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Inhibition by quinine of endothelium-dependent relaxation of rabbit aortic strips

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1 The effects of quinine sulphate, tetramethylammonium chloride (TMA) and tetraethylammonium chloride (TEA) (all blockers of the Ca^{2+}-activated K^+ channels) on the relaxations induced by acetylcholine (ACh), calcium ionophore A23187 and sodium nitrite were studied in helical strips of rabbit aorta.

2 The strips were contracted to a moderate stable tone with phenylephrine (10^{-7} M). ACh (4×10^{-9} to 10^{-6} M) as well as A23187 (10^{-8} to 3×10^{-7} M) reduced this tone in a concentration- and endothelium-dependent manner.

3 Pretreatment of the tissues with quinine (2.5×10^{-5} to 10^{-4} M) for 60 min produced a concentration-dependent inhibition of the relaxation induced by ACh. Also 90 min incubation of the strips with TMA (3×10^{-3} to 6.5×10^{-2} M) or TEA (10^{-3} to 3×10^{-2} M) inhibited the ACh-evoked relaxation in a manner similar to quinine.

4 Quinine (10^{-4} M, 60 min), TMA (6.5×10^{-2} M, 90 min) or TEA (3×10^{-2} M, 90 min) produced 5 to 10 fold reductions in the relaxant EC_{50} values of A23187 and ACh and depressed (by 40 to 95%) the maximal relaxations to the ionophore and ACh.

5 On a molar basis, quinine was more effective than the two tetraalkylammonium ions in reducing the endothelium-dependent relaxations of the aortic strips induced by ACh or A23187. The inhibitory actions were reversible after 60 to 90 min washout.

6 Exposure of the strips to either quinine (10^{-4} M, 60 min), TMA (6.5×10^{-2} M, 90 min) or TEA (3×10^{-2} M, 90 min), however, did not influence significantly the relaxations evoked by sodium nitrite, a direct smooth muscle relaxant.

7 These results suggest that stimulation of the Ca^{2+}-activated K^+ channels could be, at least partially, responsible for the endothelium-dependent relaxations induced by ACh or A23187. Their activation might not be required for the endothelium-independent relaxant effects of sodium nitrite.

Introduction

Vascular endothelial cells produce and release a factor(s) (endothelium-derived relaxing factor; EDRF) that can mediate relaxation in precontracted vessels (Furchgott & Zawadzki, 1980) and modulate vascular smooth muscle tone at rest (Griffith *et al.*, 1984). Before the discovery of EDRF, the relaxant effect of acetylcholine (ACh) was attributed to activation of the sodium-pump (Haddy, 1978; Webb & Bohr, 1978; De Mey & Vanhoutte, 1980). Recently, Bolton *et al.* (1983) suggested that EDRF might increase potassium permeability in vascular tissues. Gordon & Martin (1983) predicted the possible association of the calcium-activated potassium efflux with the endothelium-dependent vascular relaxation induced by bradykinin and adenosine triphosphate (ATP). Both ACh and the calcium ionophore A23187 are powerful endothelium-dependent relaxants and capable of evoking potassium loss (for references see Petersen & Maruyama, 1984).

The loss of K^+ from cells is conducted through transmembrane K^+ channels which are regulated by the intracellular Ca^{2+} concentration (Lattore & Miller, 1983; Peterson & Maruyama, 1984). At least three different types of Ca^{2+}-activated K^+ channels have been identified in a variety of tissues from several species. Two of these are sensitive to membrane

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voltage, show selectivity for cations but differ in K^+ conductance, whereas the third type is insensitive to membrane potential, non-selective for cations, and is activated by Ca^{2+} (Schwarz & Passow, 1983; Petersen & Maruyama, 1984). In the present study, an attempt was made to assess whether operation of the Ca^{2+}-activated K^+ channels is required for endothelium-dependent relaxation. We examined the effect of quinine and tetraalkyl-ammonium ions (tetramethylammonium chloride (TMA) and tetraethylammonium chloride (TEA)), compounds reported to block comparable ion channels in different cells (Armando-Hardy *et al.*, 1975; Atwater *et al.*, 1979; Hermann & Gorman, 1981; Walsh & Singer, 1983; Petersen & Maruyama, 1984; Bartschat & Blaustein, 1985; French & Shoukimas, 1985; Lattore, 1986), on the relaxations of endothelium-intact rabbit aortic strips induced by ACh or A23187. In addition, we studied the effect of quinine, TMA and TEA on the relaxation induced by sodium nitrite, an endothelium-independent vascular relaxant.

