J. Physiol. (1984), **351**, *pp.* 549–572
With 14 *text-figures*
Printed in Great Britain

MECHANISMS OF ACTION OF NORADRENALINE AND CARBACHOL ON SMOOTH MUSCLE OF GUINEA-PIG ANTERIOR MESENTERIC ARTERY

BY T. B. BOLTON, R. J. LANG AND T. TAKEWAKI

From the Department of Pharmacology, St George's Hospital Medical School, London SW17 0RE

(*Received 3 November 1983*)

SUMMARY

1. Membrane potential was recorded by micro-electrode in segments of small (200–500 μm o.d.) mesenteric arteries of guinea-pig. Isotonic shortening was recorded in helical strips cut from these arteries.

2. Raising the external potassium concentration, $[K^+]_o$, caused shortening and substantial depolarization. The threshold for contraction was about 30 mM which corresponded to a membrane potential of about -45 mV. Since high-potassium contractions were abolished in calcium-free solution it was suggested that they occur due to potential-sensitive calcium channels opening positive to about -45 mV.

3. Noradrenaline weakly depolarized the muscle and produced contractions resistant to calcium-free conditions. It was suggested that noradrenaline contractions are mainly caused by mechanisms other than the opening of potential-sensitive calcium channels, namely entry of calcium via other channels and release of stored calcium.

4. Carbachol had no effect on basal tension but inhibited shortening by noradrenaline or by raising $[K^+]_o$. The inhibitory effect of carbachol on tension under various conditions was associated with hyperpolarization or depolarization in a range negative to -45 mV, or no effect on potential, so that modulation of the number of open potential-sensitive calcium channels could not be evoked to explain its relaxant action.

5. Removal or destruction of the endothelium by rubbing or by distilled water perfusion left tension responses to noradrenaline or raised $[K^+]_o$ essentially unchanged. However, the inhibitory effect of carbachol on tension was attenuated and hyperpolarization of the resting artery was converted to a depolarization.

6. It was concluded that carbachol has both a strong inhibitory and a weak excitatory effect on these vascular smooth muscle cells. Membrane potential changes are not essential to its inhibitory action but may, by closing potential-sensitive calcium channels, sometimes reinforce it. Hyperpolarization by carbachol may be caused by a factor released by the action of carbachol on endothelial cells: in its absence carbachol may weakly depolarize but this alone is normally insufficient to generate tension.

INTRODUCTION

Atropine-sensitive vasodilation produced by cholinomimetic compounds has been demonstrated in a number of peripheral vascular beds (Burn & Rand, 1965; Malik & Ling, 1969) and in isolated vascular smooth muscles which have been previously constricted by sympathetic nerve stimulation, by exogenous application of noradrenaline, or by solutions of raised potassium concentration (Rand & Varma, 1970; Kuriyama & Suzuki, 1978). This vasodilator action of muscarinic agonists has been attributed, in part, to modulation of adrenergic neuromuscular transmission through the activation of muscarinic receptors on the sympathetic nerve terminals, i.e. the release of noradrenaline and the amplitude of the excitatory junctional potential are both reduced by low concentrations of acetylcholine (Steinsland, Furchgott & Kirpekar, 1973; Vanhoutte, Lorenz & Tyce, 1973; Vanhoutte, 1976; Kuriyama & Suzuki, 1981). However, in addition to their presence on sympathetic nerve endings, both excitatory and inhibitory muscarinic receptors occur elsewhere in blood vessels: contraction to acetylcholine has often been reported for the smooth muscle of larger blood vessels (Furchgott, 1955) and these include non-innervated umbilical arteries (Gokhale, Gulati, Kelkar & Kelkar, 1966) which implies a direct action. Relaxation has been described in a number of arteries (Kuriyama & Suzuki, 1978; Furchgott & Zawadzki, 1980; Takata, 1980).

The effects of exogenously applied noradrenaline on the membrane potential during contraction of vascular muscle differ from the changes in membrane potential seen during sympathetic nerve stimulation or upon exposure to raised external potassium concentration. Nerve stimulation generally produces excitatory junctional potentials which upon reaching threshold trigger an action potential and contraction (Speden, 1964, 1970; Bell, 1969; Hirst, 1977; Hirst & Neild, 1978, 1980; Holman & Surprenant, 1979; Surprenant, 1980; Kuriyama & Suzuki, 1981). Contractions to raising the external potassium concentration are associated with large depolarizations of the cells (Kuriyama & Suzuki, 1978; Mulvany, Nilsson & Flatman, 1982). On the other hand, contractions produced by exogenous noradrenaline (usually at concentrations $> 10^{-7}$ M) may be associated with no change or only a small depolarization of the membrane and with an increase, a decrease, or little change in membrane resistance (Su, Bevan & Ursillo, 1964; Droogmans, Raeymaekers & Casteels, 1977; Casteels, Kitmura, Kuriyama & Suzuki, 1977; Takata, 1980; Holman & Surprenant, 1980; Karashima, 1981; Kajiwara, Kitamura & Kuriyama, 1981), or with substantial depolarization (Trapani, Matsuki, Abel & Hermsmeyer, 1981; Harder, Abel & Hermsmeyer, 1981). The actions of exogenously applied noradrenaline can be readily reduced by α-adrenoreceptor blocking agents while only high concentrations of these (10^{-4} M) reduce the amplitude of the excitatory junction potential (Holman & Surprenant, 1980; Kajiwara *et al.* 1981; Cheung, 1982).

Contractions produced by muscarinic stimulants have been reported to be associated with depolarization (guinea-pig portal vein; Takata, 1980) with hyperpolarization (guinea-pig coronary artery; Kitamura & Kuriyama, 1979) or with no change in membrane potential or resistance (pig coronary artery; Ito, Kitamura & Kuriyama, 1979). Muscarinic-receptor-induced relaxation of vascular smooth muscle previously contracted with noradrenaline or raised external potassium solution has also been

reported, for example in rabbit and guinea-pig anterior mesenteric artery (Kuriyama & Suzuki, 1978; Takata, 1980), rabbit and rat thoracic aorta (Furchgott & Zawadzki, 1980; Davies & Williams, 1983) and canine femoral and coronary artery (Ito, Kitamura & Kuriyama, 1980; De Mey & Vanhoutte, 1981). In branches of mesenteric artery of guinea-pig (Takata, 1980) or of rabbit (Kuriyama & Suzuki, 1978), relaxation by muscarinic agents was associated with hyperpolarization, no change in membrane potential, or depolarization (see also Kuriyama & Suzuki, 1981). Muscarinic activation of an intact segment of thoracic aorta caused relaxation of an apposed strip of aorta whose endothelium had been removed whereas direct exposure of the de-endothelialized strip to muscarinic agonist was without effect (Furchgott, 1981). The inhibitory actions of a number of other 'vasodilating' agents, histamine, ATP and vasoactive intestinal polypeptide, have also been reported to be dependent on the presence of the endothelium (De Mey, Claeys & Vanhoutte, 1982; Davies & Williams, 1983).

The present experiments were undertaken to attempt to unravel the mechanisms, direct and indirect, by which noradrenaline produces contraction and carbachol (a stable muscarinic agonist) causes relaxation in guinea-pig mesenteric artery. Some of this work has been previously presented in brief (Lang & Takewaki, 1983).

METHODS

Guinea-pigs (200–500 g) were killed and the anterior mesenteric artery excised with its branches to the jejunum attached. The first or second branch of the mesenteric artery to the jejunum (0·2–0·5 mm o.d.) was dissected free of surrounding veins and connective tissues or, for some micro-electrode studies, a portion of mesentery containing the artery pinned out. This small mesenteric artery was used for all studies.

Tension recordings

Helically cut strips of the artery (0·5 mm width) were suspended in a small vertical tube and connected to an isotonic or isometric tension transducer. They were continuously perfused (at 2 ml/min) with Krebs solution warmed to 35 °C and previously bubbled with 95% O_2:5% CO_2 (Bolton, Lang & Ottesen, 1981). Strips were suspended intact or after their endothelium layer had been removed. The endothelial cells were destroyed either by the mechanical rubbing of the internal surface of the artery (Furchgott & Zawadzki, 1980), with a piece of filter paper or by perfusing the lumen of the artery with distilled water (10–30 min) before the helical strips were prepared.

Electrophysiological recordings

Intracellular recordings were made from small intact segments (0·5–1·0 cm) of the artery using glass micro-electrodes filled with 3 M-KCl and having resistances of 30–80 MΩ (Bolton, 1972). The arteries were either placed in an organ bath (0·2 ml volume) similar to that described by Hirst, Holman & Spence (1974) or in a partition chamber in which large extracellular silver–silver chloride plate electrodes were used to elicit electrotonic potentials (Abe & Tomita, 1968; Bolton, 1972).

Histology

Segments or strips of artery were examined microscopically to ascertain whether mechanical rubbing or distilled-water perfusion of the mesenteric artery was effective in removing the endothelial cells. Segments of artery were embedded in blocks of guinea-pig liver and frozen in isopentane previously cooled with liquid nitrogen. Transverse sections, 10–15 μm thick, were cut on a cryostat (freezing microtome). These sections were stained with either Toluidine Blue, or using a silver staining method kindly suggested by Dr M. Costa. Sections were stained in Toluidine Blue (0·1%) for 3–5 min, and then dehydrated and washed in xylene. To stain with silver the sections were incubated in a glucose solution (5% w/v) for 5 min, in an aqueous solution of silver nitrate

(0·4 % w/v) for 30 s, and then fixed in 4 % w/v formaldehyde (1 min). The silver was precipitated by bathing the sections in fresh photographic developer (FF Ilford Contrast) for 3 min. Sections were mounted in D.P.X. Mountant (BDH) and viewed microscopically under bright-field optics. In some cases sections were stained with both Toluidine Blue and silver nitrate.

Solutions

A modified Krebs solution of the following composition was used (mM): Na^+, 137; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 134; HCO_3^-, 15·4; $H_2PO_4^-$, 1·2; glucose, 11·4. The solution was warmed to 35–37 °C and bubbled with a 95 % O_2:5 % CO_2 gas mixture, creating a pH of 7·2. High-potassium solutions were made by substituting equimolar amounts of KCl for NaCl. Rapid applications of drugs could be made by switching the solution bathing the muscle to one containing the drug or by injecting, at a constant rate, a known concentration of the drug into the perfusing solution before the solution entered the organ bath, using a syringe pump (Harvard Apparatus). In the text the phrase 'prior application of a drug' means that the perfusing solution was changed to one containing the drug and after some period changed to another solution containing the drug at the same concentration but also containing a second drug (or raised potassium concentration). The solution was finally switched back to a drug-free solution.

