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Endothelial-dependent relaxant actions of carbachol and substance P in arterial smooth muscle

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1 In helical strips cut from the small mesenteric artery of guinea-pig (GPSMA) (0.3–0.6 mm o.d.) relaxations induced by substance P were more susceptible to damage of the endothelium by rubbing than were relaxations evoked by carbachol. Relaxations induced by 2-nicotin-amidoethyl nitrate (SG75) were unaffected by this procedure.

2 Relaxations evoked by the calcium ionophore A23187 persisted when those to substance P had been abolished by rubbing the endothelium in GPSMA, rabbit mesenteric and rabbit ear arteries. In guinea-pig pulmonary artery and aorta relaxations to A23187 were lost after this treatment.

3 Carbachol and SG75 were more effective in inhibiting phasic than tonic tension induced by noradrenaline in GPSMA, but substance P was more effective against tonic tension.

4 In the GPSMA, carbachol and substance P inhibited tension produced by noradrenaline to similar extents. However, carbachol was less, and substance P much less effective in inhibiting tension evoked by high-potassium solution than by noradrenaline.

5 Susceptibility of relaxations to blockade by haemoglobin in GPSMA was: substance P>carbachol > ATP > SG75.

6 The membrane potential of smooth muscle cells in the media of the GPSMA was recorded by microelectrode. Carbachol, but not substance P, hyperpolarized the cells both in the presence and absence of noradrenaline at concentrations which relaxed the muscle.

7 These results suggest a heterogeneity in the mechanisms of endothelial-dependent relaxations induced by various vascular relaxants.

Introduction

Acetylcholine-induced vasodilatation has been observed in both the intact animal and in isolated vascular smooth muscle preparations constricted by application of noradrenaline, by nerve stimulation, or by high potassium (Furchgott, 1955; Somlyo & Somlyo, 1970; Vanhoutte, 1977; Kuriyama & Suzuki, 1978; 1981). Relaxation by acetylcholine in a number of arteries has been shown recently to require the presence of intact endothelial cells (Furthgott & Zawadzki, 1980; Furchgott, 1981; De Mey *et al.*, 1982; De Voorde & Leusen, 1983). Responses to a number of other relaxant agents have also been shown to be dependent on the presence of intact endothelium; these include substances such as substance P, bradykinin, adenosine 5'-triphosphate (ATP), adenosine 5'-pyrophosphate (ADP), histamine and the Ca^{2+} ionophore A23187. Activation of muscarinic receptors of an intact segment of thoracic aorta relaxed a separate adjacent de-endothelialized aortic strip, while direct application of acetylcholine to the latter was without effect. Such experiments strongly suggest the release of factors from the endothelial cells (De Mey & Vanhoutte, 1981; Furchgott, 1981; Cherry *et al.*, 1982; Furchgott *et al.*, 1983; De Voorde & Leusen, 1983; Toda, 1984). Nevertheless there are a number of studies which suggest that a variety of substances (acetylcholine, ATP and bradykinin) may relax smooth muscle by a direct action (De Mey & Vanhoutte, 1981; Furchgott, 1984; Bolton *et al.*, 1984). The presence or absence of the endothelium seems likely to complicate the interpretation of electrophysiological data which have been obtained from various blood vessels. This might help to explain why contractions and relaxations associated with muscarinic receptor activation have been found to occur with hyperpolarization, depolarization, or no change in membrane potential (Kuriyama & Suzuki, 1978; 1981; Ito *et al.*, 1979; Kitamura & Kuriyama, 1979;

Takata, 1980; Bolton *et al.*, 1984). Hyperpolarization seen with carbachol has been shown to be endothelial-dependent (Bolton *et al.*, 1984).

In view of these findings, we sought to examine in more detail the role of the endothelium in the relaxation produced by various agents in a number of arteries which have been used in these electrophysiological studies. Experiments were also designed to investigate the properties of the relaxant responses induced by substance P and carbachol in the guinea-pig small mesenteric artery, in an attempt to find out whether they produce relaxations by similar or differing mechanisms.

Methods

Adult rabbits (1.5–2 kg) or guinea-pigs (300–400 g) of either sex were killed by cervical dislocation. Arteries taken from the guinea-pig were aorta (at the aortic arch), main pulmonary, and branches of the mesenteric to the jejunum (usually 1st or 2nd branch, with external diameters ranging from 0.3–0.6 mm) and from the rabbit were ear (0.6–0.8 mm) and branch of anterior mesenteric (external diameter 1 mm). They were excised and freed of surrounding veins and connective tissue under a dissecting microscope. Rings or helical strips were carefully cut to avoid unnecessary stretching or contact of instruments with the luminal surfaces so as to prevent damage of the endothelial cells (Furchgott & Zawadzki, 1980).

Tension recording

These arterial strips were suspended in a small vertical tube and connected to an isotonic or isometric transducer. They were continuously perfused with Krebs solution at 36°C (previously bubbled with 95% O_2, 5% CO_2) at a rate of 2 ml min^{-1}. An equilibrium period of 1 h was allowed after setting up each preparation under optimal basal tension (see Bolton & Clapp, 1984) viz: guinea-pig aorta and pulmonary artery (0.5 g); guinea-pig small mesenteric artery (0.25 g); rabbit mesenteric artery (0.4 g); and rabbit ear artery (0.5 g). Tension was measured either in g (isometric) or as percentage shortening, calculated by measuring the length of each arterial strip under basal tension and the change in length during drug application. When it was required, the endothelial layer was disrupted by rubbing the inner surface with a small portion of filter paper held in forceps until the paper disintegrated (Furchgott & Zawadzki, 1980).

