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Differential effects of vasodilators on the mobilization of calcium pools during contractions of rabbit ear artery induced by noradrenaline and high potassium

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The effects of some vasodilators on the mobilization of different Ca^{2+} pools during contractions produced by noradrenaline and high K^+ medium in rabbit ear artery has been investigated. Sodium nitrite was much more effective in antagonizing high K^+-stimulated Ca^{2+} fluxes through the potential-dependent Ca^{2+} channels than noradrenaline-induced mobilization of intracellular Ca^{2+} stores or Ca^{2+} fluxes through receptor-operated channels. Papaverine was only slightly more active on contractions sustained by influx of extracellular Ca^{2+} (through potential-dependent channels and receptor-operated channels) than on those sustained by intracellular Ca^{2+}. Verapamil and nifedipine were much more effective in antagonizing contractions sustained by Ca^{2+} entry through potential-dependent channels than through receptor-operated channels. Nifedipine was completely ineffective in antagonizing noradrenaline-induced mobilization of intracellular Ca^{2+} stores while verapamil had a limited inhibitory action in high concentrations. Phentolamine was equieffective in antagonizing both types of noradrenaline-induced contractions while having no effect on high K^+-induced tonic contraction.

An increase of free intracellular Ca^{2+} concentration is thought to be required both for resting tone of vascular smooth muscle (Webb & Bohr 1981) and its contractile response to vasoactive agents (Bohr 1964; Hurwitz & Suria 1971). Considerable evidence has been provided that different vasoactive agents might mobilize different Ca^{2+} pools for vascular smooth muscle contraction (Weiss 1977; Bolton 1979). High K^+ activates a transmembrane Ca^{2+} entry mechanism (potential-dependent Ca^{2+} channels) (Hudgins & Weiss 1968; Van Breemen 1969) which is functionally distinct (Bolton 1979; Meisheri et al 1981) from the transmembrane Ca^{2+} entry mechanism activated by neurohormones such as noradrenaline (receptor-operated channels) (Hudgins & Weiss 1968; Droogmans et al 1977). Furthermore, noradrenaline and other neurohormones mobilize Ca^{2+} from intracellular storage sites (Deth & Van Breemen 1974, 1977; Karaki et al 1979; Manzini et al 1982a,b). We have recently developed a method that permits the separate quantitative analysis of cellular from extracellular Ca^{2+}-dependent noradrenaline-induced contractions of rabbit ear artery (Manzini et al 1982b). Therefore we have examined whether commonly used vasodilators possess a differential pattern of activity on these processes as well as on K^+ induced contractions in comparison with the α-adrenoreceptor blocking drug phentolamine.

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METHODS

New Zealand male albino rabbits, 2·5–3 kg were heparinized (1000 u i.v.) and killed by a blow to the back of the head. A 3 cm segment of central ear artery was dissected free, cannulated at both ends with polyethylene cannulae and transferred to a 7 ml organ bath at 37 °C. The volume of the fluid was maintained constant by means of an overflow.

The segment was perfused intraluminally by means of De Saga 131900 six-channel peristaltic pump at a rate of 5 ml min^{-1}. The extraluminal fluid in the organ bath was replenished at a rate of 8 ml min^{-1} by Mariotte bottle. Both intraluminal and extraluminal fluids were gassed with 95% O_2 and 5% CO_2 and pre-heated to 37 °C.

Changes in intraluminal perfusion pressure, recorded by means of a pressure transducer attached to the inlet side of the perfusion system, were taken as an indirect measure of increases in arterial contraction above the resting tone.

After a 1 h stabilization period, the extraluminal and intraluminal normal Krebs solutions was replaced with high-K^+, Ca^{2+}-free solution which produced a rapid contraction with gradual return to resting values. Five min later, the intraluminal perfusion fluid was replaced for 3 min with high-K^+, Ca^{2+}-free solution containing noradrenaline (5 μM) which produced a contraction followed by return to resting values (Manzini et al 1982a). Subsequent restoration of both intraluminal and extraluminal fluids to normal Krebs solutions resulted in a further rapid contraction, which depends upon the presence of extracellular Ca^{2+} and is related to the concentration of the agonist previously added to the inner perfusion fluid (Manzini et al 1982b). No contraction is produced by restoration of normal Krebs solution unless intraluminal noradrenaline had been previously added to the medium (Manzini et al 1982b). This suggests that the contractile response observed in segments exposed to noradrenaline is likely to be sustained by transmembrane Ca^{2+} fluxes through receptor-operated Ca^{2+} channels without any participation of Ca^{2+} fluxes through the potential-dependent Ca^{2+} channels (Bolton 1979). Preliminary experiments showed that after 25 min of exposure of the preparation to normal Krebs solution, reproducible responses to noradrenaline were obtained and their modification by other drugs was examined by adding them in the stated concentration to the intraluminal perfusion fluid for 3 min before and during addition of noradrenaline. The effects of drugs on noradrenaline-induced contractions were assessed on the same preparations after two or more reproducible responses to noradrenaline had been recorded. Only one drug was studied on each arterial segment. Normal Krebs solution had the following composition (mM): NaCl 119, KCl 4·7, $MgSO_4$ 1·5, KH_2PO_4 1·2, $CaCl_2$ 2·5, $NaHCO_3$ 25, glucose 11. The high K^+, Ca^{2+}-free solution had the following composition (mM): NaCl 69, KCl 54·7, $MgSO_4$ 1·5, KH_2PO_4 1·2, $NaHCO_3$ 25, glucose 11, EDTA 0·77. In other experiments the effect of vasodilators on tonic contraction of the artery induced by high K^+ was investigated. After 1 h equilibration, normal Krebs solution was substituted intraluminally and extraluminally with a high K^+ (54 mM) Krebs solution prepared by replacing NaCl with equimolar quantities of KCl. This produced a phasic followed by a tonic contraction which reached steady values within 10–15 min and was suitable for testing the effect of vasodilator substances.