Methods

Rabbits of either sex weighing 2 to 3 kg were stunned by a blow to the head and exsanguinated. The chest was opened and the descending aorta removed carefully to protect the intimal layer. After excision, the aortic segment was immersed in cold Krebs solution of the following composition (mM): NaCl 113.0, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.5 and EDTA 0.03; pH 7.4. The aorta was then cleaned of adhering fat and connective tissues. Helical strips (4 × 20 mm) were cut according to the original method of Furchgott & Bhadrakom (1953), with special care taken not to cause injury to the endothelial cells. The strips were then placed in a 5 ml organ bath containing Krebs solution at 37°C and gassed with 95% O_2 plus 5% CO_2. The strip was allowed to equilibrate for 30 min before the application of resting tension. After this period, the bathing fluid was changed once and the strip was suspended under a resting tension of 2 g, one end fixed to the bottom of the bath and the other end attached to a force transducer (Ugo Basile, type DY2) connected in turn to a potentiometric recorder (Radelkis, type OH 814). The tissue was equilibrated for a further 30 min.

Fresh dilutions of drug solutions were prepared daily. Stock solutions and dilutions were such that the volumes added to the 5 ml bath never exceeded 1%.

To study relaxation responses a sustained moderate tone was induced in the untreated tissues initially by 10^{-7} M phenylephrine. Control relaxant concentration-response curves and those in the presence and after removal of quinine, TMA or TEA were determined on the same aortic strip.

After the control phase of the experiments, the strips were incubated with quinine sulphate (2.5×10^{-5}, 5×10^{-5} or 10^{-4} M, 60 min), TMA (3×10^{-3}, 10^{-2}, 3×10^{-2} or 6.5×10^{-2} M for 90 min) or TEA (10^{-3}, 3×10^{-3}, 10^{-2} or 3×10^{-2} M for 90 min); each concentration of each drug constituted a separate experiment.

Exposure to quinine depressed the contractile responsiveness of the strips to phenylephrine in a concentration- and time-dependent manner. Therefore, the concentration required to induce a tone level similar to the control was determined by cumulative addition of phenylephrine to the bath, starting 5 min after quinine injection. The optimum concentration thus obtained (10^{-6} M) was utilized as a single application, 5 min after addition of quinine, to induce a sustained tone in experiments to follow. The increased concentration of phenylephrine was without significant influence on the sensitivity and magnitude of relaxations induced by ACh or A23187. Treatment of the strips with either TMA or TEA, however, potentiated the response of the strips to phenylephrine. Therefore, 2.5×10^{-8} M phenylephrine was utilized after exposing the tissues to these agents.

Results are expressed as mean ± s.e.mean. For the comparison of mean slope values, regression lines of the apparently straight portion of the concentration-response curves were computed using grouped data. Tests for median-effective concentration differences were based on mean log values (Fleming *et al.*, 1972). The data were analysed by use of either, as appropriate, paired or unpaired Student's *t* test. When more than one mean was compared, a one way analysis of variance was employed (Snedecor & Cochran, 1967). A probability value of less than 0.05 was considered significant.

Drugs

Phenylephrine hydrochloride, acetylcholine iodide and sodium nitrite (all purchased from Sigma) were dissolved in normal saline. Solutions of tetramethylammonium chloride (TMA), tetraethylammonium chloride (TEA), 4-aminopyridine and barium chloride (all from Sigma) were made in distilled water. Quinine sulphate (Knoll Pharmaceutical Company) was dissolved in 0.1 N HCl. The total volume of this acidic solution in the bath had no apparent effect on the pH of the bathing fluid. A23187 (Calbiochem) was dissolved in dimethyl-sulphoxide (DMSO), the cumulative concentration of which in the bath was below 0.1%. This solvent had no influence on contractile or relaxation responses. Isomolar replacement of sucrose for either TMA or TEA, although causing a small increase in basal tension, had no significant effect on either the relaxation or contractile responses of the aortic strips.