Electrophysiological recordings

Guinea-pigs (200–300 g) were killed and a portion of the mesentery near the intestine excised. This was then pinned out in an organ bath (0.4 ml) and a branch of the mesenteric artery (0.2–0.3 mm o.d.) was carefully cleaned of the overlying connective tissue and vein. The muscle was superfused continuously at a rate of 2.0 ml min^{-1} with oxygenated Krebs solution warmed to 36°C. Intracellular recordings of membrane potential were made by penetrating smooth muscle cells with glass microelectrodes filled with 0.5 M KCl and having resistances between 100 and 200 MΩ (Hirst & Neild, 1981).

Solutions

A physiological (Krebs) solution of the following composition was used (mM): NaCl 120, KCl 5.9, $NaHCO_3$ 15.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.5. The pH was 7.2. Solutions containing elevated concentrations of potassium were made by replacing an equivalent amount of NaCl with KCl. A drug was applied by switching to another perfusing solution of the same ionic composition but containing the drug. The effects on tension of a brief (30s) application of a relaxant were studied in arterial strips which were first contracted by adding noradrenaline to the perfusing solution or by raising the potassium concentration, i.e. relaxations were studied on 'tonic' or 'existing' tension. The inhibitory effects of relaxants were also studied on the contractile responses ('phasic' tension) to brief applications (30 s or 1 min) of stimulant. The relaxant was added 30 s before the stimulant and was present during application of the stimulant.

In electrophysiological recordings, drug applications were made by switching to a Krebs solution containing the drug, or by injecting a small volume (at a known concentration) at a constant rate into the organ bath, using a syringe pump (Harvard Apparatus).

Preparation of haemolysate

Heparinized blood, 10–15 ml, taken from rabbit was centrifuged for 20 min at 190 *g* and the plasma supernatant discarded. Of the remaining erythrocytes 2 ml was haemolysed by the addition of 18 ml of distilled water and left for 20 min before a further 20 fold dilution was made into Krebs solution. The approximate final concentration of haemoglobin used in each experiment was 10^{-5} M.

Drugs

The following drugs were used: indomethacin N-methyl glucamine (Liometacen, Chiesi Farmaceutici); 2-nicotin-amidoethyl nitrate (SG 75, Chugai Pharmaceutical Co. Ltd); histamine dihydrochloride, noradrenaline hydrochloride (Koch-Light); prazosin

hydrochloride (Pfizer); calcium ionophore (A23187), carbachol chloride, disodium adenosine 5'-triphosphate, nordihydroguaiaretic acid (NDGA), substance P (Sigma); isoprenaline hydrochloride (Wellcome).

Treatment of results

Inhibitory or relaxant responses were expressed as the percentage change in the tension induced by the stimulant agent. Results shown in the text and figures are expressed as the mean value ± s.e.mean. Statistical analysis was by means of Student's *t* test and Mann-Whitney (Wilcoxan) rank test.

Results

Effect of substance P, carbachol and SG75 on phasic and tonic tension induced by noradrenaline in the guinea-pig small mesenteric artery

Contractions evoked by short exposures to noradrenaline were strongly inhibited by carbachol (10^{-7}–10^{-5} M). Substance P (5×10^{-10}–10^{-7} M) was generally more potent than carbachol in its inhibitory action on phasic contractions to noradrenaline, but its maximal effect was often less (Figures 1a and 2). Carbachol was somewhat less effective in reducing existing tension produced by the addition of noradrenaline to the bathing solution than it was in inhibiting phasic noradrenaline contractions. In contrast, substance P was slightly more effective on tonic than on phasic tension induced by noradrenaline (Figures 1 and 2). SG75 resembled carbachol in that it was less effective in inhibiting tonic than phasic tension but it was less potent than either carbachol or substance P in its inhibitory actions (Figure 2).

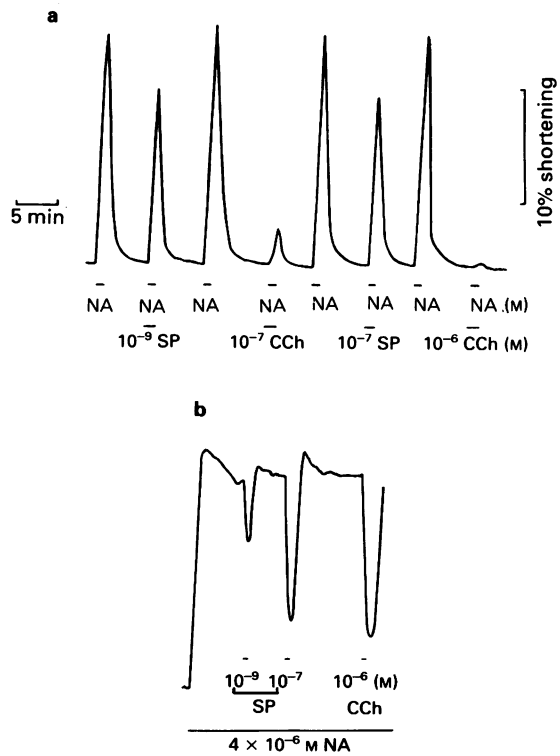

Figure 1 The effect of carbachol (CCh) and substance P (SP) on contractions induced by short applications (30 s) of noradrenaline (NA) (4×10^{-6}M) (a) or on an existing contraction (induced by NA) in a helical strip of guinea-pig small mesenteric artery (b). Substance P was more effective in relaxing existing tension than in inhibiting contraction to short applications of noradrenaline. Percentage shortening in (a) and in subsequent figures denotes the percentage change in the resting length of the arterial strip under basal tension.

