J. Physiol. (1987), **392**, *pp.* 333–348
With 9 text-figures
Printed in Great Britain

THE ROLE OF MEMBRANE DEPOLARIZATION IN THE CONTRACTILE RESPONSE OF THE RABBIT BASILAR ARTERY TO 5-HYDROXYTRYPTAMINE

BY C. J. GARLAND*

From the Department of Physiology, University College, P.O. Box 78, Cardiff CF1 1XL

(*Received 10 February 1987*)

SUMMARY

1. 5-Hydroxytryptamine (5-HT, 10^{-9}–10^{-4} M) depolarized and contracted smooth muscle cells (resting potential: $-69{\cdot}1 \pm 0{\cdot}9$ mV, $n = 112$) in isolated cylindrical segments of the rabbit basilar artery.

2. Simultaneous measurement of membrane potential and wall tension ($n = 43$, thirteen vessels) showed that the onset of 5-HT-induced depolarization coincided with the onset of smooth muscle contraction in the majority of cells studied. In addition, the onset of relaxation which followed the wash-out of 5-HT always preceded the onset of membrane repolarization by 52 ± 8 s ($n = 14$).

3. In 30% of smooth muscle cells exposed to concentrations of 5-HT greater than 10^{-6} M, fast rhythmic depolarizations (amplitude 10–20 mV) were superimposed on the developing depolarization. Rhythmic membrane depolarization was always followed by rhythmic smooth muscle contraction, which peaked 2–4 s after the peak of the fast depolarization.

4. Muscle contraction, but not depolarization, produced with concentrations of 5-HT greater than 10^{-7} M, was significantly increased by the removal of intimal–endothelial cells.

5. Smooth muscle depolarization recorded in the presence of increased extracellular K^+ ($> 5{\cdot}2$ mM) preceded the onset of smooth muscle contraction. For a similar change in membrane potential produced with either increased extracellular K^+ or 5-HT, the corresponding increase in arterial wall tension was always greater with 5-HT.

6. The depolarization and contraction induced by 5-HT was markedly reduced or abolished if extracellular Na^+ was totally replaced, isosmotically, with either sucrose or Tris at pH 7·4. Normal-sized contraction, but not depolarization, was recorded with 5-HT in Na^+-free Tris solution at pH 8.

7. These observations suggest that 5-HT-stimulated contraction in cerebrovascular smooth muscle is largely a result of mechanisms other than depolarization of the smooth muscle cell membrane which it produces. However, high concentrations of 5-HT ($> 10^{-6}$ M) can stimulate additional depolarization, which has a faster time course and rhythmic nature. Discrete depolarizations of this type are responsible for initiating additional, phasic smooth muscle contractions.

* Present address: Department of Physiology and Pharmacology, University of Southampton, Bassett Crescent East, Southampton SO9 3TU.

INTRODUCTION

5-Hydroxytryptamine (5-HT) is the major amine stored in blood platelets, and is released during blood clotting (Rand & Reid, 1951). Mammalian vascular smooth muscle is, in general, highly sensitive to the constrictor action of 5-HT. This is of functional importance in helping to limit the blood loss which follows transection or perforation of the blood vessel wall. The other major constrictor agent released by activated platelets is thromboxane A_2, which is a short-lived derivative of arachidonic acid (Hamberg, Svensson & Samuelsson, 1975). In human arteries, 5-HT is at least as potent as thromboxane A_2 in producing the contraction which follows the application of aggregating platelets (Moulds, Iwanov & Medcalf, 1984). Apart from a role in haemostasis, platelet activation and the release of 5-HT have been implicated in a number of cerebrovascular disorders. In addition, interest has been focused on the cerebrovascular action of 5-HT by the recent demonstration of a specific population of serotonergic perivascular nerve fibres supplying cerebral arteries. The ability of the 5-HT_2 antagonist ketanserin to block the contraction which follows activation of perivascular nerves in the rabbit vertebral artery, strengthens the idea that 5-HT may be acting as a neuromuscular transmitter in cerebral arteries (Griffith, Lincoln & Burnstock, 1982; Edvinsson, Degueurce, Duverger, MacKenzie & Scatton, 1983).