The vasodilators were added intraluminally in increasing concentrations, the next being given when the preceding one had produced a steady state effect.

Noradrenaline hydrochloride, Verapamil hydrochloride, papaverine hydrochloride and sodium nitrite were dissolved in double distilled water and then diluted. Phentolamine mesylate was directly diluted in Krebs or high K^+ Ca^+-free medium. A 1 mM solution of nifedipine in absolute ethanol was prepared from which a 1 μM dilution was made in high-K^+, Ca^{2+}-free medium. Care was taken to avoid exposure to the light of nifedipine and noradrenaline solutions.

Statistical analysis were performed by Student's *t*-test for paired data. The effects of drugs were evaluated by regression analysis using the least squares method. ED50 values and 95% confidence limits were calculated accordingly.

RESULTS

Effect of vasodilators on cellular Ca^{2+} dependent noradrenaline-induced contractions

Noradrenaline (5 μM) in high-K^+, Ca^{2+}-free medium produced a contractile response with a rise in perfusion pressure of 67·9 ± 5·3 mmHg (n = 33). (Fig. 1). Papaverine (30–300 μM, n = 5) produced a concentration-related reduction in amplitude of this contraction (its ED50 and relative 95% confidence

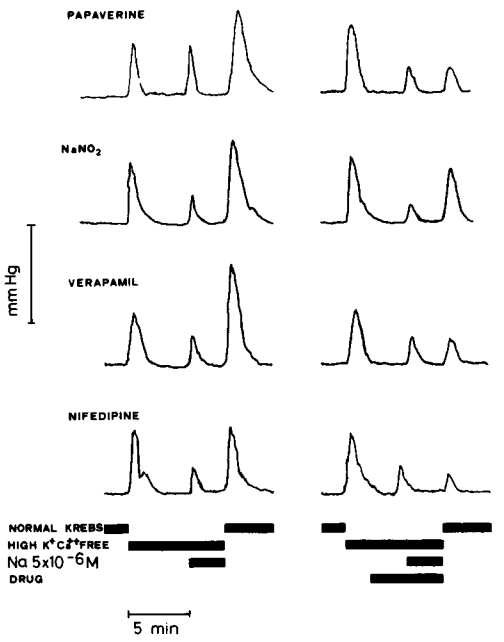

FIG. 1. Typical tracings showing the effects of several vasodilators on cellular and extracellular Ca^{2+}-dependent noradrenaline (5 μM) induced contractions. The left hand panels are preceding control observations. Vertical bar is 200 mmHg.

Table 1. Effect of vasodilator drugs on the mobilization of cellular and extracellular Ca^{2+} pools by noradrenaline and high K^+ in rabbit ear artery (ED50 ± 95% confidence limits).

	Cellular Ca^{2+} (Noradrenaline)	Extracellular Ca^{2+} (Noradrenaline)	Extracellular Ca^{2+} (High K^+)
Papaverine	110 μM (53–230 μM)	75·2 μM (68–83 μM)	18·7 μM (10–33 μM)
Sodium nitrite	>10 mM	>10 mM	1·88 mM (1·6–2·1 mM)
Verapamil	>50 μM	7·73 μM (3·6–16 μM)	1·07 μM (0·4–2·6 μM)
Nifedipine	>300 nM	57·8 nM (47·0–70·0 nM)	8·85 nM (6·5–11·0 nM)
Phentolamine	85·8 nM (44–160 nM)	93·0 nM (48–180 nM)	>100 nM

limits are shown in Table 1). Sodium nitrite, in concentrations up to 10 mM produced an inhibition not exceeding 38·8 ± 5·4% (n = 5). Verapamil (5–50 μM, n = 5) had only a limited effect (not exceeding 20% inhibition with the higher concentration tested). Nifedipine (10–300 nM) had no significant inhibitory effect (n = 5). Phentolamine (10–500 nM) (n = 5) produced a concentration-related reduction in amplitude of this contraction (Table 1).