Drugs

Acetylcholine chloride (Sigma); carbachol chloride (Sigma); disodium adenosine 5-triphosphate (Sigma); guanethedine sulphate (CIBA); noradrenaline hydrochloride (Sigma); phentolamine mesylate (CIBA); calcium ionophore A23187 (Calbiochem-Behring).

RESULTS

Mechanisms of contraction

Noradrenaline at concentrations greater than 10^{-6} M caused both contraction and detectable depolarization. Maximal contractions were recorded at 10^{-4} M-noradrenaline. The peak tensions generated by a 1 min exposure to noradrenaline were not appreciably different to those generated by longer exposures or by cumulative additions of noradrenaline to achieve the same final concentration. The resting membrane potential was usually negative to −60 mV. One or two minute exposures to noradrenaline (10^{-6} to 10^{-5} M) produced a small depolarization (< 5 mV) whereas larger concentrations produced up to 20 mV depolarization. In some experiments increasing the duration of exposure resulted in further depolarization particularly to higher concentrations and action potential discharge and slow oscillations of the potential with a period of a minute or so were produced (Figs. 1*A*, 2, 6 and 10; see also Karashima, 1981).

Increasing the external concentration of potassium ions, $[K^+]_o$, above 5·9 mM depolarized the membrane, e.g. with 84 mM-potassium the membrane was depolarized to −14 mV (Fig. 2*B*). The depolarizations to raised $[K^+]_o$ were not usually accompanied by action potentials or oscillations of the membrane potential. Contractions occurred when $[K^+]_o$ was raised above 29 mM. The maximal contraction to potassium (126 mM) in the presence of 2×10^{-6} M-phentolamine was 82 ± 10 % ($n = 6$) of the maximal contraction to 10^{-4} M-noradrenaline (Fig. 1). The peak tensions generated by increasing $[K^+]_o$ were similar whether obtained by single exposures or during cumulative additions to the same final concentration and have been averaged in Fig. 2*B*. Sometimes contractions to raised $[K^+]_o$ were reduced by phentolamine (5×10^{-6} M).

A plot of the relationship between contraction and depolarization produced by

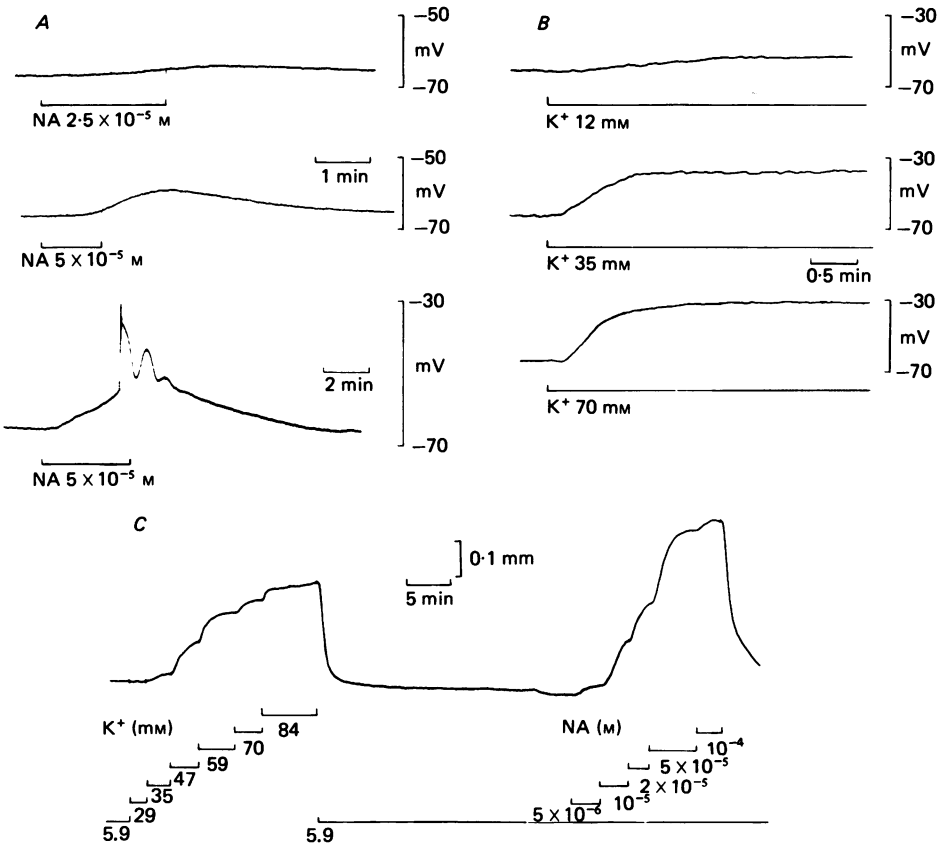

Fig. 1. Effect of noradrenaline (NA) and potassium (K^+) on the membrane potential (recorded intracellularly) (*A* and *B*) and contractile activity (*C*) of the guinea-pig mesenteric artery. Noradrenaline and raised potassium were present in the bathing solution at the concentrations shown for the periods indicated by the horizontal lines. The delays before the onset of action of noradrenaline largely reflect dead space in the perfusion system. The application of noradrenaline in the lower record of *A* produced an initial burst of action potentials, which appears as a single spike on the rising phase of a slow wave at this trace speed.

noradrenaline and by raised $[K^+]_o$ is shown in Fig. 3. Contractions to raised $[K^+]_o$ and noradrenaline have been expressed as a percentage of the contraction to 10^{-4} M-noradrenaline. Contractions to noradrenaline (1–2 min application) were associated with only relatively small depolarizations, e.g. contraction to 10^{-4} M-noradrenaline was associated with a depolarization of only 13 mV. In contrast, no contraction to raised $[K^+]_o$ was associated with a depolarization of less than 15 mV. This could be explained if potential-sensitive calcium channels open in appreciable numbers only positive to about −45 mV. High-potassium contractions were abolished in calcium-free solution, unlike noradrenaline contractions (Bolton, Lang, Takewaki & Clapp, 1983) so they are caused presumably by calcium influx through the cell membrane. Contractions to noradrenaline (1–2 min application) were associated with changes in the membrane potential *negative* to the threshold for the contractions to

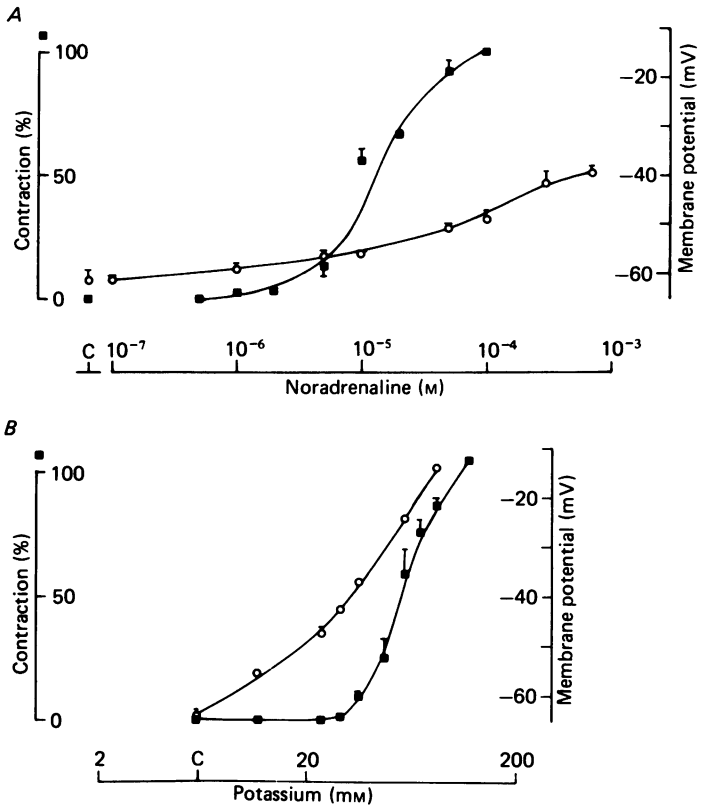

Fig. 2. Relationship between membrane potential (○) or contraction (■) (expressed as a percentage of the maximum) and the concentration of noradrenaline (*A*) or potassium (*B*). C, on the abscissa denotes mean value recorded in Krebs solution. Standard errors of the means are shown (n = 6–9). Depolarizations were measured using at least 2 min applications of noradrenaline or raised potassium concentration.

raised $[K^+]_o$ (Figs. 2 and 3); this would imply that they are not caused by the opening of appreciable numbers of potential-sensitive calcium channels. This need not mean there are no potential-sensitive calcium channels opened during the action of noradrenaline, or of small increases in $[K^+]_o$ (< 29 mM) but only that not enough are being opened to trigger a detectable contraction. For example, contraction to a low concentration of noradrenaline (10^{-6} M) which was associated with only a small change in the membrane potential (Fig. 2*A*) was enhanced in six out of eight preparations by the addition of 11 mM-external potassium, which by itself did not give rise to a contraction. However, contraction to high concentrations of noradrenaline ($> 5 \times 10^{-5}$ M) applied for long periods, evoked action potentials and slow waves which presumably involve the opening and closing of potential-operated calcium channels (Fig. 1*A*).

Effect of noradrenaline on membrane conductance

If it is assumed that the activation of a population of receptors opens ion channels, the membrane potential will move towards their equilibrium potential (ϵ, volts). A simple equivalent circuit of

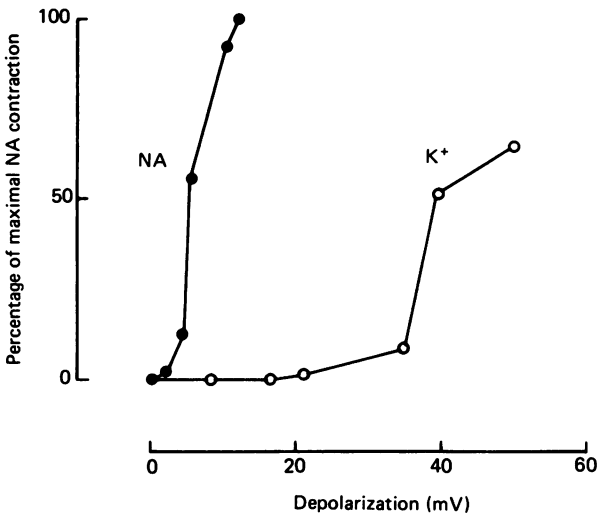

Fig. 3. Relationship between shortening and depolarization produced by noradrenaline (NA) and raised external potassium (K^+). Potassium contractions were expressed as a percentage of the maximal contraction to noradrenaline.

the membrane during receptor activation where the receptor-operated channels produce a voltage-insensitive increment, ΔG (siemens), in the resting conductance, G (siemens), which is also voltage insensitive, gives a receptor-induced change in potential of

$$\Delta V = \frac{\Delta G}{\Delta G + G}(E - \epsilon) \quad (1)$$

volts from the resting membrane potential E (volts) (Ginsborg, 1967, 1973; Bolton, 1972).