The mechanism by which 5-HT contracts arterial smooth muscle is not clear. Agonist-induced contraction of smooth muscle is the result of mechanisms both dependent on, and independent of, a change in the muscle membrane potential (Evans, Schild & Thesleff, 1958; Waugh, 1962; Keatinge, 1964). The contribution from each component varies in different smooth muscle preparations. In canine tracheal smooth muscle, the contraction produced with 5-HT is mainly a result of mechanisms independent of the change in muscle membrane potential (Coburn & Yamaguchi, 1977). In canine cerebral arteries, however, a positive correlation between smooth muscle depolarization and the level of contraction which can be produced with 5-HT led to the suggestion that smooth muscle contraction was closely coupled to the change in membrane potential (Harder & Waters, 1983). This idea is supported by the observation of rhythmic contractions stimulated in isolated coronary arteries by 5-HT. Contraction of this type may well reflect the occurrence of widespread and co-ordinated membrane depolarization (Corbett, Garland, Greenidge, Keatinge, Orteu, Salvage & Tate, 1985). Intracellular recordings from both coronary and cerebral smooth muscle cells have shown that 5-HT can induce rhythmic membrane depolarization, which may be responsible for initiating such rhythmic arterial contraction (Fujiwara & Kuriyama, 1983; Garland, 1985).

The present study was designed to clarify the role of membrane potential changes in the contraction of cerebrovascular smooth muscle which is induced by 5-HT. Intracellular records were made simultaneously with measurements of smooth muscle contraction, from cells in isolated cylindrical segments of the rabbit basilar artery. In this way, membrane electrical responses to exogenous 5-HT were recorded from smooth muscle cells contributing to the measured tension changes. Available evidence relating to the action of various exogenous agonists on vascular smooth muscle has generally involved separate experiments to measure membrane electrical

events and smooth muscle tension changes. At best, measurements have been made from separate regions of the same tissue. In the latter case, intracellular recordings are taken from an immobilized portion of tissue, in an attempt to maintain cell impalement during smooth muscle contraction (e.g. Holman & Surprenant, 1979; Fujiwara & Kuriyama, 1983; Mekata, 1984). The exception is the work of Mulvany, Nilsson & Flatman (1982), Mulvany, Nilsson, Flatman & Korsgaard (1982) and Cheung (1985), who reported the action of noradrenaline on the rat mesenteric artery and saphenous vein, respectively. These workers recorded intracellularly during tension changes produced with noradrenaline, from cells which could reasonably be expected to contribute to these tension changes.

Results obtained in the present study indicate that most of the contraction produced in cerebrovascular smooth muscle by 5-HT is the result of mechanisms other than a change in the muscle membrane potential. Higher concentrations of 5-HT can, however, stimulate fast, rhythmic membrane depolarization which will produce additional contraction in the artery wall. Some of the results have been reported in a preliminary form (Garland, 1987).

METHODS

White rabbits of either sex (2–3 kg) were anaesthetized with I.V. sodium pentobarbitone (60 mg/kg) and killed by rapid exsanguination. The brain was removed, placed in physiological salt solution (PSS) at room temperature and gassed with 95% O_2+5% CO_2. The basilar artery was carefully removed and cylindrical segments, 2 mm in length, cut from the central region. In some cases, before cutting the segments a blunt syringe needle (25 gauge) was carefully passed through the artery lumen to remove the intimal–endothelial cells. Once a segment had been cut, two tungsten wires (each of 25 μm diameter) were carefully passed through the lumen. Each wire was attached to a small plastic foot, one foot coupled to an isometric tension transducer (Harvard Biosciences, 52-9529) and the other to a microdrive (Prior, code 71). The artery segment was mounted horizontally in a tissue bath (approximate volume: 1·5 ml) and superfused (at 3 ml min^{-1}) with PSS which had been gassed with 95% O_2+5% CO_2 and warmed to 36·5±0·5 °C (Fig. 1). The segment was equilibrated for 60–90 min under a previously determined pre-load of 500 mg. The pre-load represented the peak of the averaged active length–tension curve for contraction with histamine (effective dose for 50% contraction: ED_{50}). Each segment was then contracted with histamine (10^{-6}–10^{-5} M) and the functional ability of endothelial cells to produce smooth muscle relaxation assessed with acetyl-β-methylcholine (methacholine). Methacholine (10^{-6}–10^{-4} M) relaxed the histamine contraction by 80–100%, only if the endothelial lining was intact. Histological staining (see below) was used to confirm that segments which relaxed with methacholine had undamaged endothelial cells over the majority of their luminal surface, and that in segments which failed to relax with methacholine the endothelial cells had been removed. Concentration–response curves were constructed from arterial responses to single concentrations of agonist. 5-HT, histamine and methacholine were equilibrated with the superfusate before it entered the tissue bath. Contractions were allowed to plateau, usually within 2–4 min, before readmission of agonist-free superfusate. At least 5 min separated the application of each agonist concentration, which was sufficient to allow reproducible contractile responses to be recorded throughout the course of an experiment. Where applicable, modified PSS was used as superfusate. In the experiments with K^+-rich PSS, the K^+ concentration was generally increased cumulatively.