Role of endothelium in relaxant responses to carbachol, substance P and SG75

The effects of removal of the endothelium by rubbing the intimal surface of the artery with filter paper on the inhibitory effects of carbachol, substance P and SG75 were examined in the guinea-pig small mesenteric artery. A single rub of the intimal surface of a strip always abolished the inhibition seen with substance P of both tonic (Figure 3) and phasic noradrenaline-induced tension. In contrast, higher concentrations of carbachol (10^{-5}–10^{-4} M) still inhibited both tonic (Figures 3b and 4) and phasic noradrenaline-induced tension after one rub of the endothelial layer. Further rubbing of the endothelium abolished relaxations to carbachol (Figure 3c). Bolton *et al.* (1984) also showed that rubbing had much less effect on relaxation of noradrenaline-induced tension by carbachol compared with the effect on relaxant responses to the ionophore A23187. They postulated the presence of a direct inhibitory action of carbachol on the smooth muscle. Although the present experiments do not rule out this possibility, most of the relaxation to carbachol eventually disappeared upon repeated rubbing. Damage to the endothelium sometimes unmasked an excitatory response to low (10^{-7} M) concentrations of carbachol which potentiated the response to a brief application of noradrenaline. After intimal rubbing, substance P never caused potentiation of the noradrenaline response.

The lack of inhibition shown by carbachol or substance P after rubbing could not be attributed to damage of the smooth muscle cells since inhibition of the contraction, to brief exposures of noradrenaline, by SG75 was not reduced by two or more rubbings of

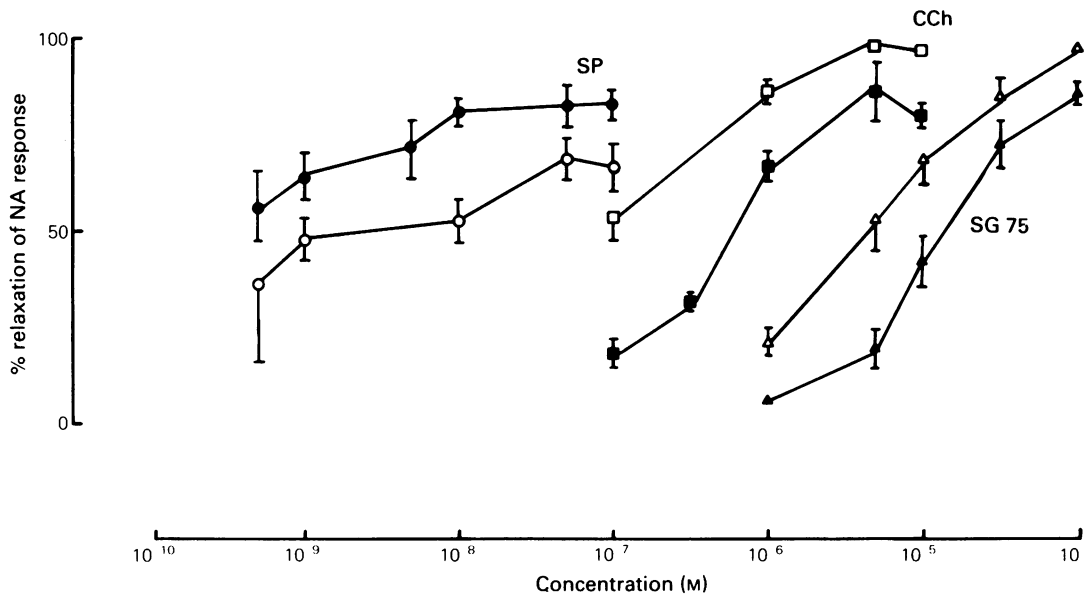

Figure 2 Summary of the effects of substance P (SP), carbachol (CCh) and SG75 on phasic (open symbols) and tonic (closed symbols) noradrenaline-induced tension in the guinea-pig small mesenteric artery. Carbachol and SG75 were more effective against existing tension. Each point represents the mean of at least five experiments. Vertical lines show s.e.mean.

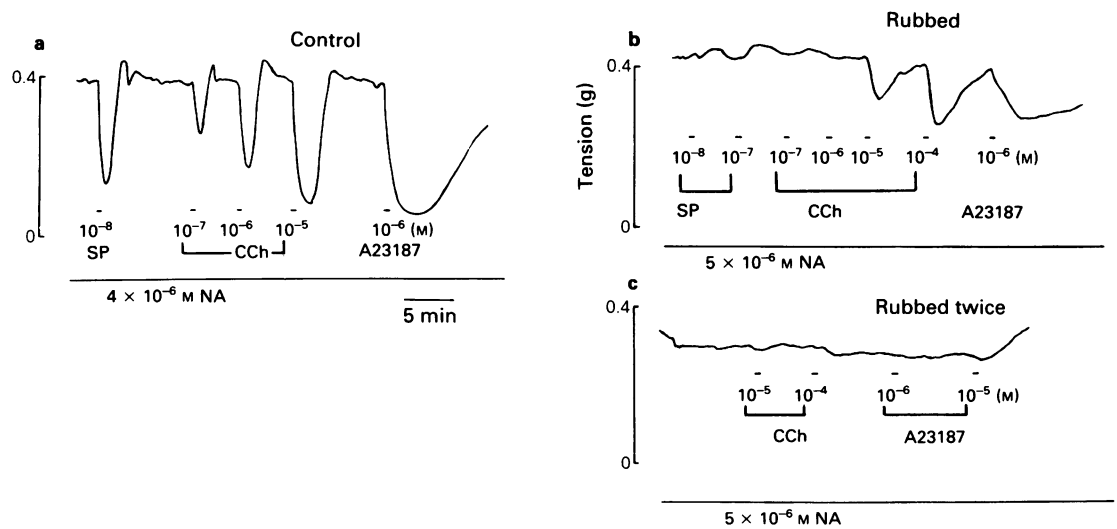

Figure 3 (a) Tonic contraction of a strip of guinea-pig mesenteric artery by noradrenaline (NA, 4×10^{-6} M) was relaxed by substance P (SP), carbachol (CCh) and A23187. (b) After rubbing the intimal surface the response to substance P was abolished, while carbachol (10^{-5}–10^{-4} M) and A23187 (10^{-6} M) still relaxed NA-evoked tension, though were much less potent. (c) A second rub of the intimal surface of the same artery abolished relaxations to carbachol and A23187. The sensitivity to noradrenaline was little changed.

