatively, on the contractile responses to (a) potassium (127 mM) or (b) prostaglandin $F_{2\alpha}$ (2.5 μM) on feline isolated meddle cerebral arteries and (c) on feline mesenteric arteries contracted by 127 mM potassium. Each point represents the mean value of 5 to 12 determinations; vertical lines show s.e.mean. For further details see Tables 1 and 2.

Table 1 Relaxation of isolated middle cerebral arteries of the cat by four different calcium antagonists

		Potassium-contracted vessels			*$PGF_{2\alpha}$-contracted vessels*	
Agent	*n*	IC_{50} (M)	I_{max} (%)	*n*	IC_{50} (M)	I_{max} (%)
Nimodipine	8	$1.9 \pm 1.5 \times 10^{-9}$	97 ± 3	7	$2.9 \pm 1.3 \times 10^{-9}$	104 ± 4
Nifedipine	8	$7.2 \pm 2.3 \times 10^{-9}$	93 ± 2	9	$2.8 \pm 1.2 \times 10^{-8}$	95 ± 3
Verapamil	5	$2.0 \pm 1.7 \times 10^{-7}$	93 ± 2	6	$3.3 \pm 1.5 \times 10^{-7}$	111 ± 18
Diltiazem	6	$1.1 \pm 1.4 \times 10^{-6}$	98 ± 4	8	$3.7 \pm 2.0 \times 10^{-7}$	96 ± 4

Contraction was induced either by 127 mM potassium buffer (9.0 ± 0.8 mN, $n = 26$) or by 2.5 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 7.2 ± 2.3 mN, $n = 30$). Mean values ± s.e.mean are given (n = number of arteries examined). I_{max} is the maximum inhibitory response of the vessel, expressed as a percentage of the induced contraction.

Table 2 Relaxation of mesenteric arteries of the cat contracted by a high potassium (127 mM) buffer solution

Agent	*n*	IC_{50} (M)	I_{max} (%)
Nimodipine	7	$1.0 \pm 2.6 \times 10^{-8}$	90 ± 4
Nifedipine	6	$1.6 \pm 0.2 \times 10^{-8}$	96 ± 4
Verapamil	5	$5.4 \pm 0.8 \times 10^{-7}$	92 ± 2
Diltiazem	5	$2.8 \pm 1.5 \times 10^{-6}$	95 ± 3

The contractions were 20.3 ± 5.3 mN ($n = 23$). I_{max} is the maximum inhibitory response of the vessel, expressed as a percentage of the induced contraction. Mean values ± s.e.mean are given (n = number of arteries examined).

Mesenteric arteries: In mesenteric arteries, potassium induced strong persistent contractions (20.3 ± 1.9 mN) of the vessels. The calcium antagonists relaxed potassium-contracted vessels concentration-dependently with the same order of potency as found in cerebral vessels, *i.e.* nimodipine > nifedipine > verapamil > diltiazem (Figure 2c).

The degree of relaxation and the mean IC_{50} values of the calcium antagonists are given in Table 2. The IC_{50} and I_{max} values are similar to those obtained in pial vessels, with the exception of nimodipine which appeared to have a lower IC_{50} value (Student's t test, $P < 0.001$) in the latter. Contractions induced by $PGF_{2\alpha}$ in mesenteric arteries were not stable enough to allow further analysis.

Discussion

Contractions induced in vascular smooth muscle by a high concentration of potassium are generally believed to be highly dependent on the concentration of extracellular calcium; these contractions are markedly decreased by the removal of calcium from the extracellular milieu (Briggs, 1962; Hinke, 1965; Hudgins & Weiss, 1968; van Breemen, 1969). In the present investigation, the concentration threshold for potassium to produce contraction was about 15 mM. This threshold seems to be lower in human cerebral arteries, where it was found recently to be about 10 mM (Brandt, Andersson, Edvinsson & Ljunggren, 1981). Potassium concentrations as high as 127 mM cause total depolarization of the cell membrane and induce very reproducible contractions.