Electrophysiology

Measurement of smooth muscle membrane potential was made using glass (Clark-electromedical capillary; 1·2 mm o.d.) intracellular microelectrodes. Electrodes were back-filled with 2 M-KCl, attached to a three-axis micromanipulator and advanced in 2 μm steps with a microprocessor-controlled stepping motor (Neurolog SCAT-01). Cell penetration was achieved by advancing the electrodes through the adventitial surface of the artery segment. Microelectrodes with a resistance

of between 80 and 120 MΩ were capable of routine, prolonged membrane impalement, and were sufficiently flexible to allow the simultaneous measurement of membrane potential during changes in wall tension. Membrane electrical events were recorded through a high-input-impedance d.c. pre-amplifier (Neurolog 102G). Data from the isometric transducer and microelectrodes was digitized, sampled at rates of 5 and 50 Hz and stored on computer disc (Garland, Robertson & Stent, 1987).

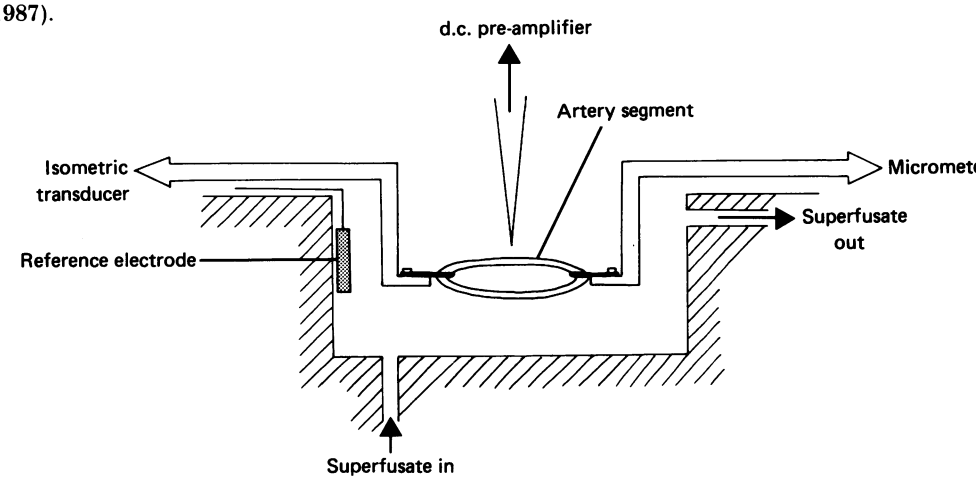

Fig. 1. Diagrammatic representation of the tissue chamber. To measure tension changes, a cylindrical segment of artery is stretched horizontally between two intraluminal tungsten wires. Individual muscle cells are impaled by a glass electrode advanced through the adventitial surface.

