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ENDOTHELIUM-DERIVED RELAXING FACTOR ALTERS CALCIUM FLUXES IN RABBIT AORTA: A CYCLIC GUANOSINE MONOPHOSPHATE-MEDIATED EFFECT

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SUMMARY

1. Measurement of tension and ^{45}Ca influx and efflux were used to study the effects of endothelium-derived relaxing factor (EDRF), sodium nitroprusside and 8-bromo-cyclic guanosine monophosphate (GMP) on contractile responses and calcium movements in aortic ring preparations of the rabbit.

2. EDRF activity, induced by stimulating endothelium-containing rings with acetylcholine, was associated with relaxation of noradrenaline-constricted rings and with a marked reduction of noradrenaline-stimulated increase in calcium influx. Sodium nitroprusside and 8-bromo-cyclic GMP had a similar effect in de-endothelialized preparations.

3. EDRF also inhibited noradrenaline-stimulated calcium efflux. Sodium nitroprusside and 8-bromo-cyclic GMP had a similar effect in de-endothelialized preparations, both in the presence and absence of extracellular calcium.

4. The vascular smooth muscle relaxant effect of EDRF and of nitrovasodilators may be effected by a cyclic GMP-mediated reduction of cytosolic calcium, through both inhibition of calcium influx and reduction of intracellular calcium release.

INTRODUCTION

The importance of the endothelium in vascular smooth muscle control has recently been recognized (Furchgott & Zawadzki, 1980). It is now apparent that endothelium produces a potent vasodilator agent which is likely to prove of major importance in mediating and co-ordinating many of the fundamental phenomena of vascular control (Furchgott, 1983; Griffith, Edwards, Collins, Lewis & Henderson, 1985*a*). Although the responsible agent, endothelium-derived relaxing factor (EDRF), has yet to be chemically identified, it is known to be an unstable, novel biological compound which is continually produced in the resting state under experimental conditions (Griffith, Edwards, Lewis, Newby & Henderson, 1984*a*; Griffith, Henderson, Hughes-Edwards & Lewis, 1984*b*) and whose production can be stimulated by a number of physiologically relevant substances (for review see Furchgott, 1983; and Griffith *et al.* 1985*a*).

The vasodilator effects both of EDRF and of nitrovasodilators appear to be mediated by activation of guanylate cyclase (Katsuki, Arnold, Mittal & Murad, 1977; Ignarro, Lippton, Edwards, Baricos, Hyman, Kadowitz & Greutter, 1981;

Rapoport & Murad, 1983; Griffith, Edwards, Lewis & Henderson, 1985*b*; Busse, Trogisch & Bassenge, 1985) and increased levels of cyclic guanosine monophosphate (GMP) in vascular smooth muscle. These in turn have been shown to be associated with reduced phosphorylation of myosin light chains (Rapoport, Draznin & Murad, 1983). Activation of cyclic GMP-dependent protein kinase by cyclic GMP has recently been shown to have no influence on the activity parameters of myosin light chain kinase, however (Hathaway, Konicki & Coolican, 1985). The mode of action of these relaxant agents may thus be primarily through reduction of the level of activating calcium within the cell with secondary dephosphorylation of myosin light chains. Possible mechanisms for reducing cytosolic calcium would be inhibition of calcium influx through one or other of its membrane 'channels' or reduction of intracellular calcium release (Rapoport *et al.* 1983). The influence of nitrovasodilators on calcium fluxes has been the subject of a number of studies (Haeusler & Thorens, 1975; Kreye, Baron, Lüth & Schmidt-Gayk, 1975; Zsoter, Henein & Wolchinsky, 1977; Hester, Weiss & Fry, 1979; Karaki, Hester & Weiss, 1980; Nakazawa & Imai, 1981; Ozaki, Shibata, Kitano, Matsumoto & Ishida, 1981; Itoh, Kuriyama & Ueno, 1983; Karaki, Nakagawa & Urakawa, 1984; Hester, 1985; Matlib, Dube, Millard, Lathrop, Baik, Sakai, Disalvo & Schwartz, 1985) with conflicting findings related possibly to differences in methodology and between different species studied. The influence of EDRF on calcium fluxes has not been studied previously. We have accordingly investigated the effect of EDRF on ^{45}Ca influx and efflux in isolated vascular preparations and compared this with the effect of nitrovasodilators.