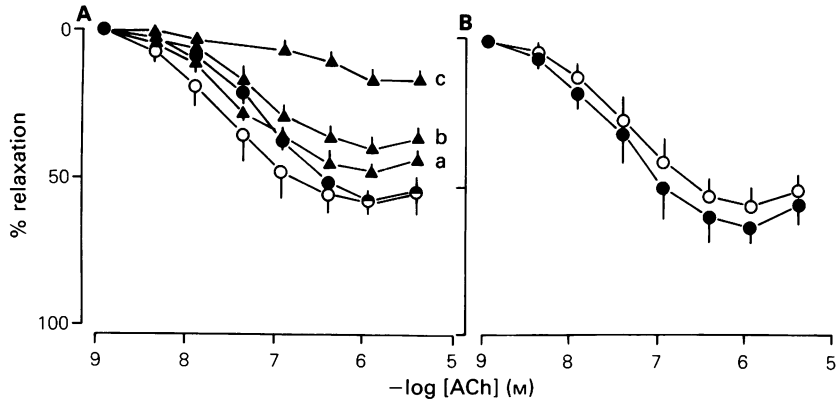

Figure 1 (A) Concentration-related inhibitory effect of quinine sulphate on the acetylcholine (ACh)-induced, endothelium-dependent relaxation in rabbit aortic strips precontracted by phenylephrine (10^{-7} M). (●) Relaxation by ACh before quinine ($n = 18$); (○) relaxation by ACh after quinine (recovery; $n = 18$); (▲) relaxant effects of ACh in the presence of 2.5×10^{-5} (a; $n = 4$), 5.0×10^{-5} (b; $n = 4$) and 10^{-4} (c; $n = 10$) M quinine. Vertical lines indicate s.e.mean. Some values of curve (a), most values of curves (b) and (c) differ significantly from the control curves. For the sake of clarity, statistical significance has not been marked.
(B) Dose-related, endothelium-dependent relaxant effect of ACh in rabbit aortic strips precontracted with 10^{-7} M (●; $n = 10$) or with 10^{-6} M (○; $n = 10$) phenylephrine. There is no significant difference between the corresponding values of the two curves.

Results

The effect of quinine on ACh-induced relaxations

Application of phenylephrine (10^{-7} M) to endothelium-intact aortic strips induced a sustained contraction (20.7 ± 1.5 mN, $n = 18$). ACh was added cumulatively (4×10^{-9} to 10^{-6} M) at the steady-state of this tone and concentration-related relaxations occurred. ACh (10^{-6} M) induced a maximal, 55%, reduction of this tone (control). Further increase in ACh concentration (3.7×10^{-6} M) resulted in contraction of the aortic strips. Incubation of the tissues with quinine (2.5×10^{-5}, 5×10^{-5} or 10^{-4} M, 60 min) depressed the response to phenylephrine.

The potency of quinine was determined by measuring the inhibitory effects of different concentrations (2.5×10^{-5}, 5×10^{-5} and 10^{-4} M, 60 min) of this agent on the endothelium-dependent relaxations of the aortic strips to ACh. These concentrations of quinine depressed significantly (Figure 1A, $P < 0.01$) the maximal relaxations of the strips in response to ACh (10^{-6} M) by 14% ($n = 4$), 32% ($n = 4$) and 75% ($n = 10$), respectively. Pretreatment of the strips with 2.5×10^{-5} M or 5×10^{-5} M quinine for 60 min increased markedly the concentration of ACh required to evoke a half-maximal relaxation (from $5.0 \pm 0.2 \times 10^{-7}$ M to $1.3 \pm 0.4 \times 10^{-6}$ M and from $4 \pm 0.1 \times 10^{-7}$ M to $1.0 \pm 0.6 \times 10^{-5}$ M; $n = 4$, $P < 0.05$ for each group, respectively); whereas the highest concentration of quinine (10^{-4} M) almost abolished the sensitivity of the strips to ACh. After repeated washout and equilibration for 60 min, the responsiveness of the tissues to phenylephrine or ACh recovered. In addition, although not significant ($P > 0.1$), the relaxant response to ACh tended to be bigger.