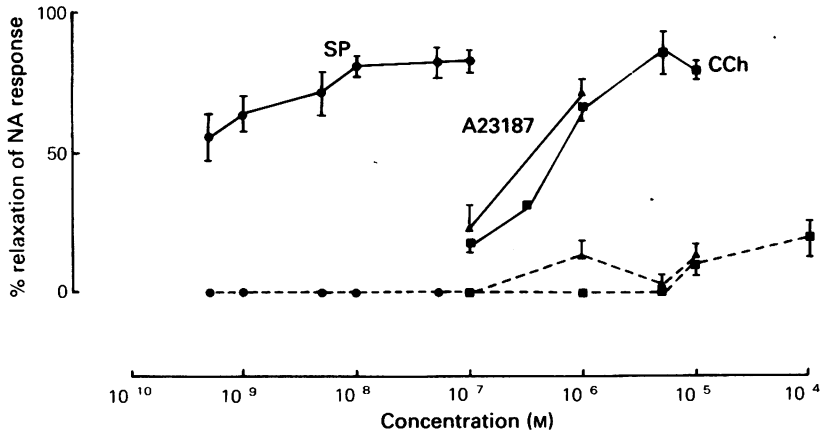

Figure 4 Summary of the effect of rubbing the intimal surface on relaxations of the guinea-pig small mesenteric artery produced by substance P (SP, ●), A23187 (▲) and carbachol (CCh, ■). Existing tension was induced by noradrenaline (NA, $1-6 \times 10^{-6}$ M). Relaxations induced by substance P were always abolished by this treatment, whereas a small inhibitory effect of carbachol and A23187 was still seen at high concentrations. Controls – solid lines; after intimal rubbing – broken lines.

the endothelium – in fact inhibition evoked by SG75 was slightly potentiated at most concentrations (Figure 5). In experiments performed on the same strips, inhibition by carbachol (10^{-6}–10^{-5} M) was abolished upon rubbing of the endothelium (Figure 5). Relaxations of noradrenaline-induced tension produced by SG75 in rabbit ear artery strips were also not affected by intimal rubbing.

Inhibition by substance P or carbachol of the contraction induced by high-potassium solutions

In view of the differences in the action of substance P and carbachol observed on noradrenaline-induced tension in the guinea-pig small mesenteric artery, we decided to look at their inhibitory effects when tension was induced by raising the potassium concentration in the bathing solution (an equal amount of sodium was removed). If carbachol and substance P acted on different receptors but brought into play the same mechanisms, it might be expected that they would inhibit contractions induced by high potassium (high-K) in a similar way. Conversely, if they acted to release different inhibitory factors, or carbachol had some additional effect, K-induced tension may be inhibited to a different extent.

Experiments showed that both substance P and carbachol were significantly ($P < 0.01$) less effective in inhibiting phasic and tonic tension induced by high-K than tension produced by noradrenaline. Contractions in response to brief applications of high-K solution were significantly ($P < 0.05$ using the Mann-Witney statistical test) more inhibited by carbachol than by substance P (see Figure 6). When substance P or carbachol was applied to arteries tonically contracted by high-K the maximum relaxation of tension seen with substance P was 46 ± 3% ($n = 4$) at 47 mM $[K^+]_o$ and 34 ± 5% ($n = 4$) at 126 mM $[K^+]_o$ compared to the corresponding values of 67 ± 3% ($n = 7$) and 62 ± 3%

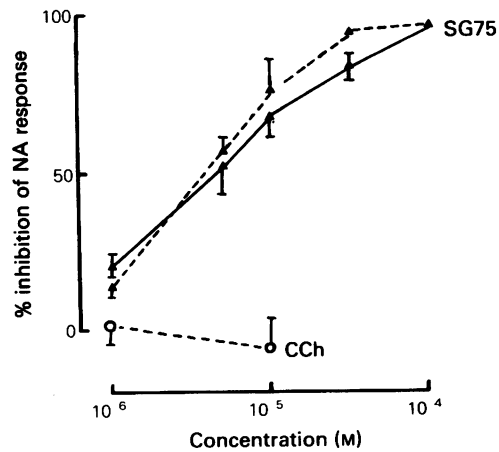

Figure 5 The inhibitory effect of SG75 on contractions to brief applications of noradrenaline (NA). Results are from five experiments where SG75 was applied to the same strip before and after endothelial rubbing. The inhibitory effect of SG75 was not reduced, whereas carbachol (CCh, 10^{-6}–10^{-5} M) had no inhibitory effect on the rubbed preparations. Solid lines – controls; broken lines – rubbed preparations.

($n = 5$) with carbachol (Figure 7). Contractions in response to short applications of 126 mM $[K^+]_o$ were inhibited 17 ± 5% ($n = 7$) by substance P (5×10^{-8} M) and 52 ± 7% ($n = 8$) by carbachol (10^{-5} M). Rubbing of the endothelium in the guinea-pig small mesenteric artery abolished inhibition by substance P and by carbachol of the tension generated by short applications of 126 mM $[K^+]_o$.