Solutions, drugs and histological methods

Most experiments were made in physiological salt solution (PSS) of the following composition (mM): NaCl, 121·8; $NaHCO_3$, 25·5; KCl, 5·2; $MgSO_4$, 1·2; $CaCl_2$, 1·6; disodium EDTA, 0·027; ascorbate, 0·114 and glucose, 9·4. In some experiments the PSS was modified either to raise the potassium concentration, or to remove extracellular sodium. Potassium-rich solutions were prepared by the replacement of NaCl with KCl, and contained 1 μM-phentolamine to block the action of neuronally released noradrenaline. To remove extracellular sodium, either sucrose or Tris was included in the PSS in place of NaCl, $NaHCO_3$ and disodium EDTA. These solutions were adjusted so as to be isosmotic with PSS and have a pH of either 7·4 or 8·0. Adjustment of pH was only necessary in the Tris solutions and was achieved after titration with HCl. This gave a final chloride concentration of 70–100 mM. Solutions were equilibrated with a mixture of 95% O_2+5% CO_2.

The drugs used were acetyl-β-methylcholine chloride (methacholine, Sigma); 5-hydroxytryptamine creatinine sulphate (Sigma); histamine acid phosphate (Sigma).

A modification of the method of Poole, Sanders & Florey (1958) was used to stain the borders of endothelial cells. Arteries were cut open along their longitudinal axis and pinned to a Sylgard block, intima uppermost. Blocks were transferred successively to solutions of (mM): formaldehyde, 650 (10 min); glucose, 277 (1 min); $AgNO_3$, 15 (3 min); glucose, 277 (1 min); $CoBr_2$, 139, in NH_4Br, 102 (10 min); glucose, 277 (1 min); formaldehyde, 1300 (at least 12 h). The tissue was then dehydrated in ethanol, cleared in xylene and examined directly by light microscopy.

Statistical comparisons

Results are expressed as mean ± S.E. Comparisons were made using the t test for unpaired groups. The Mann–Whitney U test was used in cases where data had a skewed distribution. Values of $P < 0·05$ were taken as significant with both tests.

RESULTS

Smooth muscle cells in the basilar artery had a mean resting membrane potential of $-69{\cdot}1 \pm 0{\cdot}9$ mV (mean ± S.E., $n = 112$). In the absence of stimulation the cell membrane was electrically quiescent. The addition of 10^{-11} or 10^{-10} M-5-HT failed to produce measurable smooth muscle contraction or a change in the resting membrane potential. Concentrations greater than 10^{-9} M-5-HT were associated with both membrane depolarization and smooth muscle contraction. The depolarization was smooth in form and generally simultaneous in onset with the recorded contraction ($n = 43$). In twenty-seven of the forty-three cells studied, the onset of depolarization could not be distinguished from the onset of contraction. In seven cells, depolarization occurred 2–7 s before the onset of contraction and in nine cells, 2–32 s after the onset of contraction. The time required for depolarization to plateau was $82{\cdot}2 \pm 9{\cdot}4$ s ($n = 19$). Contractions were either mono- or biphasic, with $55{\cdot}8 \pm 13{\cdot}3$ and $79{\cdot}4 \pm 16{\cdot}0$ s ($n = 19$) required for the respective responses to plateau. These times were not significantly different from the time required for the depolarization to plateau. Representative traces are shown in Fig. 2. The size of depolarization and contraction increased with each tenfold increase in the concentration of 5-HT; 10^{-4} M-5-HT producing a mean depolarization of $18{\cdot}0 \pm 2{\cdot}9$ mV and mean contraction of 756 ± 158 mg (Fig. 3).

The application of 5-HT in concentrations above 10^{-6} M was often followed by rhythmic oscillations in membrane potential. The amplitude of these oscillations varied between 10 and 20 mV, and occurred in 30% of cells. Rhythmic membrane depolarization was always followed by rhythmic smooth muscle contraction, with an amplitude of between 80 and 210 mg; both rhythmic events were superimposed on the normal depolarization and contraction, and had a peak-to-peak separation of 2–4 s (Fig. 4).

Wash-out of PSS containing 5-HT was followed by smooth muscle relaxation which preceded the onset of membrane repolarization by a mean of 52 ± 8 s, see Fig. 2 ($n = 14$). Muscle relaxation preceded membrane repolarization with each concentration of 5-HT which had previously produced a measurable contraction.