The contractile response of vascular smooth muscle to potassium consists of two phases: one initial and rapid; the other slow in onset and long-lasting. Both phases are considered to depend on calcium associated with extracellular or surface membrane pools (van Breemen, Farinas, Gerba & McNaughton, 1972; Deth & van Breemen, 1974). Calcium inflow is believed to occur through potential-sensitive channels in the membrane; these channels can be blocked by calcium antagonists (Bolton, 1979).

The present results showed that the responses to potassium in both cerebral and mesenteric vessels were decreased to less than 10% after 30 min in a calcium-free medium (Figure 1). These results illustrate the dependency on extracellular calcium for contraction, emphasised further by the fact that replacement of calcium induces contractions which reach their initial tension value at a concentration of 4 mM. Both nifedipine and nimodipine, as expected,

suppressed the contractile effect caused by reintroducing calcium into the bathing fluid. The small contractions induced by potassium in the absence of calcium were further reduced by nifedipine and nimodipine, suggesting that these drugs not only inhibit the influx of extracellular calcium during the depolarization by potassium, but may also have intracellular effects (Church & Zsotér, 1980). They may attenuate the release of intracellularly bound calcium or interact with, for example, calmodulin (Boström, Ljung, Mårdh, Forsén & Thulin, 1981). Data supporting an additional site of action of calcium antagonists in vascular smooth muscle have been obtained in studies on human mesenteric (Mikkelsen, Andersson & Lederballe Pedersen, 1979) and pial vessels (Brandt *et al.*, 1981). Another calcium antagonist, verapamil, has been shown recently to have its main site of action at the inner side of the cell membrane of rat heart cells, a site different from that of Mn^{2+} (Payet, Schanne, Ruiz-Ceretti & Demers, 1980). Thus, calcium antagonists may well turn out to have several sites of action.

A number of previous studies have demonstrated the contractile effect of $PGF_{2\alpha}$ both in cerebral (Toda, 1976; Edvinsson, Hardebo, McCulloch & Owman, 1978; Uski, Edvinsson & Owman, 1981) and peripheral vessels (Nakano, 1968; Greenberg & Sparks, 1969). The maximum contractile effect of $PGF_{2\alpha}$ corresponds well with that of potassium in pial arteries although a longer time is taken to reach the maximum response. In the present, as well as in previous studies (Edvinsson *et al.*, 1978), the contraction induced by $PGF_{2\alpha}$ on the feline middle cerebral artery remained stable for at least 30 min and this allowed relaxation to be studied. The four calcium antagonists relaxed both potassium- and $PGF_{2\alpha}$-contracted vessels concentration-dependently and completely (Figure 2). This is in contrast to recent findings on human cerebral arteries (Brandt *et al.*, 1981), where potassium-contracted vessels relaxed almost completely, while $PGF_{2\alpha}$-contracted arteries were relaxed by only 60%. Shimizu, Ohta & Toda (1980) have also shown that in dog cerebral arteries, verapamil relaxed potassium-contracted arteries more than $PGF_{2\alpha}$-contracted vessels. This difference may indicate that, in human and dog cerebral vessels but not in feline vessels, the $PGF_{2\alpha}$-induced contractions are less dependent on the influx of extracellular calcium across the arterial smooth muscle cell membrane, than contractions evoked by depolarization of the vessels by potassium.

The potency differences between the calcium antagonists accord with the literature (Fleckenstein, 1977; Mikkelsen *et al.*, 1979; Shimizu *et al.*, 1980). Nimodipine had the highest potency, and diltiazem the lowest; there were no differences between the maximum vasodilator effects obtained. Nimodipine and nifedipine have been suggested as selective calcium antagonists for cerebral vessels (Towart & Kazda, 1980) and have been shown to have greater effects on cerebral than peripheral arteries in the dog (Allen & Banghart, 1979; Shimizu *et al.*, 1980). A significant difference in potency on pial vessels between nimodipine and nifedipine was observed in the present investigation, irrespective of the contractile agent used (potassium or $PGF_{2\alpha}$); nimodipine being 4–10 times more potent than nifedipine. On the other hand, nifedipine had a similar potency in cerebral and mesenteric arteries contracted by potassium and in mesenteric arteries, nifedipine and nimodipine were equipotent. Thus, nimodipine had a certain degree of selectivity for cerebral vessels giving further support to the view that species and regional differences exist in the action and potency of calcium antagonists.

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