The concentration-response curves for ACh (4×10^{-9} to 10^{-6} M) in the presence of 10^{-7} or 10^{-6} M phenylephrine were not significantly different (Figure 1B, $P > 0.1$, $n = 10$).

The effects of tetraalkylammonium ions on ACh-induced relaxations

The concentration-response curves in Figure 2A and B illustrate the effect of 90 min pretreatment with different concentrations of TMA (3×10^{-3}, 10^{-2}, 3×10^{-2} and 6.5×10^{-2} M) and TEA (10^{-3}, 3×10^{-3}, 10^{-2} and 3×10^{-2} M) on the relaxations of rabbit aortic strips to ACh (4×10^{-9} to 10^{-6} M). At all concentrations tested TMA, but not TEA, induced a fast transient contraction of each strip. On a molar basis, both compounds induced a comparable concentration-related inhibition of the relaxations to ACh. As these figures illustrate, following treatment with different concentrations of either TMA or TEA, the concentration-response curves for ACh were significantly displaced and the maximum effect decreased. Even in the presence of one of the lower concentrations of TMA (10^{-2} M) and TEA (3×10^{-3} M), the median effective concentrations of

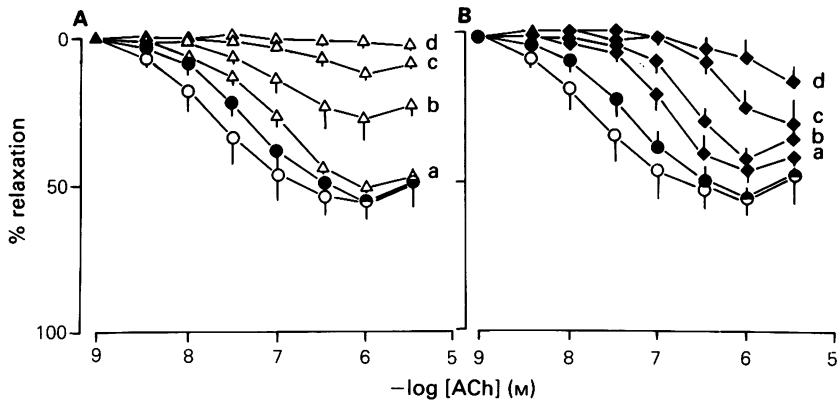

Figure 2 (A) Dose-related inhibitory effect of tetramethylammonium (TMA) on the acetylcholine (ACh)-induced, endothelium-dependent relaxation in rabbit aortic strips precontracted with phenylephrine (2.5×10^{-8} M). (●) Relaxation to ACh before TMA treatment ($n = 16$); (○) relaxant action ACh after washout of TMA ($n = 16$). (△) Relaxation produced by ACh in the presence of 3×10^{-3} (a; $n = 4$), 10^{-2} (b; $n = 4$), 3×10^{-2} (c; $n = 4$) and 6.5×10^{-2} (d; $n = 4$) M TMA.
(B) Inhibitory effect of tetraethylammonium (TEA) on the ACh-induced, endothelium-dependent relaxation in aortic strips of rabbits. For (●) and (○) see (A). (◆) Relaxant action of ACh in the presence of 10^{-3} (a; $n = 4$), 3×10^{-3} (b; $n = 4$), 10^{-2} (c; $n = 4$) and 3×10^{-2} (d; $n = 4$) M TEA. For both (A) and (B): some values of curves (a) and most values of curves (b), (c) and (d) differ significantly from the corresponding control data. To avoid confusion statistical significance has not been indicated. Vertical lines relate to s.e.mean.

ACh were increased from $7.0 \pm 0.3 \times 10^{-7}$ M to $8.0 \pm 0.2 \times 10^{-6}$ M ($P < 0.01$) and from $5.0 \pm 0.3 \times 10^{-7}$ M to $1.4 \pm 0.1 \times 10^{-6}$ M ($P < 0.01$), respectively.