METHODS

Artery preparation

Male New Zealand White rabbits (2–2·5 kg) were killed by a blow to the neck. The thoracic aorta was removed and placed in 500 ml of oxygenated (100% O_2), HEPES-buffered physiological saline of the following composition (mM): NaCl, 140; KCl, 4·6; $MgCl_2$, 1; $CaCl_2$, 1·5; HEPES, 5; glucose, 10; pH, 7·3, at 37 °C. Connective tissue and fat were dissected away and the vessel ends trimmed off. The remaining vessel was cut into rings 2–3 mm long. Endothelium was removed from some rings by gently rubbing the intimal surface with filter paper. The presence (>90%) or absence (<5%) of endothelium was confirmed in representative rings by *en face* silver staining (Poole, Sanders & Florey, 1958). Rings were suspended at each end by fine silk thread from a stainless-steel needle for ease of handling. All preparations were equilibrated for 90 min before an experiment.

Measurement of ^{45}Ca influx

Calcium influx was measured by the method of Meisheri and colleagues (Meisheri, Palmer & van Breemen, 1980). Following 90 min equilibration, rings with and without endothelium were placed in separate 75 ml aliquots of oxygenated buffer at 37 °C. Rings were then exposed to short pulses (1·5 or 3 min) of ^{45}Ca (specific activity 1·5–2 μCi/ml), quickly removed, lightly blotted and placed for 45 min in ice-cold buffer in which $CaCl_2$ had been replaced by 2 mM-EGTA. Rings were removed, blotted with Whatman (No. 5) paper, weighed and placed in 3 ml of 5 mM-EDTA overnight to release intracellular ^{45}Ca. The following day 10 ml of triton-containing scintillant (Pico-fluor TM30) was added and the radioactivity was counted in a liquid scintillation counter (Philips PW 4540). Calcium influx during the period of exposure to ^{45}Ca can be calculated from the increase in calcium content.

Measurement of ^{45}Ca efflux

Calcium efflux was measured by the method of Aaronson and van Breemen (Aaronson & van Breemen, 1981). Rings (mounted on stainless-steel needles) were transferred to oxygenated buffer

at 37 °C and labelled with ^{45}Ca (specific activity 1·5–2 μCi/ml buffer) for 3 h. They were then transferred to ice-cold buffer containing 6·5 mM-$CaCl_2$ (to prevent alterations in membrane function) and 5 mM-EGTA (to remove extracellular ^{45}Ca label) for 45 min. The rings were then returned to normal buffer at 37 °C and the efflux of intracellular ^{45}Ca was measured over consecutive 5 min periods for 60 min, by transferring the rings into separate 3 ml aliquots of buffer. After 60 min the tissues were blotted, weighed and the residual tissue label was measured as described for the calcium influx experiments. Each aliquot of buffer was counted for ^{45}Ca in a liquid scintillation counter as described above. The tissue radioactivity at the start of the efflux and at each time point was calculated by cumulatively adding the counts in each sample to the residual tissue ^{45}Ca. Results are expressed as a fraction of initial tissue ^{45}Ca content, and are given as the rate of loss of ^{45}Ca per minute.

Noradrenaline transiently increases ^{45}Ca efflux in the presence or absence of extracellular calcium (Deth & Lynch, 1981; Loutzenhiser & van Breemen, 1981) indicating that the increase in ^{45}Ca efflux is a measure of intracellular mobilization of calcium. Noradrenaline (10^{-5} M) was added 30 min after the start of the efflux studies in all experiments and the effect of the agents on the noradrenaline-stimulated ^{45}Ca efflux was investigated.