The contractions produced by 5-HT were not modified indirectly by the release of noradrenaline from sympathetic nerve endings, as they were not reduced in the presence of 10^{-8} M-ICS 205–930. This compound is a 5-HT_3-receptor antagonist, which inhibits the 5-HT-stimulated release of noradrenaline from sympathetic nerve endings in the rabbit heart, with a pA_2 value of 10·6 (Richardson, Engel, Donatsch & Stadler, 1985).

Depolarization and contraction with 5-HT after removal of intimal–endothelial cells

In the absence of a functional endothelium, unstimulated smooth muscle cells were again electrically quiescent. However, their resting membrane potential, $> -66{\cdot}6 \pm 1{\cdot}0$ mV ($n = 101$), was reduced, although this reduction was not statistically significant. Disruption of the endothelium did not alter the size of contraction which could be produced in the artery segments with 100 mM-K^+, at the end of an experiment. The respective responses were 1939 ± 133 mg ($n = 9$) in unrubbed segments, and 1747 ± 179 mg ($n = 8$) in rubbed segments of basilar artery (difference not significant). In basilar artery segments whose endothelium had been destroyed, the

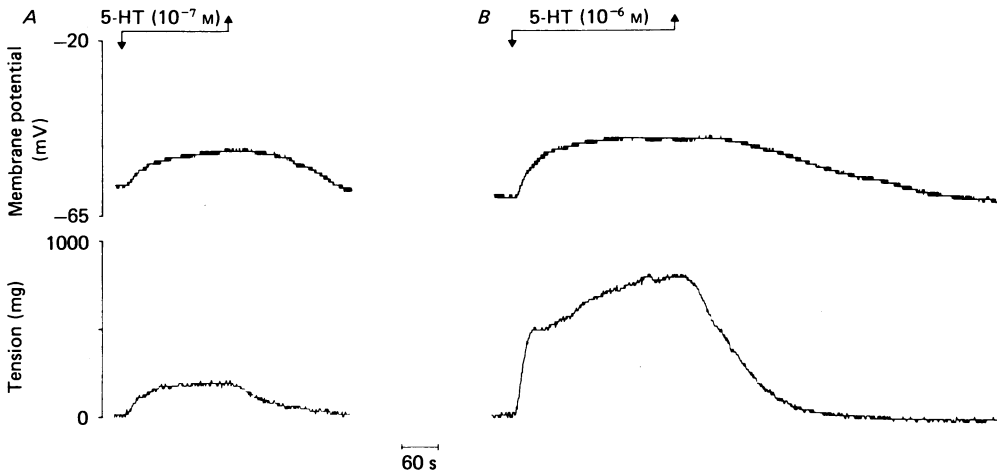

Fig. 2. Responses of the basilar artery to 10^{-7} (*A*) and 10^{-6} M-5-HT (*B*). Membrane depolarization (upper trace) and muscle contraction (lower trace) are simultaneous in onset. During the wash-out of 5-HT, membrane repolarization begins after muscle relaxation. Digitized data.