The effect of quinine on A23187-induced relaxation

Intact aortic strips precontracted with 10^{-7} M phenylephrine (mean tension 20 ± 1.5 mN) were relaxed concentration-dependently by A23187 over a concentration range of 10^{-8} to 3×10^{-7} M, the latter concentration producing a 68% reduction in phenylephrine-induced tone. Concentrations of A23187 exceeding 3×10^{-7} M reversed the relaxation to contraction. Pretreatment of the tissues with quinine (10^{-4} M, 60 min) reduced significantly the sensitivity to A23187 (median effective concentrations: $1.0 \pm 0.03 \times 10^{-7}$ M before and $2.0 \pm 0.4 \times 10^{-6}$ M after quinine application, $P < 0.01$, $n = 10$), as well as the maximum relaxant effect of A23187 (3×10^{-7} M) by 44% (Figure 3, $n = 10$, $P < 0.01$). The elevated concentration of phenylephrine (10^{-6} M), which was needed to produce a tone to match that seen in the absence of quinine, did not change the relaxant potency of A23187 (data not shown). After removal of quinine and equilibration of the tissues for at least 60 min, A23187 (10^{-8} to 3×10^{-7} M) elicited relaxations that were equal in size to those of the control phase (Figure 3).

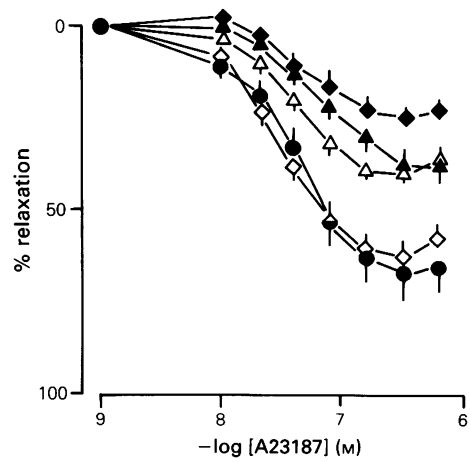

Figure 3 The effects of the Ca^{2+}-activated K^+ channel inhibitors quinine sulphate (10^{-4} M (▲), $n = 10$), tetraethylammonium (6.5×10^{-2} M (△), $n = 49$) or tetraethylammonium (3×10^{-2} M (◆), $n = 4$) on the A23187-induced, endothelium-dependent relaxation in rabbit aortic strips. (●) Represent the effect of the ionophore before the addition of the inhibitors ($n = 18$) and (◇) indicate the recovery of the relaxant action of A23187 after washout of the inhibitors ($n = 18$). For clarity statistical significance has not been indicated. Vertical lines denote s.e.mean.

The effects of tetraalkylammonium ions on the A23187-induced relaxations

Pretreatment of the aortic strips for 90 min with either TMA (6.5×10^{-2} M) or TEA (3×10^{-2} M) potentiated the response of the tissues to phenylephrine. In addition to the displacement of the concentration-response curves (Figure 3), there was a reduction of maximal response and a significant decrease in sensitivity (EC_{50}: from $8.5 \pm 0.03 \times 10^{-8}$ M to $4.0 \pm 0.1 \times 10^{-7}$ M and from $9.7 \pm 1 \times 10^{-8}$ M to $5.4 \pm 3 \times 10^{-5}$ M, $P < 0.01$, $n = 4$ for each group, respectively) of the aortic strips to A23187. TMA showed a 41% inhibition, while TEA elicited a 56% reduction of the maximal relaxation responses of the strips to this agonist. In both cases, after repeated washout and a 90 min rest, the responses of the strips to A23187 were restored.

The effect of quinine and tetraalkylammonium ions on sodium nitrite-induced relaxations