In the present experiments a rectangular current pulse was applied to an intact segment of artery in a partition chamber to evoke an electrotonic potential, recorded intracellularly, P (volts) in size. If the recording is made close to the point of injection, and assuming the artery can be well described in the longitudinal direction by an infinite cable then with certain assumptions the following approximation holds

$$\Delta V = [E - \epsilon][1 - (P'/P)^2], \quad (2)$$

where P' is the amplitude of the electrotonic potential during receptor activation (Ohashi, 1971; Bolton, 1972).

There was no consistent change in the amplitude of the electrotonic potential during the action of noradrenaline at concentrations below 10^{-5} M (Fig. 4*B*). On some occasions there was an apparent small increase in the electrotonic potential, on other occasions a small decrease. Noradrenaline, at concentrations which produced depolarizations greater than 5 mV, decreased the electrotonic potential (Fig. 4*B*). However depolarizations of the membrane similar in amplitude to the noradrenaline depolarizations, produced by passing constant electrical currents, were associated with a similar decrease in the electrotonic potential (Fig. 4*C*). Karashima (1981) described an increase in resistance, while Kuriyama & Makita (1983) reported a decrease, upon application of noradrenaline at higher concentrations to small guinea-pig mesenteric arteries. In our experiments, only at depolarizations greater

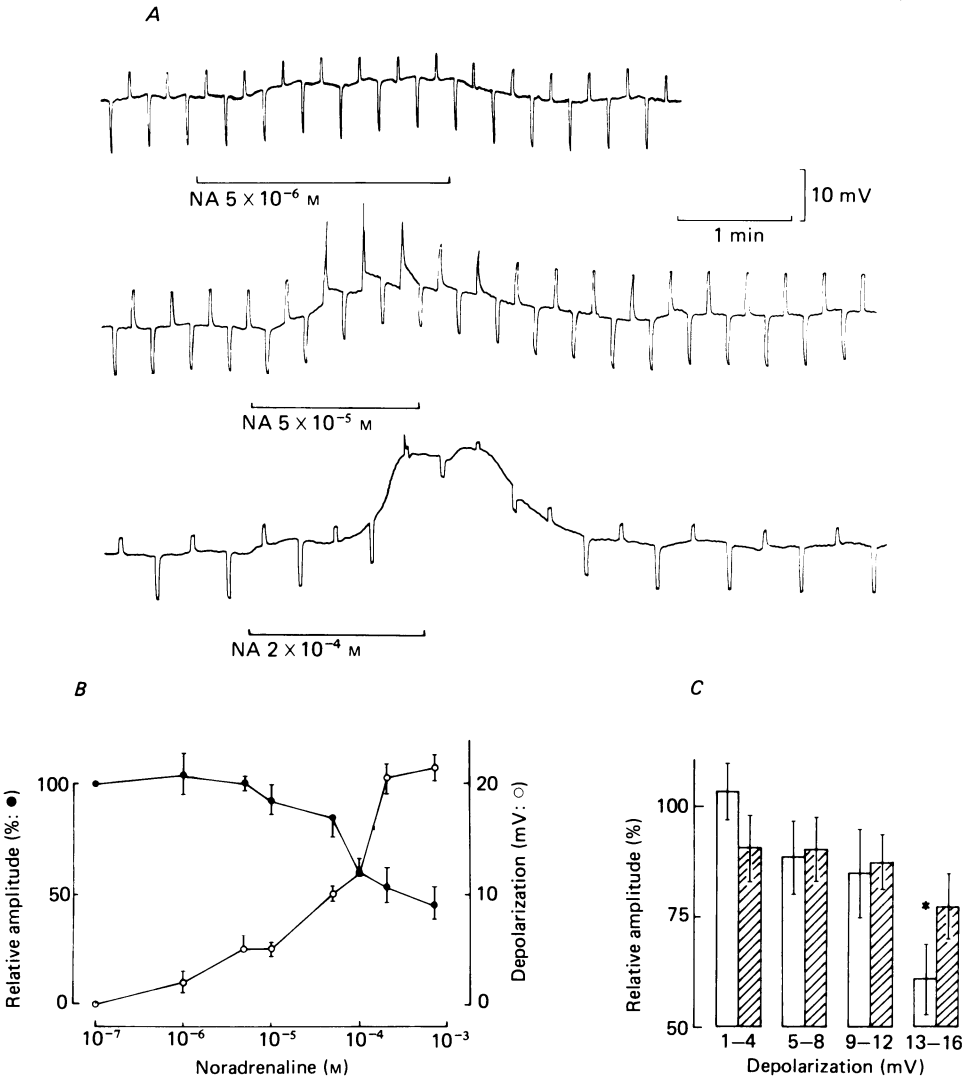

Fig. 4. *A* and *B*, effect of noradrenaline (NA) on the membrane potential and on the size of the electrotonic potential (expressed as a percentage of that recorded in control solution) elicited by alternating depolarizing or hyperpolarizing current pulses applied by large external plates in a partition chamber. *C*, only depolarizations to noradrenaline greater than 12 mV were associated with a reduction in the hyperpolarizing electrotonic potential which was significantly different from the reduction in the electrotonic potential observed during depolarizations induced by passing constant electrical current. Each point represents the mean of five to twelve experiments.

than 12 mV was the reduction in the electrotonic potential during the depolarization to noradrenaline greater than the reduction during the application of constant current (Fig. 4*C*). The reduction in the amplitude of the electrotonic potential during the application of small depolarizing currents implies that there is some opening during these small depolarizations of potential-sensitive channels, though not necessarily channels which allow the entry of calcium ions.

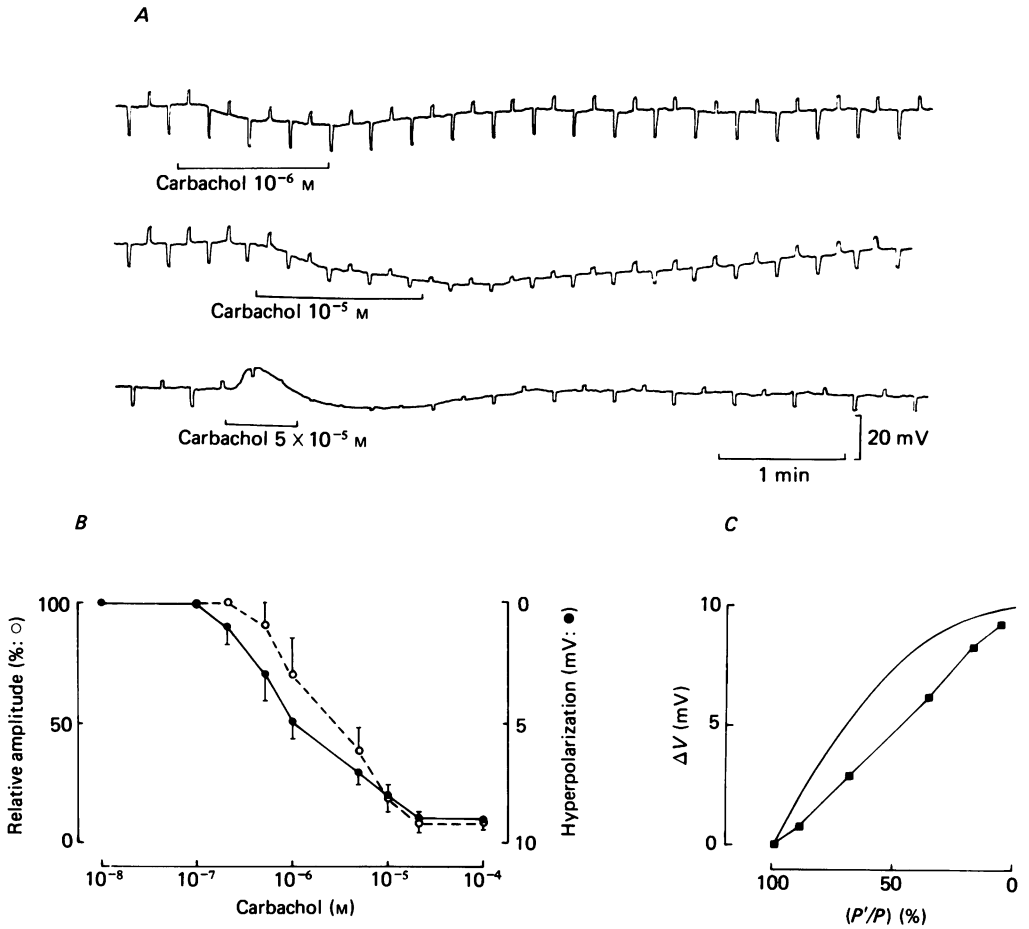

Fig. 5. *A*, effect of carbachol on the membrane potential and the size of the electrotonic potential. In *B* the peak of the hyperpolarization (●) to carbachol and the associated reduction in the electrotonic potential (○) are plotted against the concentration of carbachol. *C*, the recorded hyperpolarization to carbachol for all relative reductions in the electrotonic potential (■) was always less than that predicted by $\Delta V = (E-E_K)(1-(P'/P)^2)$ (continuous line; eqn. (2), see text) which assumes a conductance increase only to potassium ions. This may indicate the occurrence of conductance change to ions other than potassium during the action of carbachol. Each point represents the mean of five to eleven experiments.

Effects of carbachol

Carbachol (10^{-7} to 10^{-4} M) had little or no effect on resting tension or length of the mesenteric artery. Concentrations greater than 10^{-7} M usually produced hyperpolarization associated with reduction in the size of electrotonic potentials (Fig. 5*A*). At higher concentrations (10^{-5} to 10^{-4} M) biphasic or triphasic responses were seen, depolarization preceding or following hyperpolarization. Both phases were associated with a reduction in the size of the electrotonic potential. The hyperpolarization and relative size of the electrotonic potential are plotted against the concentration of carbachol in Fig. 5*B*.