Mechanical responses

Ring segments were prepared as described above. They were opened immediately after dissection (and in some preparations endothelium was removed) and mounted longitudinally in a 15 ml organ bath containing oxygenated buffer at 37 °C. One end was attached to a hook and the other by silk thread to a transducer (Ether type UF1 2 oz). Isometric responses were recorded on a Lectromed multitrace 8 (Ormed Ltd., Welwyn Garden City, Herts.) chart recorder. Maximum force development in response to constrictor agents occurred at resting tensions of 1·0–1·2 g which was therefore used in all preparations, with frequent readjustments during the equilibration period of 60–90 min until stress relaxation no longer occurred. Responses to single concentrations of the chosen agonist were obtained in strips with and without endothelium.

Experimental protocols

Effect of endothelium on noradrenaline-stimulated ^{45}Ca influx. Noradrenaline (10^{-5} M) was added 4·5 min before the ^{45}Ca pulse. Acetylcholine (10^{-6} M) was added 3 min after the noradrenaline, i.e. 1·5 min before the ^{45}Ca pulse. To test whether endothelium-dependent effects were due to EDRF, known inactivators of EDRF (Griffith *et al.* 1984*a*) – dithiothreitol (5×10^{-4} M), potassium borohydride (5×10^{-4} M) or phenidone (5×10^{-4} M) – were added 30 s after acetylcholine, allowing a 1 min interaction time before the ^{45}Ca pulse. Experiments were also performed in which the cyclooxygenase inhibitor flurbiprofen (10^{-5} M) was present during the 90 min equilibration period. All agents added were then present throughout the subsequent experiments including the ^{45}Ca pulse.

Effects of nitroprusside and 8-bromo-cyclic GMP on noradrenaline-stimulated ^{45}Ca influx. For nitroprusside experiments, noradrenaline (10^{-5} M) was added 8 min before the ^{45}Ca pulse and nitroprusside (10^{-5} M) was added 3 min later, i.e. 5 min before the ^{45}Ca pulse. For 8-bromo-cyclic GMP experiments, noradrenaline (10^{-5} M) was added 13 min before the ^{45}Ca pulse and 8-bromo-cyclic GMP was added 3 min later, i.e. 10 min before the ^{45}Ca pulse. Comparable control experiments were performed in which nitroprusside and 8-bromo-cyclic GMP were added 5 and 10 min, respectively, before the ^{45}Ca pulse in the absence of noradrenaline.

Effect of endothelium on noradrenaline-stimulated ^{45}Ca efflux. Acetylcholine (10^{-6} M) was added 5 min before noradrenaline. In some experiments flurbiprofen (10^{-5} M) was added to all solutions used from the time of tissue preparation. As in influx experiments, all agents added were then present throughout the subsequent experiment. Efflux experiments to investigate the action of EDRF could not be performed in the absence of extracellular calcium because EDRF release is dependent on the presence of extracellular calcium (Griffith *et al.* 1985*a*; Long & Stone, 1985; Griffith, Edwards, Newby, Lewis & Henderson, 1986).

Effect of nitroprusside and 8-bromo-cyclic GMP on noradrenaline-stimulated ^{45}Ca efflux. Nitroprusside (10^{-5} M) or 8-bromo-cyclic GMP (10^{-3} M) were added 5 and 10 min respectively before the addition of noradrenaline. Experiments with nitroprusside and 8-bromo-cyclic GMP were performed also in the absence of extracellular calcium by replacing the $CaCl_2$ in the buffer with 2 mM-EGTA 10 min before the addition of noradrenaline.

Effect of endothelium on mechanical responses to acetylcholine in noradrenaline-constricted preparations. Preparations with and without endothelium were pre-constricted with noradrenaline (10^{-5} M). Acetylcholine (10^{-6} M) was added during the tonic phase of contraction *ca.* 3 min after the noradrenaline. To test whether endothelium-dependent effects were due to EDRF, potassium borohydride (5×10^{-4} M) was added when relaxation had reached its maximum *ca.* 5 min after the acetylcholine.