Endothelium-independent relaxations of aortic strips were evoked by sodium nitrite (3×10^{-6} to 9×10^{-4} M). Exposure of the strips to quinine (10^{-4} M, 60 min) neither altered the median effective concentrations ($5.0 \pm 0.6 \times 10^{-5}$ M in the absence and $4.0 \pm 1.0 \times 10^{-5}$ M in the presence of quinine, $P > 0.1$, $n = 7$) nor the threshold and maximal responses to sodium nitrite. On the other hand, treatment of the strips with tetraalkylammonium ions (6.5×10^{-2} M TMA or 3×10^{-2} M TEA for 90 min) caused the sodium nitrite-induced relaxations to develop initially more slowly relative to the control. However, this exposure of the strips to these organic ions did not antagonize the relaxations evoked by sodium nitrite. In addition, the concentrations of sodium nitrite required to induce threshold as well as half-maximal effects (EC_{50}: $5.0 \pm 0.1 \times 10^{-5}$ M, $5.0 \pm 0.4 \times 10^{-5}$ M before and $8.0 \pm 0.9 \times 10^{-5}$ M, $6.0 \pm 0.2 \times 10^{-5}$ M after application of TMA and TEA, respectively) were not affected significantly (Figure 4, $P > 0.05$, $n = 4$ for each group).

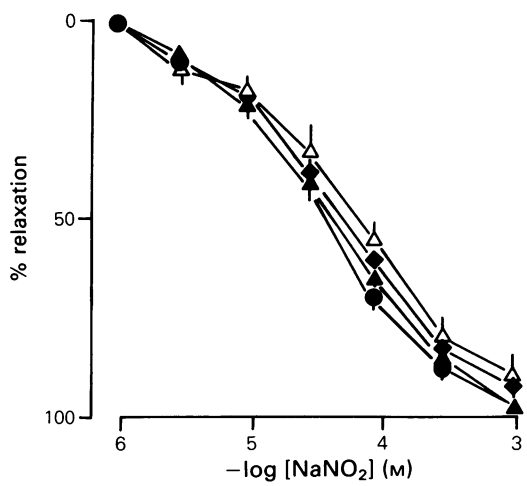

Figure 4 Concentration-related relaxant effect of sodium nitrite in rabbit aortic strips in the absence (●; $n = 18$) and presence of quinine sulphate (10^{-4} M (▲), $n = 10$), tetraethylammonium (3×10^{-2} M (◆), $n = 4$) or tetramethylammonium (6.5×10^{-2} M (△), $n = 4$). Vertical lines indicate s.e.mean.

Effects of 4-aminopyridine and $BaCl_2$

Two other blockers of the Ca^{2+}-activated K^+ channel, 4-amino-pyridine and $BaCl_2$ (Hermann & Gorman, 1979; Bartschat & Blaustein, 1985) were also studied. However, 4-aminopyridine (10^{-5} to 10^{-4} M) induced spontaneous phasic contractions which made the quantitative determination of relaxation impossible, whilst $BaCl_2$, at concentrations (2.5 to 5×10^{-2} M) sufficient to block Ca^{2+}-activated channels (Hermann & Gorman, 1979) produced a high, sustained tone 2–3 times greater than, and thereby not comparable to, the size of contractions that were induced by phenylephrine when testing quinine, TMA and TEA.

Discussion

In this study quinine depressed the endothelium-dependent relaxation induced by ACh or A23187 but did not affect the relaxant activity of sodium nitrite, an endothelium-independent vasodilator. The site and mechanism of action of quinine are far from being clarified. In theory, this substance might have interfered with the ACh- and A23187-induced release of EDRF from the endothelial cells and/or antagonized the relaxant effect of this factor on the vascular smooth muscle cells. However, there is general agreement that ACh and A23187 both release EDRF, and both EDRF and nitrite relax vascular smooth muscle, most probably by stimulating soluble guanylate cyclase and increasing guanosine 3′:5′-cyclic monophosphate (cyclic GMP) levels (Furchgott & Jothianandan, 1983; Rapoport & Murad, 1983). Therefore our finding that quinine inhibits endothelium-dependent relaxation to ACh and A23187, but not the endothelium-independent relaxation to sodium nitrite may indicate that the endothelium and not the smooth muscle is the site of action of quinine. It is also conceivable that quinine inactivates the EDRF released from the endothelium. Such inactivation by several compounds was described by Griffith *et al.* (1984).