We also examined the effect of substance P and carbachol on tonic tension induced by high-K solution in the rabbit ear artery with intact endothelium. Relaxant responses were of a similar magnitude whether tension was generated by 21 mM $[K^+]_o$ (just above threshold for contraction) or by 4×10^{-7} M histamine. However, relaxations induced by substance P and carbachol were both significantly less in 57 mM $[K^+]_o$. Carbachol (10^{-5} M) and substance P (5×10^{-8} M) relaxed arteries tonically contracted with high-K by 26 ± 10%, ($n = 5$) and 36 ± 10% ($n = 4$), respectively. This result is in contrast to that in the guinea-pig small mesenteric artery where carbachol was significantly more effective than substance P in inhibiting tension induced by high-K solutions. It may be that, in contrast to the situation in the guinea-pig small mesenteric artery, carbachol and substance P act via a similar mechanism in the rabbit ear artery.

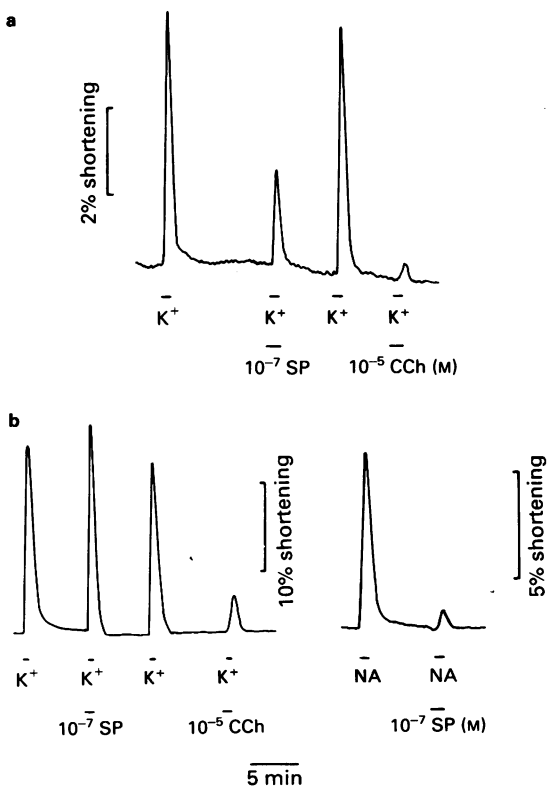

Figure 6 Inhibition by substance P (SP) and carbachol (CCh) of contractions to short applications of noradrenaline (NA) (2×10^{-6}M) and high-K (a, 47 mM; b, 126 mM) in the guinea-pig small mesenteric artery. Substance P (10^{-7} M) inhibited contractions to 47 mM but not 126 mM $[K^+]_o$, whereas carbachol (10^{-5} M) was a potent inhibitor of both. In the same strip, substance P inhibited contractions to a brief application of noradrenaline to a greater extent than those to high-K.

Effect of indomethacin and haemoglobin on inhibitions produced by carbachol and substance P

In an attempt to elucidate the possible mechanisms involved in the inhibitory effects of substance P and carbachol, we examined the effects of indomethacin (a cyclo-oxygenase inhibitor) and haemoglobin (a guanylate cyclase inhibitor) on relaxations produced by substance P and carbachol.

Nordihydroguaiaretic acid (a lipoxygenase inhibitor) itself severely reduced tension in the guinea-pig small mesenteric artery. For this reason we were unable to examine its effects on relaxations to other agents. In experiments using indomethacin, substance P and carbachol were given before and in the presence of the inhibitor, at least 35 min being allowed between applications. Results showed that the ability of carbachol and substance P to inhibit tension evoked by noradrenaline was not significantly affected by addition of indomethacin (3×10^{-5} M). This result does not support the idea that these endothelium-dependent relaxations are mediated by a product(s) of the cyclo-oxygenase pathway (cf Furchgott, 1981).

Since there are reports connecting increased levels of cyclic GMP with endothelial-dependent relaxations (e.g. Rapaport & Murad, 1983), we looked at the effect of haemoglobin, which is known to block guanylate cyclase (Mittal *et al.*, 1978), on relaxations elicited by substance P, carbachol, ATP and SG75. In the presence of haemolysate (haemoglobin at about 10^{-5} M) relaxations to substance P (10^{-8}–10^{-7} M) and carbachol (10^{-6}–10^{-5} M) were abolished, but substantial relaxations to ATP and SG75 still remained. Relaxations induced by substance P were most sensitive to blockade by haemoglobin, whereas carbachol was slightly more resistant (not shown) which may be because carbachol has a small direct effect as well (Figure 8). The relaxant action of ATP, which is known to have both direct and endothelial-dependent actions on smooth muscle (Bolton *et al.*, 1984), was significantly ($P < 0.01$) less inhibited by haemoglobin, compared to substance P and carbachol. The dose-response curve to SG75 was also shifted to the right by haemoglobin but a substantial relaxation of 65 ± 4% ($n = 10$) was seen to 10^{-4} M SG75. The shift in the dose-response curve suggests that SG75 may, as part of its mechanism of relaxation, increase cyclic GMP