Assuming that there was only a conductance change to potassium ions during the peak of the hyperpolarization to carbachol (Kuriyama & Suzuki, 1978) the change in potential (ΔV) for various changes in the membrane conductance (estimated from the decrease in the size of the electrotonic potential) can be predicted from eqn. (2). Assuming a membrane potential of −60 mV and potassium equilibrium potential

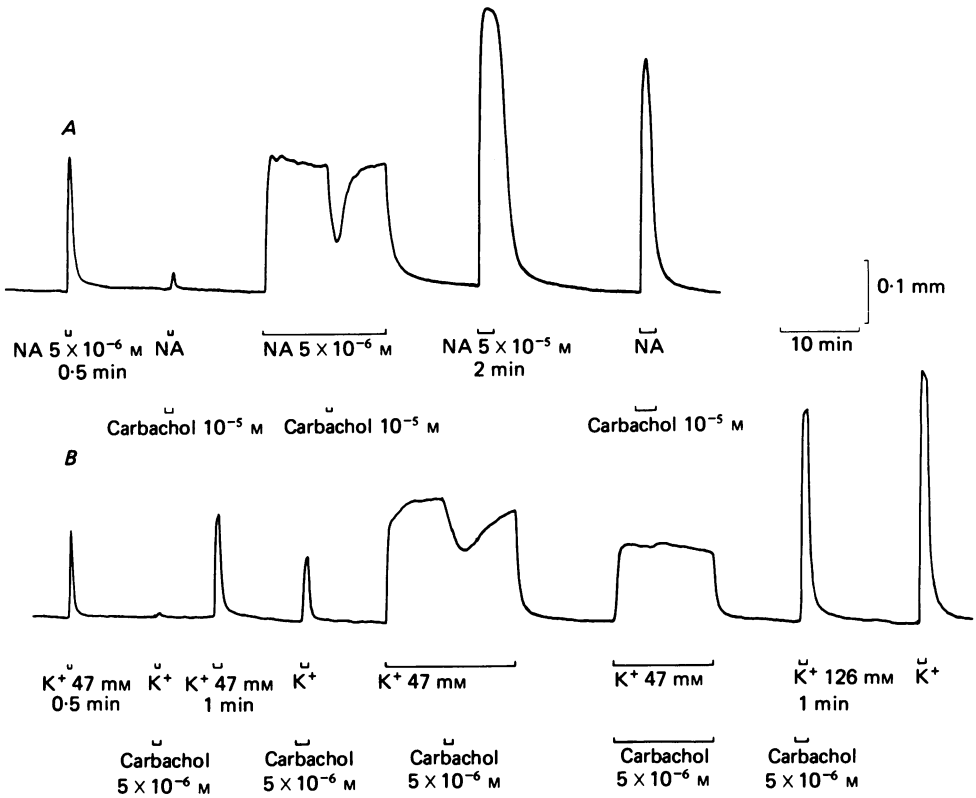

Fig. 6. *A*, action of carbachol (10^{-5} M) on contractions to short (0·5 min) or sustained exposures to noradrenaline (5×10^{-6} M). Contractions to noradrenaline were reduced under all conditions. *B*, contractions to raised external potassium (47 mM) applied for 0·5 min were strongly inhibited by the prior application of carbachol (5×10^{-6} M). Contraction to longer exposures to raised external potassium (47 mM) or sustained contractions to raised potassium were reduced to a lesser extent.

$E_K = -70$ mV (Kuriyama & Suzuki, 1978; Takata, 1980) the predicted ΔV is plotted against the relative amplitude of the electrotonic potential in Fig. 5*C*. At all P'/P the recorded hyperpolarization to carbachol (■—■) was less than predicted. This may occur because there is an increase in conductance to an additional ion or ions which have an equilibrium potential positive to E_K. This conductance change is presumably responsible for the depolarization sometimes recorded before or after the hyperpolarization to carbachol (see also Kuriyama & Suzuki, 1978).

Action on noradrenaline responses. Contractions induced by short exposures (0·5–1 min) to noradrenaline (10^{-6} to 10^{-4} M) were strongly inhibited by prior

introduction of carbachol (10^{-7} to 10^{-5} M). If tension was first induced by noradrenaline, carbachol strongly relaxed the artery but the inhibitory effect was less (Fig. 6*A*). Carbachol had somewhat less inhibitory effect on contractions induced by high concentrations (5×10^{-5} M) of noradrenaline. Depolarization to noradrenaline was virtually abolished either by the prior application of carbachol or its application during a sustained depolarization to noradrenaline (Fig. 7*A*).

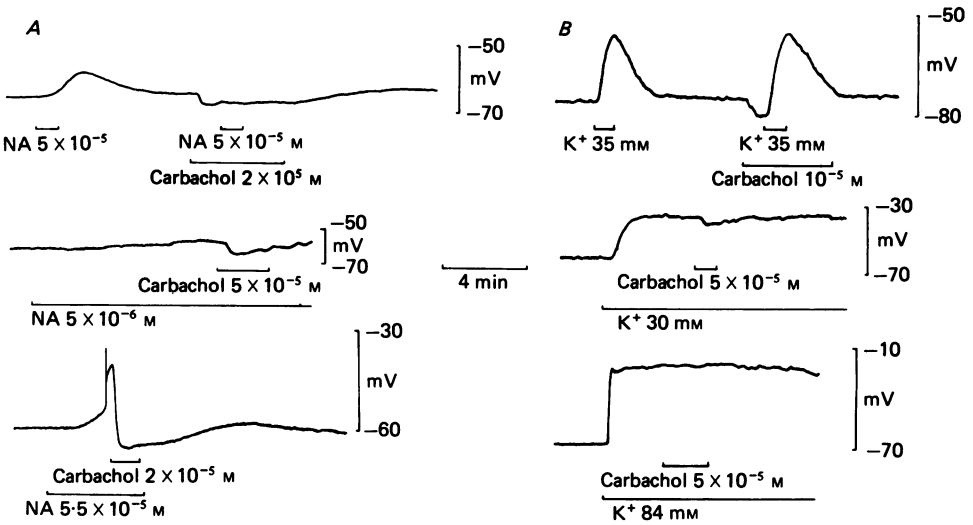

Fig. 7. *A*, depolarizations to noradrenaline (NA) (5×10^{-5} M) are virtually abolished by the prior application of carbachol (2×10^{-5} M) as were the depolarizations to sustained exposure to either 5×10^{-6} M- or $5{\cdot}5 \times 10^{-5}$ M-noradrenaline. *B*, the depolarization to raised external potassium (11–35 mM) was only slightly reduced when carbachol (10^{-5} M) was added before or during a sustained exposure to raised potassium. Depolarizations to raised external potassium (> 40 mM) were little affected by carbachol.

Action on responses to raised $[K^+]_o$. Contractions induced by short exposure (0·5 min) to raised $[K^+]_o$ (up to 126 mM) were reduced more than responses to longer exposures by prior or simultaneous application of carbachol (10^{-7} to 10^{-5} M). Prior application of carbachol reduced contractions to raised $[K^+]_o$ more than simultaneous application. Also, carbachol was less effective at inhibiting tension generation to 126 mM-external potassium than to lower concentrations (Fig. 6*B*). In normal solutions containing 5·9 mM-external potassium, carbachol hyperpolarized the membrane. A small hyperpolarization occurred generally in 30 mM-external potassium but above this concentration carbachol was without detectable effect on membrane potential when added in the presence of an already raised $[K^+]_o$ (Fig. 7*B*). Phentolamine (5×10^{-6} M) or guanethidine (5×10^{-6} to 10^{-5} M) did not noticeably affect the inhibitory effects of carbachol on tension generated by raised $[K^+]_o$.

Role of the endothelium

Inhibitory responses of large blood vessels to muscarinic agonists seem to involve the release of some factor or factors from endothelial cells which then act to relax

the smooth muscle cells, since after destruction or removal of the endothelium the inhibitory action of these agonists is lost or they produce contraction (Furchgott & Zawadzki, 1980). We confirmed that relaxation of transverse strips of rabbit thoracic aorta, produced by carbachol application during an established noradrenaline contraction, was abolished or converted to contraction upon rubbing off the

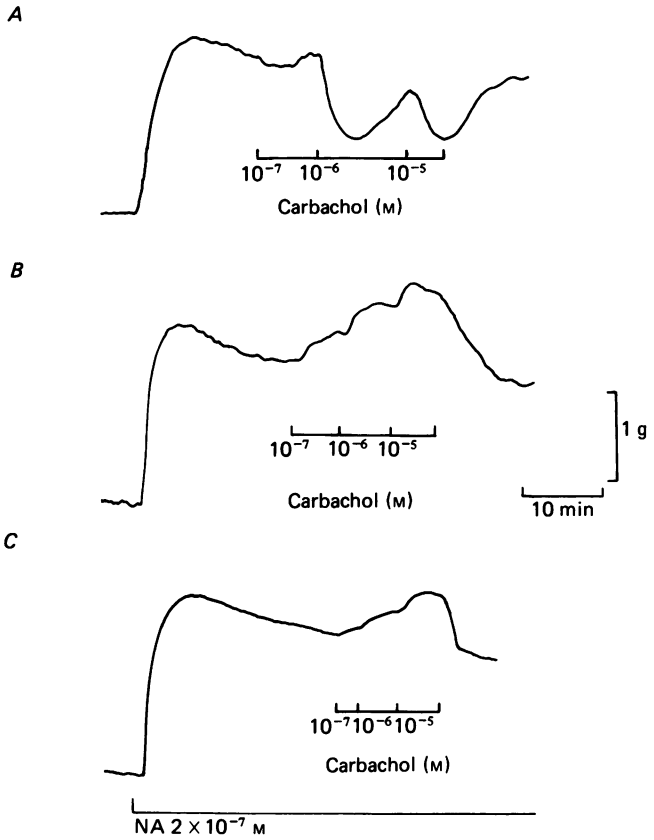

Fig. 8. *A*, contractions of intact strips of rabbit thoracic aorta to noradrenaline (NA) (2×10^{-7} M) were relaxed by increasing concentrations of carbachol (10^{-7} to 10^{-5} M). These relaxations to carbachol were converted to contraction if the endothelium was removed by either *B*, perfusing the lumen with distilled water (20–30 min) before the strips were prepared, or *C*, by rubbing the luminal surface after the strips were prepared.

endothelial cells. We further showed that perfusion of the lumen of the aorta with distilled water for 20–30 min before preparing transverse strips had an effect similar to rubbing off the endothelium, implying that distilled-water treatment destroyed the endothelial cells (Fig. 8).