Effects of nitroprusside and 8-bromo-cyclic GMP on mechanical responses in noradrenaline-constricted preparations. Preparations without endothelium were pre-constricted with noradrenaline (10^{-5} M). Nitroprusside (10^{-5} M) or 8-bromo-cyclic GMP (10^{-3} M) were added *ca.* 3 min later during the tonic phase of contraction.

Statistical analysis

Results are expressed as mean ± S.E. Data are compared using Student's *t* test for unpaired data. Differences are considered significant where $P < 0.05$.

Materials and drugs

Acetylcholine chloride, noradrenaline hydrochloride, dithiothreitol, potassium borohydride, phenidone and sodium nitroprusside were obtained from the Sigma Chemical Co., UK. 8-bromo-cyclic GMP was obtained from Boehringer, F.R.G. Sodium flurbiprofen dihydrate was obtained from the Boots Co., UK. ^{45}Ca (specific activity 2·0 mCi/ml) was obtained from Amersham International Ltd., UK.

RESULTS

Effect of endothelium on noradrenaline-stimulated calcium influx

Noradrenaline increased calcium influx into preparations with and without endothelium (Fig. 1*A*). This noradrenaline-induced increase in calcium influx was prevented by the calcium channel blocking agent verapamil (Fig. 1*B*). Calcium influx was slightly but significantly higher with than without endothelium both in the absence and presence of noradrenaline. As previously reported (Collins, Griffith, Henderson & Lewis, 1985), this endothelium-dependent increment was abolished by flurbiprofen but not by the EDRF inhibitors dithiothreitol, potassium borohydride or phenidone (Griffith *et al.* 1984*a*) (data not shown). Acetylcholine reduced noradrenaline-stimulated calcium influx in the presence of endothelium but had no effect in the absence of endothelium (Fig. 1*A*). Acetylcholine did not alter calcium influx in the absence of noradrenaline, either with or without endothelium (data not shown). The endothelium-dependent acetylcholine-induced reduction of noradrenaline-stimulated calcium influx was inhibited by dithiothreitol, potassium borohydride and phenidone; conversely it was slightly enhanced by flurbiprofen. None of these agents influenced noradrenaline-stimulated calcium influx in the absence of endothelium.

Effects of nitroprusside and 8-bromo-cyclic GMP on noradrenaline-stimulated calcium influx

Nitroprusside and 8-bromo-cyclic GMP each reduced noradrenaline-induced calcium influx in preparations without endothelium (Fig. 2). They did not influence calcium influx in preparations in the absence of noradrenaline (data not shown).