The importance of potassium efflux in the endothelium-dependent relaxation was suggested by Bolton *et al.* (1983); they concluded that EDRF increases potassium permeability in several blood vessels including rabbit aorta. Thus, substances that reduce potassium outflow may counteract vascular relaxation. We assumed that quinine, by blocking Ca^{2+}-activated K^+ channels (Schwarz & Passow, 1983; Bartschat & Blaustein, 1985), might be such a substance. We also assumed that quinine might have inhibited the relaxations in response to ACh and A23187 by blocking the voltage-insensitive Ca^{2+}-activated K^+ channels. This assumption can be supported by the finding of Hermann & Gorman (1981) who reported that external quinine blocks the Ca^{2+}-activated K^+ current but acts intracellularly to block voltage-dependent K^+ current in excitable cells. The requirement of extracellular Ca^{2+} (Singer & Peach, 1982), and a rise in the level of ionised Ca^{2+}, following stimulation by A23187, within the endothelial cells (Zawadzki *et al.*, 1980; Gordon & Martin, 1983), for triggering endothelium-mediated vascular smooth muscle relaxation have been suggested. If the availability of Ca^{2+} *per se* is a prerequisite for the endothelium-mediated relaxation, as suggested by these authors, then it is probable that stimulation of the Ca^{2+}-activated K^+ channel could be an additional criterion for the release of the relaxant factor from endothelial cells.

The secretory process in several exocrine glands is thought to be associated with the stimulation of the Ca^{2+}-activated K^+ channels (Petersen & Maruyama, 1984). If such a mechanism operates in vascular endothelium as well, it is plausible to assume that quinine, a blocker of these K^+ channels, may inhibit the secretion of EDRF. The finding that tetraethylammonium, another blocker of the Ca^{2+}-activated K^+ channels, inhibited the ACh-induced relaxation of rat aorta (Rapoport *et al.*, 1985) is consistent with our assumption.

To support our hypothesis for the mechanism of action of quinine we have also studied the effects of TMA (3×10^{-3} to 6.5×10^{-2} M), TEA (10^{-3} to 3×10^{-2} M), and other blockers of the Ca^{2+}-activated K^+ channels (Walsh & Singer, 1983; Bartschat & Blaustein, 1985; Lattore, 1986), on the endothelium-dependent relaxation of the aortic strips. Treatment of the strips with either TMA or TEA depressed the ACh- and A23187-induced relaxations in a concentration-dependent manner, whereas it had no significant effect on the nitrite-induced relaxation. We suppose that these findings provide additional evidence for the requirement of the operation of the Ca^{2+}-activated K^+ channels for the endothelium-mediated relaxations by ACh or A23187. However, in contrast to quinine, TMA and TEA enhanced the response of the vascular smooth muscle cells to phenylephrine. Despite this difference, the blockers elicited qualitatively similar depressant actions on the endothelium-dependent relaxations of the strips. Isomolar replacement of the organic salts by sucrose (French & Wells, 1977) on the other hand, induced a small increase in basal tension and a 5% reduction in both EDRF- and nitrite-evoked relaxations (data not shown). It is assumed that a small component of the inhibitory actions of these blockers, particularly at higher concentrations, could also be partly due to the prevailing hyperosmolarity.

During incubation of the strips with quinine, TMA or TEA, we observed that the ACh-induced endothelium-dependent vasorelaxation was more readily (by 75%, 95% and 71%) inhibited than that due to A23187 (by 44%, 41% and 56%). Although the explanation for this difference remains unclear, it could be the impact of the larger magnitude of relaxation evoked by the ionophore ($P < 0.05$). It has also been observed that quinine reduced, while TEA enhanced, the sensitivity of the aortic strips to phenylephrine. The blockade of α-adrenoceptors (Mecca *et al.*, 1980) and of Ca^{2+} transport across the membrane (Couturier *et al.*, 1980) by quinine; and the depolarization (Benham *et al.*, 1985) and/or enhanced noradrenaline release (Kirpekar, 1978) by TEA (unfortunately, data on such effects of TMA are missing in the literature), may well explain the reduced or enhanced (respectively) contractile responsiveness of rabbit aortic strips to phenylephrine.

In conclusion, we propose that stimulation of the Ca^{2+}-activated K^+ channels may contribute to the endothelium-dependent relaxations induced by ACh or A23187, while their activation may not be required for the nitrite-induced vasorelaxation. To explain the nature of the interrelationship between EDRF and these K^+ channels further investigations need to be undertaken.

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