In guinea-pig small mesenteric arteries, rubbing the luminal surface of helically cut strips was shown histologically to remove endothelial cells. In strips which were not rubbed the endothelial cells and their nuclei could be clearly seen within the internal elastic lamina in sections stained with Toluidine Blue or silver nitrate. After rubbing

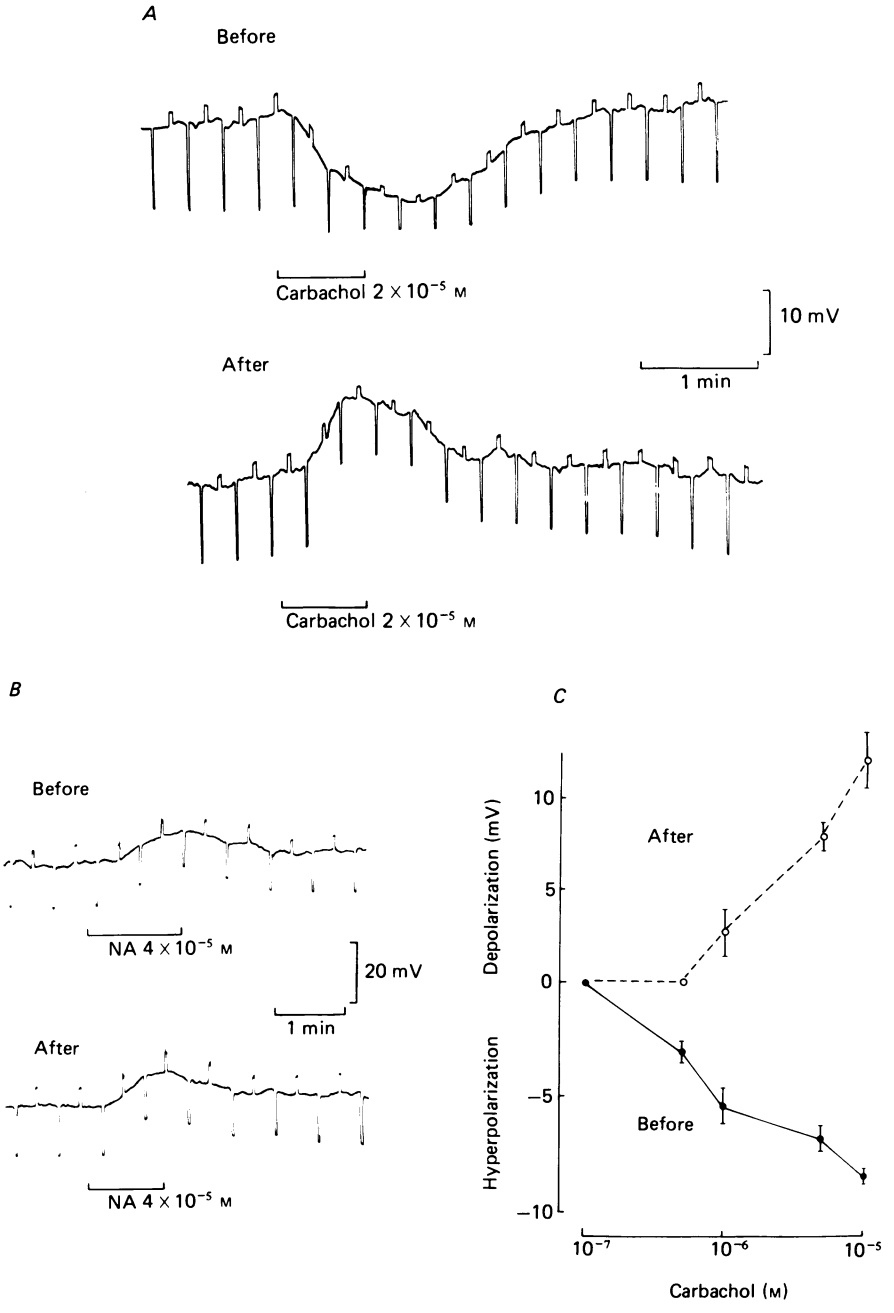

Fig. 9. *A*, membrane responses to carbachol of a previously cannulated mesenteric artery, mounted in a partition chamber, before and after a 30 min luminal perfusion with distilled water. *B*, the depolarization and associated change in electrotonic potential to noradrenaline (NA) (4×10^{-5} M) in the same artery were essentially unchanged by the perfusion procedure. *C*, averaged results showing that the hyperpolarizing action of carbachol (10^{-7} to 10^{-5} M) was converted to a depolarizing action after distilled water perfusion. Each point represents the mean of five or six experiments.

these were absent. Distilled water perfusion before cutting strips also removed the endothelial cells leaving some cellular debris and a few scattered nuclei.

Rabbit mesenteric arterial tree from its root at the aorta to the border of the intestine was perfused with physiological salt solution. Atropine (10^{-7} or 10^{-6} M) was added to the resultant perfusate which was dripped over a helical strip of rabbit thoracic aorta which had been previously de-endothelialized by mechanical rubbing. Injection of carbachol (10^{-7} or 10^{-6} M) into the mesenteric tree caused relaxation of the atropinized de-endothelialized rabbit thoracic aortic strip previously contracted with noradrenaline (10^{-7} to 10^{-6} M). Similar results were obtained if rabbit aortic strips were used instead of mesenteric vessels. However, no relaxation of atropinized, de-endothelialized, rabbit aortic strip was obtained if guinea-pig mesenteric arterial tree was substituted for rabbit. These results support the idea of the release of some relaxing factor from the endothelial cells of rabbit vessels.

Carbachol and noradrenaline responses

After removal of the endothelium, carbachol was still without effect on *resting* tension or length of guinea-pig small mesenteric artery strips. However, the hyperpolarizing action of carbachol seen in normal strips was converted to a depolarization. The effect of noradrenaline on membrane potential and size of electrotonic potential was essentially unchanged (Fig. 9).

The depolarization to carbachol after distilled water treatment was also associated with a decrease in the amplitude of the electrotonic potential. An estimate of the equilibrium potential for this depolarization can be made by rearranging eqn. (2) such that

$$\epsilon = E - \frac{\Delta V}{[1-(P'/P)^2]}. \tag{3}$$

In the present experiments the equilibrium potential for the carbachol depolarization was calculated as $-52 \pm 0{\cdot}9$ mV (mean $\pm$ standard error of mean, $n = 10$). A threefold reduction in the membrane resistance $(P'/P)^2$ was estimated to occur during the action of 5×10^{-6} M-carbachol for example.

Effect of carbachol on noradrenaline responses. After removal of the endothelium by rubbing or distilled water treatment, relaxation of established noradrenaline-induced tension by muscarinic agonists still occurred although it was reduced (Fig. 10*B*). Contractions to brief exposures (1 min) to noradrenaline (10^{-6} to 10^{-5} M) were still substantially reduced by carbachol in these preparations (Fig. 10*A*). These results imply that there is an appreciable direct inhibitory action of carbachol via muscarinic receptors on smooth muscle cells in these small arteries in contrast to the rabbit aorta (Fig. 8). This was somewhat surprising since the hyperpolarizing action of carbachol was converted to a depolarization by removal of the endothelium. However, depolarization to carbachol was less than 12 mV after removal of the endothelium (Fig. 9*C*), presumably not enough to cause appreciable opening of the potential-sensitive calcium channels which normally engenders tension.

After removal of the endothelium, carbachol always inhibited noradrenaline-induced tension. However in the presence of lower concentrations of noradrenaline, carbachol depolarized the membrane slightly. In higher concentrations ($5{\cdot}5 \times 10^{-5}$ M) of nor-

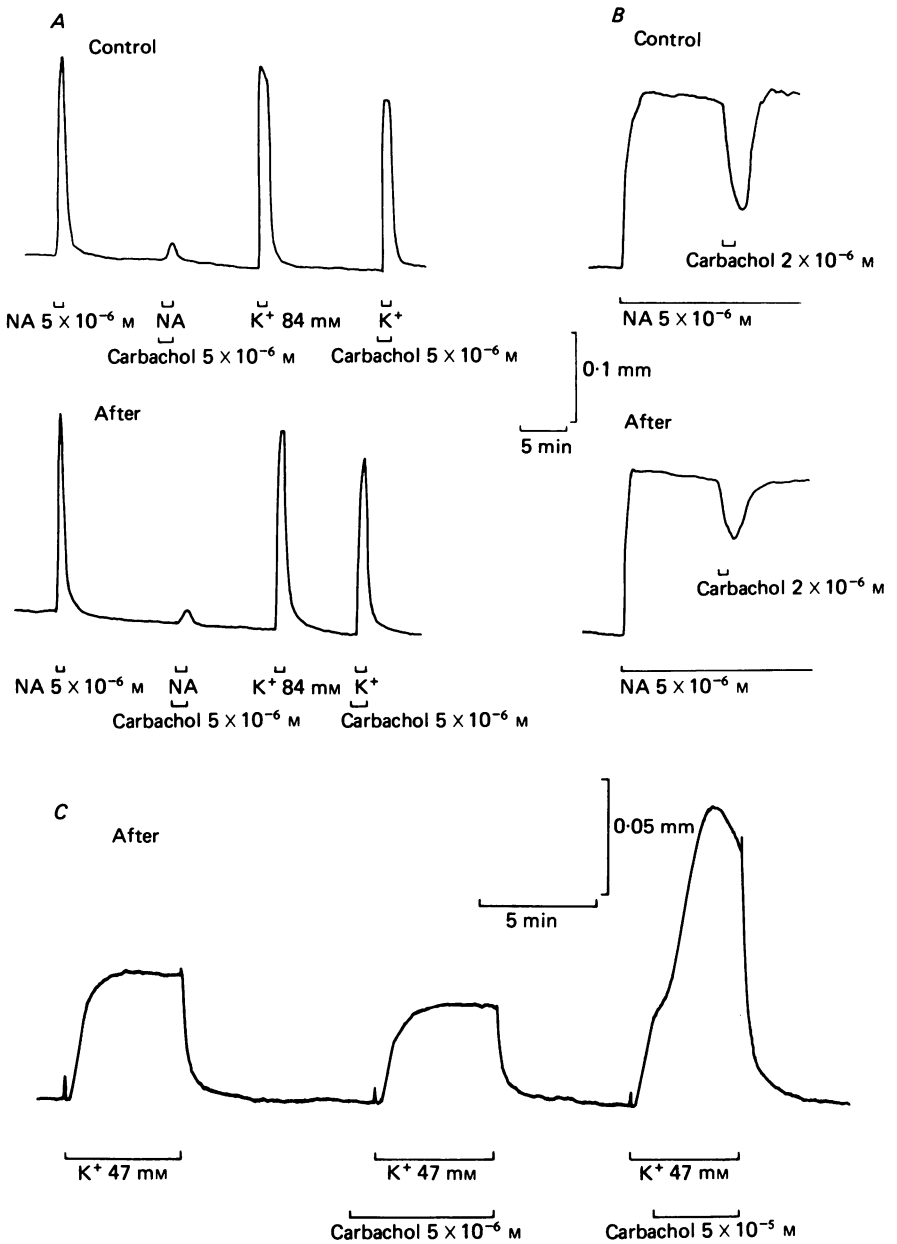

Fig. 10. *A*, carbachol (5×10^{-6} M) reduced contractions to noradrenaline (NA) (5×10^{-6} M) and to raised external potassium (K^+) (84 mM) both before and after the endothelium had been removed by mechanical rubbing. *B*, carbachol (2×10^{-6} M)-induced relaxation of sustained contractions to noradrenaline (5×10^{-6} M) still occurred if the mesenteric arteries were perfused with distilled water before the preparation of the helical strips. The difference between the responses of these two preparations is within experimental variation. *C*, after the removal of the endothelium the prior application of carbachol (5×10^{-6} M) reduced contractions to raised external potassium (< 50 mM) but enhanced contractions to raised potassium if added after the raised potassium solution.