Effect of endothelium on noradrenaline-stimulated calcium efflux

Efflux of ^{45}Ca was transiently increased by noradrenaline, in the presence or

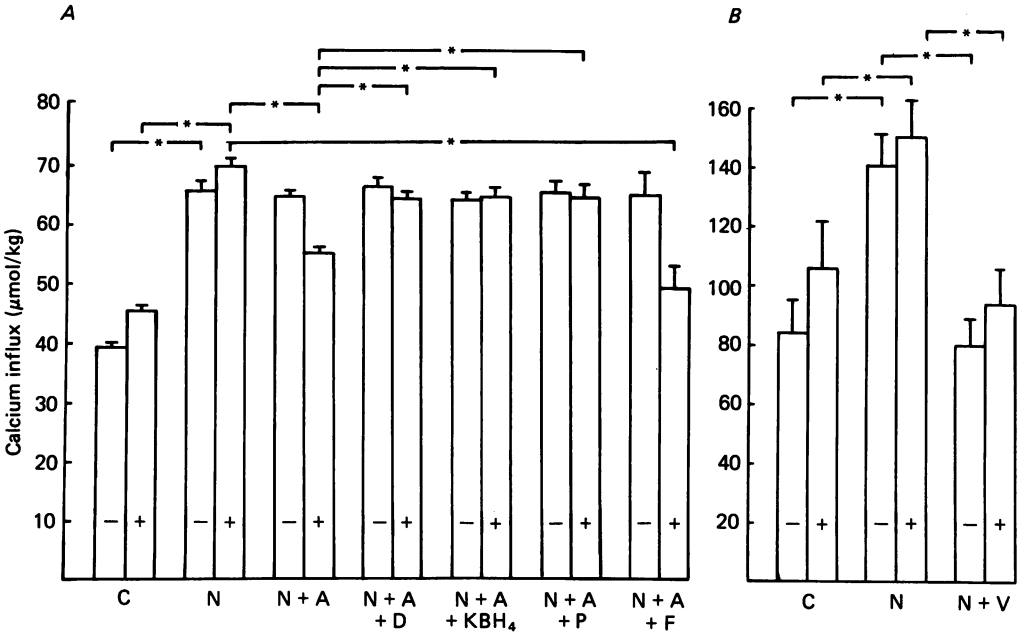

Fig. 1. Calcium influx into noradrenaline-stimulated preparations with (+) and without (−) endothelium. Preparations were incubated with ^{45}Ca for 1·5 min (*A*), 3 min (*B*) and pre-incubated with agents (for times indicated below), the agent being present also during incubation with ^{45}Ca. Abbreviations; C: control, no added agent; N: noradrenaline (10^{-5} M), 4·5 min; A: acetylcholine (10^{-6} M), 1·5 min; D: dithiothreitol (5×10^{-4} M), 1 min; KBH_4: potassium borohydride (5×10^{-4} M), 1 min; P: phenidone (5×10^{-4} M), 1 min; F: flurbiprofen (10^{-5} M), 90 min; V: verapamil (10^{-5} M), 30 min. Bars indicate mean ± S.E. of mean, $n \geqslant 12$. $^*P < 0{\cdot}05$.

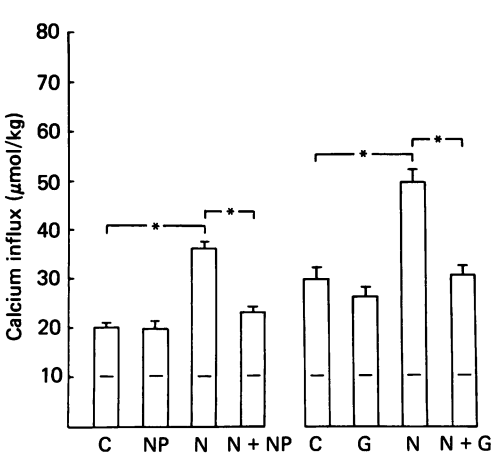

Fig. 2. Influence of nitroprusside and 8-bromo-cyclic GMP on calcium influx into resting, or noradrenaline-stimulated endothelium-free preparations. ^{45}Ca incubation for 1·5 min. Experimental protocol and conventions as in Fig. 1. Noradrenaline (N) was added 3 min before the addition of nitroprusside (NP) (10^{-5} M) or of 8-bromo-cyclic GMP (G) (10^{-3} M). NP was added 5 min before ^{45}Ca and G was added 10 min before ^{45}Ca. Bars indicate mean ± S.E. of mean, $n \geqslant 18$. $^*P < 0{\cdot}05$.