Effect of vasodilators on extracellular Ca^{2+} dependent noradrenaline-induced contractions

Restoration of normal Krebs solution in noradrenaline-pretreated (5 μM) arterial segments resulted in a transient contraction with a peak increase in perfusion pressure of 169·6 ± 6·9 mmHg (n = 33). (Fig. 1). Sodium nitrite up to 10 mM did not exceed 28·1 ± 3·8% inhibition. Papaverine (30–300 μM), verapamil (1–50 μM), nifedipine (10–300 nM) and phentolamine (10–500 nM) produced concentration-related inhibitions of this contraction (their relative ED50 values and 95% confidence limits are shown in Table 1).

Effect of vasodilators on high K^+-induced tonic contractions

Exposure of rabbit ear artery segments to high K^+ medium (54 mM) produced a steady tonic contraction with an increase in perfusion pressure of 114·9 ± 12·3 mmHg (n = 33). Sodium nitrite (0·3–10 mM), papaverine (10–300 μM), verapamil (0·1–30 μM) and nifedipine (1–30 nM) produced concentration-dependent inhibitions of this contraction (n = 5 for each drug tested). Their ED50 value and relative 95% confidence limits are shown in Table 1. Phentolamine (0·1 μM, n = 4) was ineffective.

DISCUSSION

Vasodilator drugs are thought to produce relaxation of vascular smooth muscle by interfering with Ca^{2+} movements responsible for initiating or maintaining the contractile state. The experimental procedure employed in this study deals with the separate quantitative analysis of the effects of some commonly used vasodilators on: (a) noradrenaline-induced mobilization of intracellularly stored Ca^{2+} (Deth & Van Breemen 1974; Karaki et al 1979; Manzini et al 1982a,b), (b) noradrenaline-induced increase in transmembrane Ca^{2+} conductance (Steinsland et al 1973; Manzini et al 1982b) presumably through receptor-operated Ca^{2+} channels (Droogmans et al 1977; Bolton 1979), and (c) transmembrane Ca^{2+} influx through channels opened by K^+-induced depolarization of the cell membrane (Bolton 1979; Meisheri et al 1981).

Quite obviously, α-adrenoreceptor blocking agents are expected to be equieffective in antagonizing cellular and extracellular Ca^{2+}-dependent noradrenaline-induced contractions while having little effect on those elicited by high K^+ medium. Our results with phentolamine, a well known unselective α-adrenoreceptor blocker confirm this hypothesis.

Despite the widespread clinical use of nitrocompounds, the mechanism through which they exert their vasodilator action has not been elucidated. In particular, little attention has been paid thus far to the analysis of the effects of nitrocompounds in terms of differential inhibition of Ca^{2+} mobilization from different pools in vascular smooth muscle. Our results indicate that, although sodium nitrite produces a generalized depression of Ca^{2+} mobilization, its relaxant effect can be fully estimated on contractions which are likely to be dependent upon a transmembrane influx of Ca^{2+} through the potential-dependent channels. Since the vasodepressor action of nitrocompounds is thought to be linked to an elevation in intracellular concentrations of cGMP (Ignarro et al 1981; Gruetter et al 1981; Kukovetz et al 1979) it could be speculated that cGMP-mediated modulation of intracellular free Ca^{2+} concentrations is preferentially coupled to Ca^{2+} entry into the cells through activation of potential-dependent channels. The vascular smooth muscle relaxant effects of papaverine have been ascribed to its inhibitory action on phosphodiesterases (Kukovetz & Poch 1970; Poch & Kukovetz 1971) and consequent rise in intracellular concentration of cAMP (Takayanagi et al 1978; Anderson 1972) leading to a reduction of free intracellular Ca^{2+} available for contraction (Bohr & Webb 1978). If this were true, papaverine should have been equieffective in antagonizing the three types of contraction recorded in our

experimental conditions. However papaverine was more effective in antagonizing contractions sustained by transmembrane fluxes of Ca^{2+} through receptor-operated and potential dependent Ca^{2+} channels compared with those dependent upon the mobilization of intracellularly stored Ca^{2+}. This observation agrees well with recent findings indicating that the relaxant properties of papaverine are at least in part attributable to a Ca^{2+} entry blocking action (Huddart & Saad 1980; Imai & Kitagawa 1981).

Two main conclusions can be drawn from our results with verapamil and nifedipine: (a) these agents are more effective in blocking Ca^{2+} influx activated by high K^+ than that by noradrenaline, while having only barely detectable effect on mobilization of intracellular Ca^{2+} stores, and (b) their differential selectivity in blocking Ca^{2+} entry through Ca^{2+} channels activated by high K^+ and noradrenaline is a further demonstration of their different mechanism of action at smooth muscle level (see also Kondo et al 1980; Mikkelsen et al 1979; Maggi et al 1982; Hay & Wadsworth 1982; Flaim 1982). However the greater ability of verapamil compared with nifedipine in antagonizing noradrenaline-induced than high K^+-induced contractions could be attributed to the higher affinity of verapamil for α-adrenoceptors (Nayler et al 1982).

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