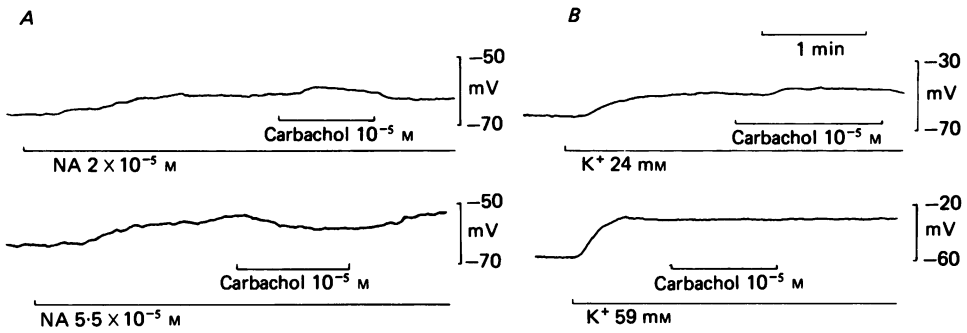

Fig. 11. *A*, carbachol (10^{-5} M) in the presence of a low concentration of noradrenaline (NA, 2×10^{-5} M) induced a further small depolarization of the mesenteric artery previously perfused with distilled water, but hyperpolarized the membrane if greater depolarization was produced with high concentrations of noradrenaline ($5{\cdot}5 \times 10^{-5}$ M). *B*, carbachol also depolarized the membrane further when the external potassium (K^+) was raised to 24 mM. In concentrations of external potassium greater than 50 mM, carbachol had little effect on the membrane potential.

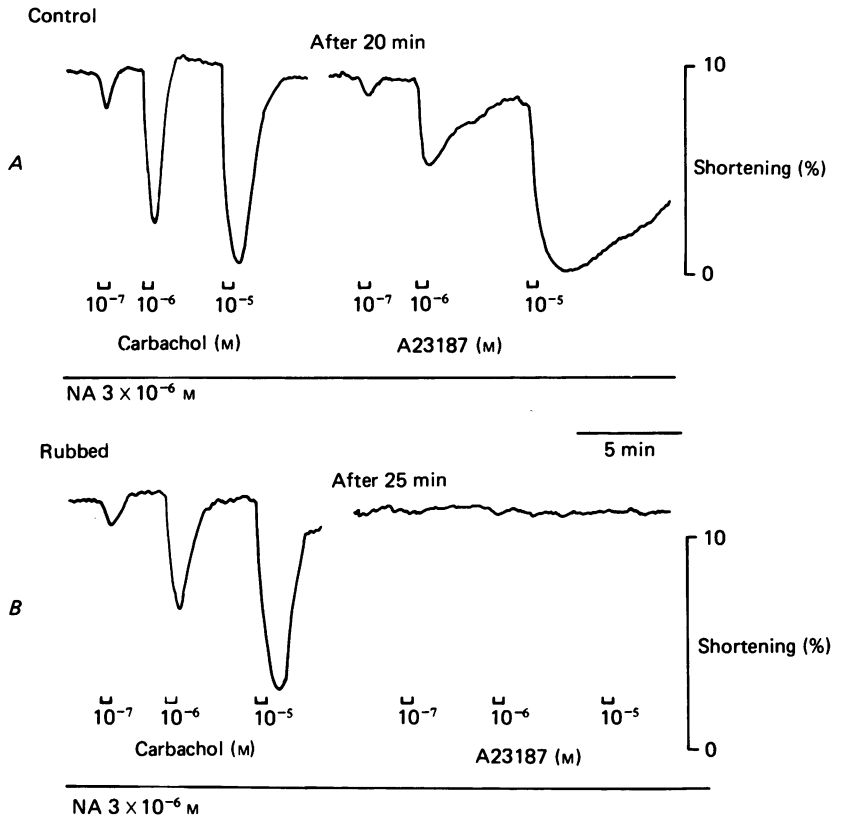

Fig. 12. Abolition of relaxations to the ionophore A23187 in strips of guinea-pig small mesenteric artery by rubbing the intimal surface. *A*, responses to carbachol and A23187 are shown in an intact strip. *B*, after rubbing the intimal surface the response of the same strip to carbachol was little changed but responses to A23187 were abolished. In the upper record 20 min of the trace have been removed, in the lower record 25 min.

adrenaline or when greater depolarization was produced, carbachol still hyperpolarized the membrane (Fig. 11*A*).

Action of carbachol on responses to raised $[K^+]_o$. Muscarinic agonists always inhibited tension induced by raising $[K^+]_o$ when the endothelium was intact. However, after its removal, simultaneous application of carbachol often potentiated the contraction

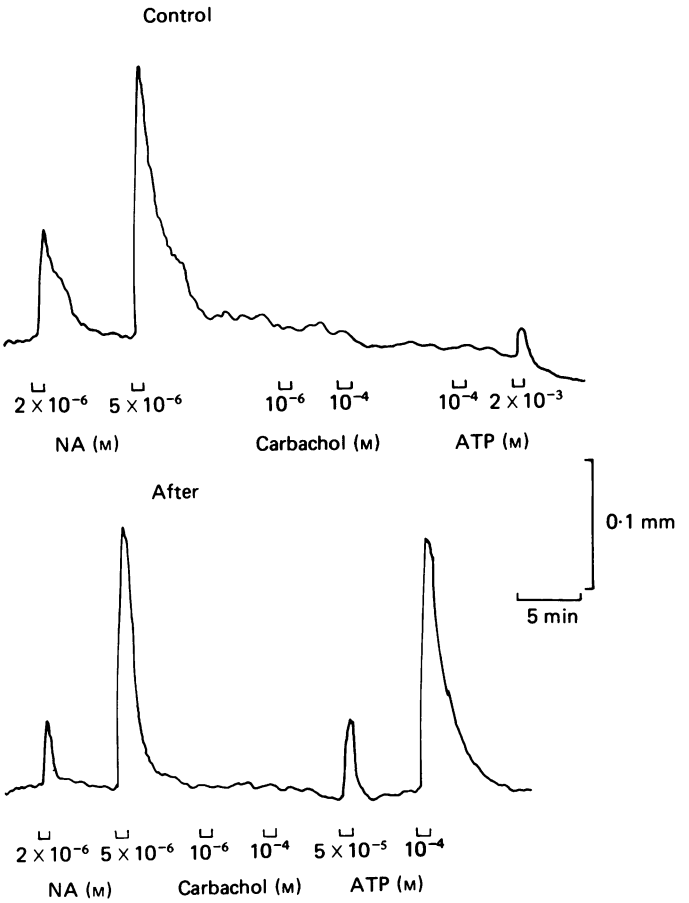

Fig. 13. ATP ($> 10^{-4}$ M) contracted intact strips of mesenteric artery only weakly and carbachol (10^{-6} to 10^{-4} M) was without effect. After removal of the endothelium by rubbing, ATP (5×10^{-5} and 10^{-4} M) strongly contracted the same artery, while the effects of carbachol (10^{-6} and 10^{-4} M) or noradrenaline (NA) (2×10^{-6} and 5×10^{-6} M) were little changed.

to threshold concentrations (20–50 mM) of raised $[K^+]_o$. In the presence of 20–50 mM $[K^+]_o$, carbachol (2×10^{-6} to 5×10^{-5} M) produced strong contraction. Prior application of carbachol reduced contractions to subsequent application of 30–50 mM-external potassium (Fig. 10*C*). Carbachol inhibited tension generated by 60 mM or more external potassium.

The potentiation by carbachol of the contractions to just threshold concentrations of raised $[K^+]_o$ was associated with a summation of the depolarizations produced by

carbachol application and raised $[K^+]_o$. In an $[K^+]_o$ greater than 50 mM, carbachol still hyperpolarized the membrane slightly or was without detectable effect (Fig. 11*B*).

The persistence of carbachol relaxations in small guinea-pig mesenteric arteries after treatments which removed the endothelial cells implied that in contrast to the

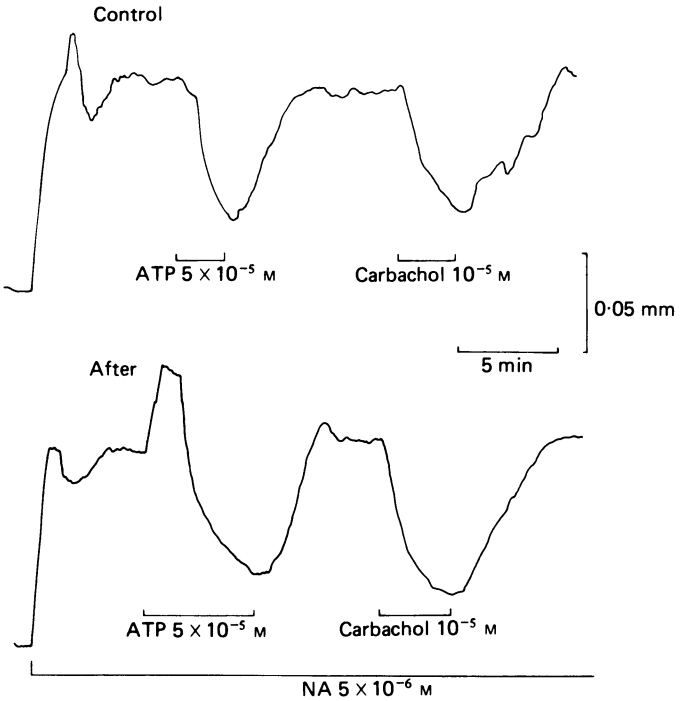

Fig. 14. Contractions to noradrenaline (NA) (5×10^{-6} M) were strongly relaxed by carbachol (10^{-5} M) and by ATP (5×10^{-5} M) both before and after destruction of the endothelium. An initial contraction to ATP also occurred after the removal of the endothelium.

rabbit aorta and some other larger blood vessels (Furchgott, 1981) muscarinic agonists may relax guinea-pig small mesenteric arteries by a direct action on the vascular smooth muscle cells. M. J. Mulvany and J. G. De Mey (personal communication) have found that small mesenteric arteries from the rat resemble the rabbit aorta. We therefore did further experiments on both rat and guinea-pig small mesenteric arteries. We confirmed that the rat vessels differed from the guinea-pig in that carbachol relaxation of existing noradrenaline-induced tension depended upon the integrity of the endothelium. Upon the suggestion of Dr R. F. Furchgott, we also looked at the action of the ionophore, A23187, which produces a powerful endothelial-dependent relaxation in many blood vessels (Furchgott, 1981). This compound also relaxed guinea-pig mesenteric strips if the endothelium was intact but not if the intimal surface was rubbed (Fig. 12). In these, higher concentrations of A23187, ($0{\cdot}5$–1×10^{-4} M) sometimes produced contraction.