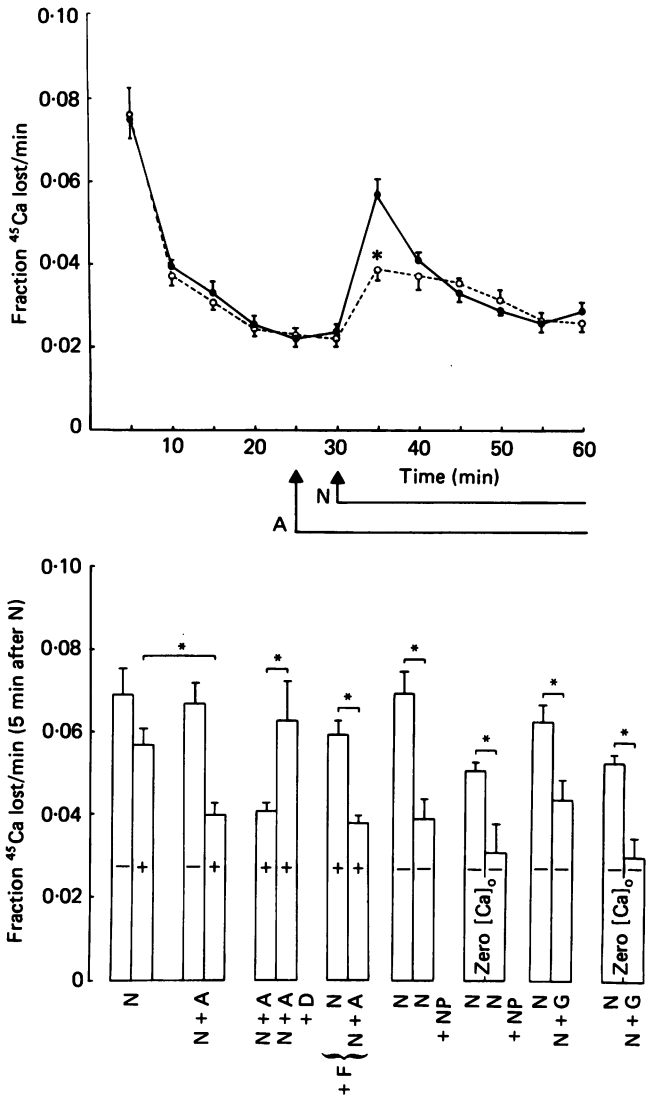

Fig. 3. ^{45}Ca efflux rates from rabbit aortic preparations. Upper panel: efflux rates from preparations with endothelium, as influenced by the addition of noradrenaline (N) (10^{-5} M) and as additionally influenced by acetylcholine (A) (10^{-6} M) added at times shown and present in subsequent aliquots of buffer. Continuous line = only N added; dashed line = N + A added. Mean ± S.E. of mean, $n \geqslant 5$. *$P < 0.05$ cf. efflux rate during 0–5 min after adding only N, i.e. point of maximum divergence of traces.

Lower panel: effect of various agents on N-stimulated ^{45}Ca efflux rates from preparations with (+) or without (−) endothelium, agents being first added at times before addition of N as indicated. Protocol is illustrated in upper panel; presented are rates 0–5 min after adding N. No agent significantly influenced ^{45}Ca efflux rate before or 10 min after addition of N. N: noradrenaline (10^{-5} M); A: acetylcholine (10^{-6} M), 5 min; D: dithiothreitol (5×10^{-4} M), 5 min; F: flurbiprofen (10^{-5} M), 255 min; NP: sodium nitroprusside (10^{-5} M), 10 min; G: 8-bromo-cyclic GMP (10^{-3} M), 10 min; buffer contained 1·5 mM-$CaCl_2$ except where shown (zero $[Ca]_o$). Bars indicate mean ± S.E., $n \geqslant 6$, *$P < 0{\cdot}05$.

absence of extracellular calcium (Fig. 3). This effect of noradrenaline was inhibited ($P < 0{\cdot}01$) by acetylcholine in preparations with endothelium but not in its absence. The endothelium-dependent acetylcholine effect was blocked by dithiothreitol but not by flurbiprofen.

Effect of nitroprusside and 8-bromo-cyclic GMP on noradrenaline-stimulated calcium efflux

Noradrenaline-stimulated ^{45}Ca efflux was inhibited by nitroprusside and by 8-bromo-cyclic GMP in preparations without endothelium, both in the presence and absence of extracellular calcium (Fig. 3).