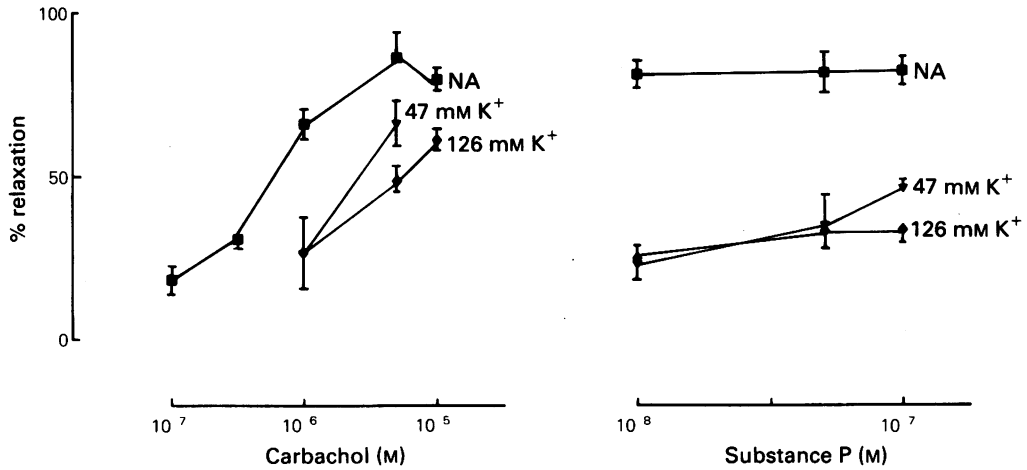

Figure 7 Inhibitory effect of substance P and carbachol on tonic tension evoked by high-K solution and by noradrenaline (NA, $1-5 \times 10^{-6}$ M) in the guinea-pig small mesenteric artery. Carbachol or substance P were applied either on the same strip or strips taken from the same artery and run in parallel experiments. Carbachol was more effective than substance P at inhibiting tension evoked by 47 mM $[K^+]_o$ or 126 mM $[K^+]_o$.

via a direct action on the smooth muscle.

As haemoglobin was an effective blocker of endothelium-dependent relaxations by substance P and carbachol, it may be that these relaxations are mediated via increases in cyclic GMP caused by the action of endothelial-derived factors on the smooth muscle cells.

Substance P and carbachol in other arteries

We examined the effects of substance P and carbachol in a number of different arteries both from guinea-pig and rabbit to determine the role of the endothelium in the relaxations produced by both agents. In the rabbit ear artery, both carbachol and substance P relaxed tension evoked by histamine (4×10^{-7} M) (Figure 9a). The maximum relaxation seen with substance P (10^{-7} M) was $88 \pm 4\%$ ($n = 5$) and with carbachol (10^{-5} M) $89 \pm 8\%$ ($n = 4$). In preparations subjected to a single rub of the intimal surface there was complete loss of the relaxation responses to carbachol and substance P at all concentrations (Figure 9b). This was unlike the guinea-pig small mesenteric artery, where carbachol relaxations were more resistant to rubbing of the endothelium. In the same rubbed preparations, the potent relaxing effect of isoprenaline (in the presence of 2×10^{-7} M prazosin) was unchanged or slightly reduced. The response was little further affected upon a second rubbing of the intimal surface.

In the guinea-pig aorta, concentrations of carbachol up to 10^{-5} M were ineffective at eliciting relaxation of preparations contracted with noradrenaline. Relaxa-

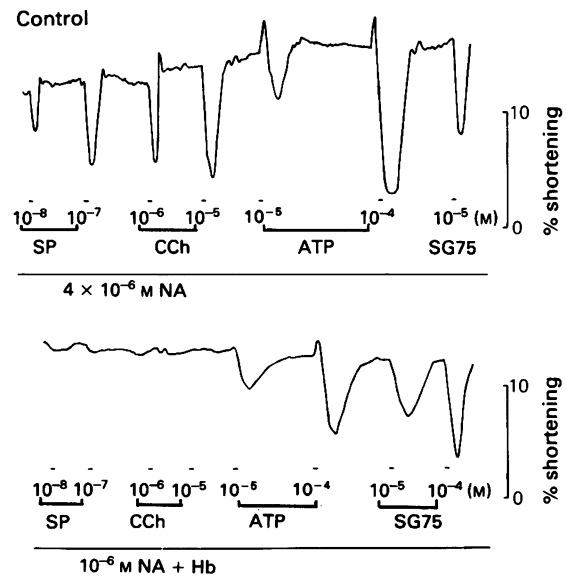

Figure 8 Effect of haemoglobin (Hb, approximately 10^{-5} M) on relaxations produced by substance P (SP), carbachol (CCh), ATP and SG75 in the guinea-pig small mesenteric artery. Haemoglobin abolished relaxations seen with carbachol and substance P, but was much less effective at inhibiting the relaxations to ATP and SG75. In the presence of haemoglobin, the sensitivity to noradrenaline (NA) was increased 4 fold.

tions to substance P and ATP were also small in this preparation, the average maximum relaxation was 28% (at 10^{-6} M) and 25% (at 10^{-4} M), respectively. However, strong relaxations in response to the ionophore A23187 were observed and these were readily abolished upon rubbing.

In the guinea-pig pulmonary artery, carbachol was a weak relaxant of noradrenaline-induced contraction; at 5×10^{-5} M carbachol the maximum relaxation was 18%. However, substance P (10^{-8}–10^{-7} M) and ATP (10^{-6}–10^{-4} M) strongly relaxed noradrenaline-evoked contraction. Rubbing of the endothelium abolished relaxations induced by substance P and carbachol, as well as those to the ionophore A23187; but ATP still relaxed the contracted arteries, though to a lesser extent.

In the rabbit small mesenteric artery, relaxations to carbachol were not always abolished upon destruction of the endothelium. Endothelial damage often unmasked an excitatory response to carbachol followed by relaxation.