Actions of ATP

ATP (greater than 10^{-4} M) only weakly contracted the resting artery with intact endothelium (Fig. 13) but 10^{-5} to 10^{-4} M-ATP strongly relaxed noradrenaline contractions after an initial small contraction which occurred more often after destruction of the endothelium (Fig. 14). Measurements of membrane potential revealed a small depolarization upon application to normal arteries which was greater after removal of the endothelium. After loss of the endothelium, the resting artery became more sensitive to the contractile effect of ATP (Fig. 13).

DISCUSSION

The depolarization associated with activation of α-adrenoreceptors by noradrenline was clearly much less than when a similar contraction was evoked by raising $[K^+]_o$. Similar observations have been made in vascular muscle from a number of other arteries (Su *et al.* 1964; Mekata & Niu, 1972; Casteels *et al.* 1977; Droogmans, Raeymaekers & Casteels, 1977) and there is a striking similarity to results obtained in rat small mesenteric artery (Mulvany *et al.* 1982). The greater sensitivity of high $[K^+]_o$ than of noradrenaline contractions to calcium-free conditions supports the notion that high $[K^+]_o$ depolarizes the membrane until a point is reached where sufficient potential-sensitive channels open and admit calcium into the cell. Contraction follows.

In the case of noradrenaline the depolarization even with larger concentrations would seem to be often insufficient to reach threshold for opening of these postulated channels in appreciable numbers; also, the resistance of noradrenaline contractions to calcium-free conditions implies some other mechanism or mechanisms. Since a proportion of the noradrenaline contractile response is more rapidly lost in calcium-free conditions, at least some of the tension generation could result from calcium entry into the cell through receptor-operated channels (Bolton, 1979, 1983). The residual component resistant to calcium-free conditions may represent the release of stored calcium (Casteels & Droogmans, 1981). The choice of small mesentery artery for these experiments was fortunate in that the threshold for the opening of appreciable numbers of potential-sensitive calcium channels was considerably positive, 15 mV or so, to the resting membrane potential, so that there was a clear separation of the membrane potentials associated with equally sized contractions to high $[K^+]_o$ and to noradrenaline. In some other arteries the threshold $[K^+]_o$ for contraction is less so that this separation is less or non-existent and, furthermore, the opening of receptor-operated channels has been reported to produce a large depolarization (Harder *et al.* 1981; Trapani *et al.* 1981). This would bring into play the opening of potential-sensitive calcium channels so that in these arteries three types of calcium mechanism at least would seem to be involved in tension generation to noradrenaline.

In preparations of the guinea-pig small mesenteric artery, which are unusually sensitive to the depolarizing action of noradrenaline, or when high concentrations are applied, calcium entry through potential-sensitive channels may contribute to an appreciable extent to tension generation and reinforce other mechanisms. In particular, the appearance of action potentials and, to a lesser extent, slow waves, presumably

indicates the rhythmic opening of ionic channels, including potential-sensitive calcium channels. Cyclical changes in potential, either slow waves or spikes, were seldom if ever seen with raised $[K^+]_o$ in guinea-pig small mesenteric artery – although it is significant that they have been described in response to activation of other types of receptor, e.g. vasopressin (Karashima, 1981). Rhythmic or cyclic changes in potential in arterial muscle seem to occur upon activation of receptors but not in response to electrical or high $[K^+]_o$ depolarizations except inasmuch as these procedures may release noradrenaline from nerve endings (Mekata, 1979). Rhythmical potential changes evoked by receptor activation in both vascular and visceral (Bolton, 1971) muscle have been described. Their occurrence presumably indicates an effect of receptor activation on the properties of potential- and time-dependent membrane channels which is not caused simply by the depolarization produced (Bolton, 1972, 1979). It is also important to remember that activation of perivascular nerves evokes a transient depolarization of the membrane, the excitatory junction potential (e.j.p.), and that it seems that nerve-evoked contraction occurs when the e.j.p. reaches threshold for the opening of potential-sensitive calcium channels, i.e. when a regenerative or graded action potential is triggered (Speden, 1964; Holman & Surprenant, 1979; Surprenant, 1980; Kuriyama & Suzuki, 1981). Presumably the e.j.p. represents the action of nerve-released noradrenaline on adrenoreceptors, although its resistance to α-blockers may indicate that other types of adrenoreceptor (Hirst & Neild, 1980, 1981) or other transmitters (G. Burnstock & P. Sneddon, personal communication) are involved. However, the observation that contractions to low concentrations of noradrenaline could be potentiated by small increases in $[K^+]_o$ which depolarize in the range considerably *negative* to the threshold potential for high $[K^+]_o$ contractions, implies either some opening of potential-sensitive calcium channels in the range near the resting membrane potential, or more complicated interactions of raised $[K^+]_o$ and α-receptor activation.

Activation of muscarinic receptors in arteries often causes relaxation but frequently will only do so if the endothelial lining of the blood vessel is intact (Furchgott, 1981). Small guinea-pig mesenteric arteries studied here showed evidence of a hyperpolarizing factor emanating from the endothelium since the normal hyperpolarization to carbachol was converted to a depolarization following destruction of the endothelium. However, we were unable to detect this factor in donor–recipient experiments similar to ones in which we could detect a factor released from rabbit aortic endothelium. At least in our experiments it cannot be excluded that the procedures used to destroy the endothelium may have had other effects, for example on the vascular smooth muscle cells, so altering their response to carbachol. However, several lines of evidence support our belief that a direct action as well as an endothelial inhibitory factor is important in muscarinic-receptor-mediated relaxation of contractions of guinea-pig small mesenteric artery. Cutting of helical strips from arteries normally damages the endothelial cells (Furchgott, 1981) although in our experiments on unrubbed guinea-pig small mesenteric artery strips, histological examination and the relaxation by the ionophore A23187 indicated the persistence of substantial endothelium in most strip preparations. Nevertheless, rubbing the intimal surface or distilled water perfusion were shown to be effective in rabbit aorta in abolishing carbachol relaxations, and histological examination of guinea-pig small mesenteric

artery strips confirmed the efficacy of these methods in removing endothelium in this artery also. Finally, the loss of the relaxing action of A23187 provided further support. Thus a component, though not all, of the inhibitory action of carbachol via muscarinic receptors seems to be directly on the smooth muscle cells of the guinea-pig small mesenteric artery.

If noradrenaline elicits contraction of arterial smooth muscle by mechanisms which involve potential-sensitive opening of calcium channels to only a minor extent, then by the same token the inhibition of this tension by carbachol must involve some mechanism other than a reduction in the numbers of open potential-sensitive calcium channels. Thus, even though muscarinic receptor activation hyperpolarized under most conditions, it is difficult to suppose that this hyperpolarization has more than a minor effect on tension for two reasons: it occurs in a potential range negative to the threshold for high $[K^+]_o$ contractions, and no change in potential occurred during inhibition of high $[K^+]_o$ tension (see also Kuriyama & Suzuki, 1978). Nevertheless, hyperpolarization by carbachol may on occasions close potential-sensitive calcium channels and so reinforce its relaxant effect produced primarily in other ways. The main mechanism of carbachol's inhibitory action is at present obscure, especially as it inhibits both high $[K^+]_o$ and noradrenaline contractions which we believe utilize calcium from different sources.

Muscarinic receptor activation relaxed the smooth muscle of small mesenteric arteries which was already contracted by noradrenaline or by raised $[K^+]_o$. However contractions to either agent were more strongly inhibited if carbachol was added first; contractions to brief exposures (30 s) to contractile agents were particularly strongly inhibited. These differential effects of carbachol may reflect some aspect of the kinetics of the inhibitory mechanism activated by carbachol, and of the activation of channels opened during the phasic and tonic components of the contractions to raised $[K^+]_o$ and to noradrenaline.

The depolarization to carbachol and the enhancement of just-threshold contractions to raised $[K^+]_o$ by carbachol after the removal of the endothelial cells suggest the presence of excitatory muscarinic receptors on the smooth muscle cells. The effects on tension of activation of these receptors, however, were generally masked by those of activating muscarinic receptors on smooth muscle and on endothelium with an inhibitory effect. After destruction of the endothelium, activation of excitatory receptors by carbachol produced depolarization which, in combination with the depolarization produced by normally subthreshold concentrations of $[K^+]_o$, could elicit large contractions.

Muscarinic receptor activation with carbachol in the presence of an intact endothelium produced either hyperpolarization or a complex membrane response of depolarization followed, or preceded, by hyperpolarization. The peak hyperpolarizations to carbachol were smaller than those predicted if the change in membrane conductance, estimated by the change in the electrotonic potential, was to potassium ions alone (Fig. 4) suggesting an additional underlying increase in the membrane conductance. This conductance change is revealed after destruction of the endothelium when a depolarizing response to carbachol is recorded (Fig. 9). The depolarization to carbachol under these conditions had an estimated reversal potential of -50 to -55 mV which was confirmed during exposures to low and to higher concentrations

of noradrenaline (Fig. 11) perhaps suggesting a change in the membrane conductance to a number of ions, sodium, potassium, even calcium, during the action of carbachol (c.f. Casteels *et al.* 1977). A conductance increase to chloride alone, however, cannot be excluded. Thus a direct inhibitory action on the smooth muscle cells not primarily involving a conductance change seems to be reinforced by a hyperpolarizing inhibitory factor from the endothelium which opens potassium channels: in its absence a weak direct excitatory depolarizing action of muscarinic receptor activation due to ion channel opening is unmasked.

This work was supported by the Medical Research Council.

REFERENCES

ABE, Y. & TOMITA, T. (1968). Cable properties of smooth muscle. *J. Physiol.* **196**, 87–100.