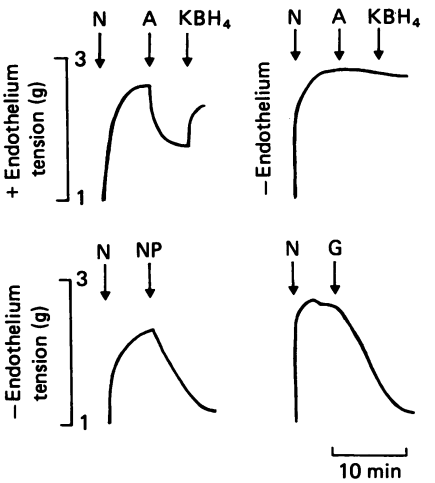

Fig. 4. Representative traces comparing relaxant responses in rabbit aortic preparations pre-constricted with noradrenaline (N) (10^{-5} M). Upper panel: responses to acetylcholine (A) (10^{-6} M) in the presence and absence of endothelium. Relaxation in endothelialized strips is reversed by the EDRF inhibitor potassium borohydride (KBH_4) (5×10^{-4} M). Lower panel: responses to sodium nitroprusside (NP) (10^{-5} M) and 8-bromo-cyclic GMP (G) (10^{-3} M) in preparations without endothelium.

Effect of endothelium on mechanical responses to acetylcholine in noradrenaline-constricted preparations

Fig. 4 (upper panel) illustrates the effect of acetylcholine in noradrenaline-constricted preparations. Relaxation occurred only in those preparations with an intact endothelium, tension falling from 2007 ± 75 mg by 958 ± 74 mg (i.e. by $48 \pm 2\%$) ($n = 11$). This acetylcholine-induced endothelium-dependent relaxation was inhibited by the EDRF inhibitor, potassium borohydride (5×10^{-4} M) ($n = 6$). In preparations without endothelium, acetylcholine induced either no change in tension or a slight further increase ($n = 6$).

Effect of nitroprusside and 8-bromo-cyclic GMP on mechanical responses in noradrenaline-constricted preparations

Fig. 4 (lower panel) illustrates the effects of nitroprusside and 8-bromo-cyclic GMP in noradrenaline-constricted preparations without endothelium. Both agents relaxed

the pre-constricted preparations completely, tension falling by from 1493 ± 165 mg by 1580 ± 106 mg (i.e. by 106 ± 11 %) with nitroprusside and from 1873 ± 48 mg by 1973 ± 92 mg (i.e. by 105 ± 5 %) with 8-bromo-cyclic GMP.

DISCUSSION

In the present study, ^{45}Ca flux measurements have been used to investigate the influence of EDRF on calcium movements in aortic ring preparations of the rabbit. The effect of EDRF has been compared with that of sodium nitroprusside, and 8-bromo-cyclic GMP.

The short period of ^{45}Ca influx used in this method is considered to provide a valid measure of calcium influx in vascular smooth muscle because calcium efflux is negligible during this time in such preparations (van Breemen, Aaronson, Cauvin, Loutzenhiser, Mangel & Saida, 1982). Previously, measurements of ^{45}Ca flux across smooth muscle cell membranes have been confounded by the presence of extracellular calcium (bound and free). The method of van Breemen *et al.* (1982) used in this study overcomes this problem by the removal of extracellular calcium with EGTA in the cold, a procedure which leaves intracellular calcium largely intact (van Breemen *et al.* 1982). This was confirmed in our hands in a series of methodological studies where we compared ^{45}Ca loss from rabbit aortic rings placed in ice cold EGTA solution for 45 min or in physiological saline at 37 °C. The rate of ^{45}Ca loss from the tissue in ice cold EGTA solution was 54 % ($P < 0.01$) of that in the physiological saline at 37 °C as previously found by van Breemen *et al.* (1982). The EDTA method here used to measure intracellular ^{45}Ca has been shown to give similar results to ashing (Meisheri *et al.* 1980) or to tissue digestion (P. Collins, unpublished observation); it is reliable, simple and relatively inexpensive.

In the present study, measurements of ^{45}Ca influx and efflux were made in untethered aortic rings not under passive stretch. Mechanical measurements were, however, made in tethered preparations to which was applied a resting tension of 1–1·2 g. Using identical techniques and preparations to the present investigation, Gleason, Ratz & Flaim (1985) have shown that measurements of ^{45}Ca influx in resting or agonist-constricted preparations are unaffected by the presence of resting tension. Their results therefore confirm the validity of correlating ^{45}Ca influx data from preparations with no resting tension with mechanical measurements obtained from preparations under tension.