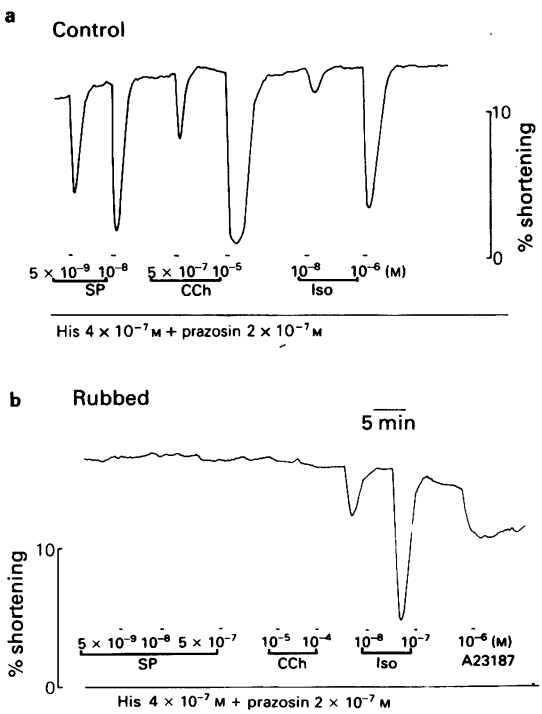

Figure 9 (a) Responses to substance P (SP), carbachol (CCh) and isoprenaline (Iso) on a strip taken from the rabbit ear artery with intact endothelium. (b) After rubbing the intimal surface, the relaxation responses of the same strip to isoprenaline were little changed, but relaxation responses to carbachol and substance P were abolished. The ionophore A23187 relaxed the ear artery despite the disappearance of the responses to substance P and carbachol.

A23187

It has been shown by Furchgott (1981) that the ionophore A23187 produces powerful endothelial-dependent relaxations in many blood vessels. In the guinea-pig pulmonary artery and aorta, relaxations to A23187 (10^{-7}–10^{-5} M) totally disappeared upon rubbing of the endothelium. In the guinea-pig and rabbit mesenteric artery and rabbit ear artery relaxations to the ionophore were abolished at low concentrations ($< 10^{-6}$ M) and substantially reduced at higher concentrations. However, relaxations, though generally slow in nature, could often be seen after responses to substance P (and sometimes carbachol) had disappeared (Figure 9b). Sometimes biphasic responses were observed. It is likely that A23187 has effects on smooth muscle cells as well as on the endothelium.

Effects of carbachol and substance P on membrane potential

In view of the differences in the action of substance P and carbachol in the guinea-pig small mesenteric artery, the effects of these two agents on the membrane potential of smooth muscle cells in this artery were examined. It was of interest to see whether substance P caused an endothelial-dependent hyperpolarization of the membrane, previously described for carbachol in this artery (Bolton *et al.*, 1984). Intracellular recordings of membrane potential were made in response to applications of substance P or carbachol at concentrations that produced maximal relaxation of noradrenaline-evoked tension. The resting membrane potential was -61.4 ± 1.4 mV ($n = 24$). Application of substance P (10^{-6} M) for 1 min did not modify the membrane potential (Figure 10b). In the same cell (not shown) raising $[K^+]_o$ to 47 mM for the same period of time depolarized the membrane to -35 mV. Also, in the same preparation carbachol (10^{-5} M) hyperpolarized the membrane by some 11 mV (Figure 10a). In five experiments substance P did not produce any significant hyperpolarization, the membrane potential was -56.0 ± 2.0 mV in the absence and -56.1 ± 2.5 mV ($n = 5$) in the presence of substance P. Control injections of Krebs solution had a similar lack of effect on the membrane potential. Carbachol (10^{-5} M) hyperpolarized the membrane from -62.8 ± 2.2 mV to -69.2 ± 2.1 mV ($n = 6$). Substance P was always added first so that its lack of effect on the membrane potential could not be explained by failure of the endothelial factor releasing mechanism to recover. The effects of carbachol and substance P on the membrane potential in the presence of noradrenaline are also shown in Figure 10. Substance P (10^{-7} M)

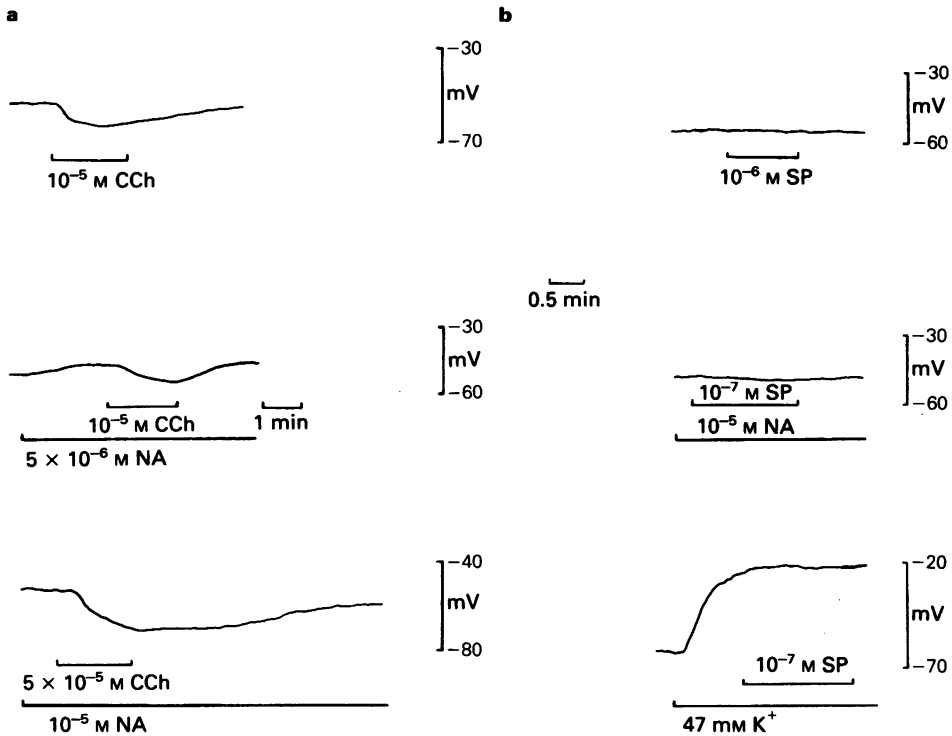

Figure 10 Effects of (a) carbachol (CCh) and (b) substance P (SP) on the membrane potential (recorded intracellularly) in the guinea-pig small mesenteric artery. Carbachol and substance P were present in the bathing solution for the period (1–1.5 min) indicated by the horizontal bar. Carbachol hyperpolarized the membrane in normal solution and in the presence of noradrenaline (NA, 5×10^{-6} or 10^{-5} M). The effect of substance P on the membrane potential was negligible and no different from the effects of control injections of vehicle solution.