BELL, C. (1969). Transmission from vasoconstrictor and vasodilator nerves to single smooth muscle cells of guinea-pig uterine artery. *J. Physiol.* **205**, 695–708.

BOLTON, T. B. (1971). On the nature of the oscillations of the membrane potential (slow waves) produced by acetylcholine or carbachol in intestinal smooth muscle. *J. Physiol.* **216**, 403–418.

BOLTON, T. B. (1972). The depolarizing action of acetylcholine or carbachol in intestinal smooth muscle. *J. Physiol.* **220**, 647–671.

BOLTON, T. B. (1979). Mechanism of action of transmitters and other substances on smooth muscle. *Physiol. Rev.* **59**, 606–718.

BOLTON, T. B. (1983). Mechanism of action of transmitters and other substances on vascular and non-vascular smooth muscle. In *Vascular Neuroeffector Mechanisms: 4th International Symposium*, ed. BEVAN, J. A. *et al.*, pp. 47–55. New York: Raven Press.

BOLTON, T. B., LANG, R. J. & OTTESEN, B. (1981). Mechanism of action of vasoactive intestinal polypeptide on myometrial smooth muscle of rabbit and guinea-pig. *J. Physiol.* **318**, 41–55.

BOLTON, T. B., LANG, R. J., TAKEWAKI, T. & CLAPP, L. H. (1983). Autonomic receptors and cell membrane potential. In *Mechanisms of Vasodilatation*, 3rd International Symposium, ed. VANHOUTTE, P. M. & VATNER, S. F. Basel: S. Karger (in the Press).

BURN, J. H. & RAND, M. J. (1965). Acetylcholine in adrenergic transmission. *A. Rev. Pharmac.* **5**, 163–182.

CASTEELS, R. & DROOGMANS, G. (1981). Exchange characteristics of the noradrenaline-sensitive calcium store in vascular smooth muscle cells of rabbit ear artery. *J. Physiol.* **317**, 263–279.

CASTEELS, R., KITAMURA, K., KURIYAMA, H. & SUZUKI, H. (1977). Excitation–contraction coupling in the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol.* **271**, 63–69.

CHEUNG, D. W. (1982). Two components in the cellular response of rat tail arteries to nerve stimulation. *J. Physiol.* **328**, 461–468.

DAVIES, J. M. & WILLIAMS, K. I. (1983). Relaxation of the rat aorta by vasoactive intestinal polypeptide is endothelial cell dependent. *J. Physiol.* **343**, 65*P*.

DE MEY, J. G. & VANHOUTTE, P. M. (1981). Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. *J. Physiol.* **316**, 347–355.

DE MEY, J. G., CLAEYS, M. & VANHOUTTE, P. M. (1982). Endothelium-dependent inhibitory effects of acetylcholine, adenosine triphosphate, thrombin and arachidonic acid in the canine femoral artery. *J. Pharmac. exp. Ther.* **222**, 166–173.

DROOGMANS, G., RAEYMAEKERS, L. & CASTEELS, R. (1977). Electro and pharmacomechanical coupling in the smooth muscle cells of the rabbit ear artery. *J. gen. Physiol.* **70**, 129–148.

FURCHGOTT, R. F. (1955). The pharmacology of vascular smooth muscle. *Pharmac. Rev.* **7**, 183–265.

FURCHGOTT, R. F. (1981). The requirement for endothelial cells in the relaxation of arteries by acetylcholine and some other vasodilators. *TIPS* **2**, 173–176.

FURCHGOTT, R. F. & ZAWADZKI, J. V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature, Lond.* **288**, 373–376.

GINSBORG, B. L. (1967). Ion movements in junctional transmission. *Pharmac. Rev.* **19**, 289–316.

GINSBORG, B. L. (1973). Electrical changes in the membrane in junctional transmission. *Biochim. biophys. Acta* **300**, 289–317.

GOKHALE, S. D., GULATI, O. D., KELKAR, L. V. & KELKAR, V. V. (1966). Effect of some drugs on human umbilical artery in vitro. *Br. J. Pharmac. Chemother.* **27**, 332–346.

HARDER, D. R., ABEL, P. W. & HERMSMEYER, K. (1981). Membrane electrical mechanism of basilar artery constriction and pial artery dilation by norepinephrine. *Circulation Res.* **49**, 1237–1242.

HIRST, G. D. S. (1977). Neuromuscular transmission in arterioles of guinea-pig submucosa. *J. Physiol.* **273**, 263–275.

HIRST, G. D. S., HOLMAN, M. E. & SPENCE, I. (1974). Two types of neurones in the myenteric plexus of duodenum in the guinea-pig. *J. Physiol.* **236**, 303–326.

HIRST, G. D. S. & NEILD, T. O. (1978). An analysis of excitatory junctional potentials recorded from arterioles. *J. Physiol.* **280**, 87–104.

HIRST, G. D. S. & NEILD, T. O. (1980). Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle. *Nature, Lond.* **283**, 767–768.

HIRST, G. D. S. & NEILD, T. O. (1981). On the mechanism of action of prazosin at the sympathetic nerve–muscle junction of arterioles of the guinea-pig. *Br. J. Pharmac.* **74**, 189*P*.

HOLMAN, M. E. & SURPRENANT, A. M. (1979). Some properties of the excitatory junction potentials recorded from saphenous arteries of rabbits. *J. Physiol.* **287**, 337–351.

HOLMAN, M. E. & SURPRENANT, A. (1980). Effects of tetraethylammonium chloride on sympathetic neuromuscular transmission in saphenous artery of young rabbits. *J. Physiol.* **305**, 451–465.

ITO, Y., KITAMURA, K. & KURIYAMA, H. (1979). Effects of acetylcholine and catecholamines on the smooth muscle cell of the porcine coronary artery. *J. Physiol.* **294**, 595–611.

ITO, Y., KITAMURA, K. & KURIYAMA, H. (1980). Nitroglycerine and catecholamine actions on smooth muscle cells of the canine coronary artery. *J. Physiol.* **309**, 171–183.

KAJIWARA, M., KITAMURA, K. & KURIYAMA, H. (1981). Neuromuscular transmission and smooth muscle membrane properties in the guinea-pig ear artery. *J. Physiol.* **315**, 283–302.

KARASHIMA, T. (1981). Effects of vasopressin on smooth muscle cells of guinea-pig mesenteric vessels. *Br. J. Pharmac.* **72**, 673–684.

KITAMURA, K. & KURIYAMA, H. (1979). Effects of acetylcholine on the smooth muscle cell of isolated main coronary artery of the guinea-pig. *J. Physiol.* **293**, 119–133.

KURIYAMA, K. & MAKITA, Y. (1983). Modulation of noradrenergic transmission in the guinea-pig mesenteric artery: an electrophysiological study. *J. Physiol.* **335**, 609–627.

KURIYAMA, H. & SUZUKI, H. (1978). The effects of acetylcholine on the membrane and contractile properties of smooth muscle cells of the rabbit superior mesenteric artery. *Br. J. Pharmac.* **64**, 493–501.

KURIYAMA, H. & SUZUKI, H. (1981). Adrenergic transmissions in the guinea-pig mesenteric artery and their cholinergic modulations. *J. Physiol.* **317**, 383–396.

LANG, R. J. & TAKEWAKI, T. (1983). Role of endothelium in the relaxation of small arteries of guinea-pig produced by activation of muscarinic receptors. *J. Physiol.* **343**, 64*P*.

MALIK, K. U. & LING, G. M. (1969). Modifications by acetylcholine of the response of rat mesenteric arteries to sympathetic stimulation. *Circulation Res.* **25**, 1–9.

MEKATA, F. (1979). Studies of the electrical excitability of aorta smooth muscle of rabbit. *J. Physiol.* **293**, 11–21.

MEKATA, F. & NIU, H. (1972). Biophysical effects of adrenaline on the smooth muscle of the rabbit common carotid artery. *J. gen. Physiol.* **59**, 92–102.

MULVANY, M. J., NILSSON, H. & FLATMAN, J. A. (1982). Role of membrane potential in the response of rat small mesenteric arteries to exogenous noradrenaline stimulation. *J. Physiol.* **332**, 363–373.

OHASHI, H. (1971). The relative contribution of K and Cl to the total increase of membrane conductance produced by adrenaline on the smooth muscle of guinea-pig taenia coli. *J. Physiol.* **212**, 561–575.

RAND, M. J. & VARMA, B. (1970). The effects of cholinomimetic drugs on responses to sympathetic nerve stimulation and noradrenaline in the rabbit ear artery. *Br. J. Pharmac.* **38**, 758–770.

SPEDEN, R. N. (1964). Electrical activity of single smooth muscle cells of the mesenteric artery produced by splanchnic nerve stimulation in the guinea-pig. *Nature, Lond.* **202**, 193–194.

SPEDEN, R. N. (1970). Excitation of vascular smooth muscle. In *Smooth Muscle*, ed. BÜLBRING, E., BRADING, A. F., JONES, A. W. & TOMITA, T., pp. 558–588. London: Arnold.

STEINSLAND, O. S., FURCHGOTT, R. F. & KIRPEKAR, S. M. (1973). Inhibition of adrenergic neurotransmission by parasympathomimetics in the rabbit ear artery. *J. Pharmac. exp. Ther.* **184**, 346–356.

SU, C., BEVAN, J. A. & URSILLO, R. C. (1964). Electrical quiescence of pulmonary artery smooth muscle during sympathomimetic stimulation. *Circulation Res.* **15**, 20–27.

SURPRENANT, A. (1980). A comparative study of neuromuscular transmission in several mammalian muscular arteries. *Pflügers Arch.* **386**, 85–91.

TAKATA, Y. (1980). Regional differences in electrical and mechanical properties of guinea-pig mesenteric vessels. *Jap. J. Physiol.* **30**, 709–728.

TRAPANI, A., MATSUKI, N., ABEL, P. W. & HERMSMEYER, K. (1981). Norepinephrine produces tension through electromechanical coupling in rabbit ear artery. *Eur. J. Pharmac.* **72**, 87–91.

VANHOUTTE, P. M. (1976). Inhibition by acetylcholine of adrenergic neurotransmission in vascular smooth muscle. In *Physiology of Smooth Muscle*, ed. BÜLBRING, E. & SHUBA, M. F., pp. 369–377. New York: Raven Press.

VANHOUTTE, P. M., LORENZ, R. R. & TYCE, G. M. (1973). Inhibition of norepinephrine – ^{3}H release from sympathetic nerve endings in veins by acetylcholine. *J. Pharmac. exp. Ther.* **185**, 386–394.