EDRF activity was demonstrable only when EDRF was stimulated by acetylcholine, the basal EDRF activity being relatively small in these preparations (Griffith *et al.* 1984*b*). The extent of inhibition of noradrenaline-stimulated ^{45}Ca influx by acetylcholine (67 ± 8 %) was generally similar to the extent of inhibition of tension by acetylcholine (48 ± 2 %) in these endothelialized noradrenaline-stimulated preparations.

As in the case of EDRF, the extent of inhibition of ^{45}Ca influx by nitroprusside and by 8-bromo-cyclic GMP in noradrenaline-stimulated preparations (83 ± 2 and 90 ± 13 % respectively) was generally similar to the extent of inhibition of tension (106 ± 11 and 105 ± 5 % respectively) in these noradrenaline-constricted prepara-

tions. Our findings are similar to those recently reported by Matlib *et al.* (1985) using a similar experimental protocol but studying the effects of isosorbide dinitrate on bovine coronary artery preparations.

The similar findings with EDRF and nitrovasodilators both of which increase cyclic GMP, and with 8-bromo-cyclic GMP, suggest that it is cyclic GMP which mediates the effects.

The inhibition of ^{45}Ca efflux by EDRF, sodium nitroprusside and 8-bromo-cyclic GMP remained true in the absence of extracellular calcium where this could be studied that is, with nitroprusside and with 8-bromo-cyclic GMP, EDRF production is calcium dependent (Griffith *et al.* 1985*a*; Long & Stone, 1985; Griffith *et al.* 1986). Since these interventions were made *after* the ^{45}Ca labelling of intracellular stores, an indirect effect through reduced loading of intracellular stores seems unlikely. It has been argued by others that decreased efflux of ^{45}Ca might alternatively be due to increased calcium sequestration, on the basis of an associated observation that nitroprusside depressed phasic contraction in noradrenaline-stimulated rat aortic ring preparations in the absence of extracellular calcium (Lincoln, 1983). The transience of the effect in the present study, however, argues against a sustained increase in calcium sequestration, which would also seem unlikely on theoretical grounds in that it would lead to intracellular accumulation of calcium. A third possible explanation is reduced efflux of the noradrenaline-induced rise in intracellular free calcium. However, this too seems unlikely in view of recent evidence that cyclic GMP, by activating cyclic GMP-dependent protein kinase, stimulates rather than inhibits sarcolemmal calcium extrusion ATPase in vascular smooth muscle (Suematsu, Hirata & Kuriyama, 1984; Popescu, Panoiu, Hinescu & Nutu, 1985). The most likely explanation for the data is therefore that EDRF, sodium nitroprusside and 8-bromo-cyclic GMP reduce the noradrenaline-induced intracellular release of calcium (Deth & van Breemen, 1974; Loutzenhiser & van Breemen, 1981).

A possibility has been that the increased cyclic GMP through its protein kinase (Fiscus, Rapoport & Murad, 1983), mediates dephosphorylation of myosin light chains (Rapoport *et al.* 1983) which is known to be associated with mechanical relaxation of vascular smooth muscle. Hathaway *et al.* (1985), however, showed that cyclic GMP-dependent protein kinase does not phosphorylate an active site on myosin light-chain kinase nor alter its requirement for calmodulin. Our data indicate that cyclic GMP mediates a reduction of calcium influx and of the intracellular release of calcium, implying that it reduces the intracellular calcium concentration available for contraction. The recent demonstration by Morgan & Morgan (1984) that sodium nitroprusside can reduce free cytosolic calcium levels in potassium depolarized strips of ferret portal vein provides direct support for this view. Dephosphorylation of myosin light chains may therefore be secondary to a reduction of intracellular free calcium concentration, as suggested by Johnson & Lincoln (1985) since calcium is known to be an essential component of myosin light chain kinase activation (Aksoy & Murphy, 1983).

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