had little or no effect on the membrane potential in the presence of 10^{-5} M noradrenaline. In contrast, carbachol repolarized the membrane back to near resting level when the cell was depolarized by either 5×10^{-6} or 10^{-5} M noradrenaline. Substance P had no significant effect on membrane potential in the presence of 47 mM $[K^+]_o$. These results indicate that substance P relaxes arteries in the absence of any significant changes in the membrane potential. Carbachol appears to have an additional endothelial-dependent hyperpolarizing action on the membrane which might serve to reinforce other endothelial-dependent effects which do not involve a conductance change, since carbachol-induced relaxations were still observed in 126 mM $[K^+]_o$ when changes in membrane potential do not occur (Bolton *et al.*, 1984).

Discussion

The results described in this paper support the proposal that vascular relaxants release endothelial-derived factors (Furchgott & Zawadzki, 1980). Experiments showed that the presence of intact endothelium was required for at least a large component of the observed relaxations to substance P, carbachol, and A23187 in a number of arteries including the guinea-pig small mesenteric artery. However, there were certain differences between the actions of substance P and carbachol which may be related to the existence of more than a single mechanism or pathway (Furchgott, 1984) by which relaxations are produced in the guinea-pig small mesenteric artery by these agents.

Damage to the endothelium had more effect on the relaxations evoked by substance P than on those evoked by carbachol in guinea-pig small mesenteric artery. Often a light rub of the endothelium abolished relaxation to substance P whereas further rubbing was required to abolish relaxation to higher concentrations of carbachol. Substance P was less effective than carbachol at inhibiting tension evoked by raising the potassium concentration, although these drugs were equally effective at inhibiting existing tension

produced by noradrenaline. Also, substance P was more effective at inhibiting tonic rather than phasic noradrenaline-induced tension whereas carbachol and SG75 inhibited phasic tension more strongly. Although haemoglobin largely inhibited relaxations to substance P and carbachol, the latter were noticeably more resistant to the effect of haemoglobin. A simple hypothesis which might explain these differences is that, in the guinea-pig small mesenteric artery carbachol and substance P release different substances from the endothelium. Other studies in the literature suggest heterogeneity in the mechanism of endothelial-dependent relaxations by different agents in the same artery (De Mey *et al.*, 1982; Singer & Peach, 1983). Also Gordon & Martin (1983), in studying rubidium efflux from endothelial cells of the pig aorta, found that ATP, bradykinin and A23187 all increased efflux whereas acetylcholine did not, indicating a different pathway for the latter. Differences in the effect of inhibitors on relaxation induced by methacholine and A23187 in aorta have been described by Singer & Peach (1983).

If carbachol and substance P do release different inhibitory factors from the endothelium, it would not only explain the different effects of these two drugs on tension, but also the dissimilar electrophysiological responses produced by their application to arteries with intact endothelium. To explain these and other results it would have to be supposed not only that carbachol releases an inhibitory factor (or factors) from the endothelium which inhibits tension generation by a potential-independent mechanism (since relaxation of tension engendered by high $[K^+]_o$ occurs without change in membrane potential, Bolton *et al.*, 1984), but also, that this factor, or another released at the same time, acts to hyperpolarize the membrane in low $[K^+]_o$ solutions. Such an effect, by closing any potential-sensitive calcium channels which might be open, would reinforce the potential-independent mechanism (Bolton, 1979). Substance P seems not to release the hyperpolarizing factor in effective quantities, although it is a potent relaxant of noradrenaline-induced tension.

Experiments with haemoglobin showed that it inhibited relaxations with the following order of potency: substance P > carbachol > ATP > SG75. This different susceptibility may indicate that agents produce relaxation by a number of pathways. Relaxation induced by substance P and carbachol were almost abolished by haemoglobin in the guinea-pig small mesenteric artery, suggesting that endothelial-dependent relaxations involve increased levels of cyclic GMP. There is a correlation between increases in cyclic GMP levels and endothelial-dependent relaxations (Rapport & Murad, 1983). Relaxations induced by ATP were partially reduced by either rubbing the endothelium or by haemoglobin, suggesting that relaxation occurs in part via an endothelial-dependent mechanism and in part via a mechanism which presumably does not involve changes in cyclic GMP. The action of SG75 was unaffected by rubbing the endothelium and was only partially inhibited by haemoglobin, indicating that more than one mechanism is involved in the direct relaxant action of SG75 (see Itoh *et al.*, 1981).

Carbachol apparently acts on both excitatory and inhibitory receptors on smooth muscle (Kuriyama & Suzuki, 1978; Kitamura & Kuriyama, 1979; Bolton *et al.*, 1984) so that the final tension response will be determined by the balance between these two effects. Damage to the endothelium favours the excitatory effect of carbachol and removal of the endothelium converts the hyperpolarizing action of carbachol to a depolarization (Bolton *et al.*, 1984). However, in the rabbit ear artery, responses to substance P and carbachol were similarly affected by endothelial damage and these drugs were equally potent in inhibiting tension induced by high-K solution. This may indicate that these drugs release the same endothelial factor(s) in this artery, in contrast to the suggested diversity in the guinea-pig small mesenteric artery.

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