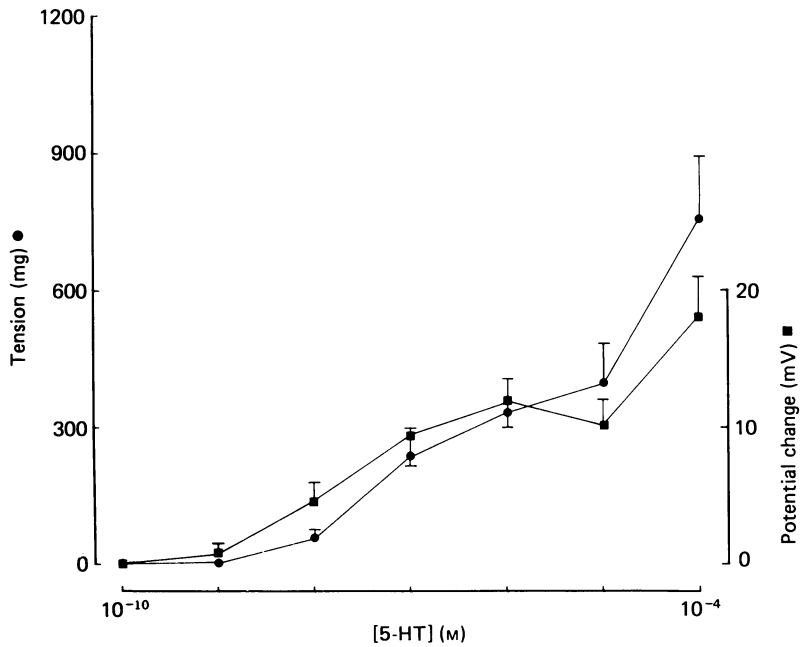

Fig. 3. Concentration–response curves showing increasing depolarization and contraction in the basilar artery, produced with increasing concentrations of 5-HT. Points are the mean of seven to eleven observations for depolarization (■), and seven to sixteen observations for contraction (●). The endothelium was functional in this series.

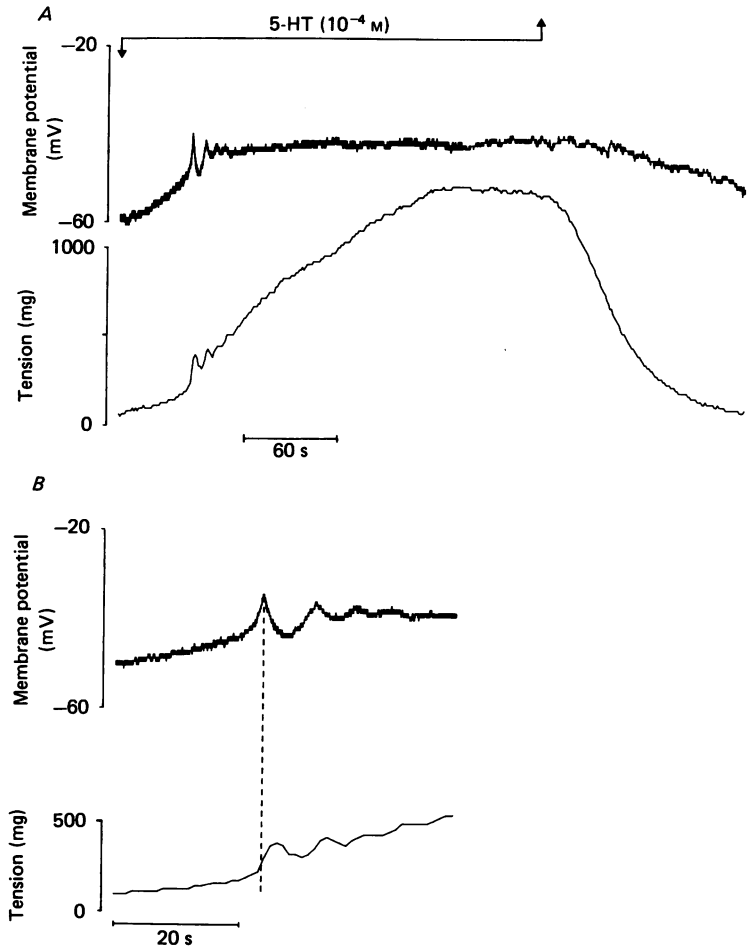

Fig. 4. *A*, depolarization and contraction produced by 10^{-4} M-5-HT, showing oscillations in both membrane potential and smooth muscle contraction. *B*, same cell at greater resolution, showing that the rhythmic oscillations in membrane potential precede the rhythmic increases in tension. Digitized data.

amplitude of membrane depolarization with 5-HT was not significantly changed (Fig. 5). Depolarization was still smooth in form, and the onset of relaxation still preceded the onset of membrane repolarization. However, the size of contraction produced with 5-HT was increased, significantly so with concentrations of between 10^{-6} and 10^{-4} M (Fig. 6).

Membrane and tension responses with increased K^+

Smooth muscle contraction and depolarization was recorded during cumulative or non-cumulative superfusion with PSS which contained increasing concentrations of K^+. Phentolamine (1 μM) was present throughout these experiments, to block the

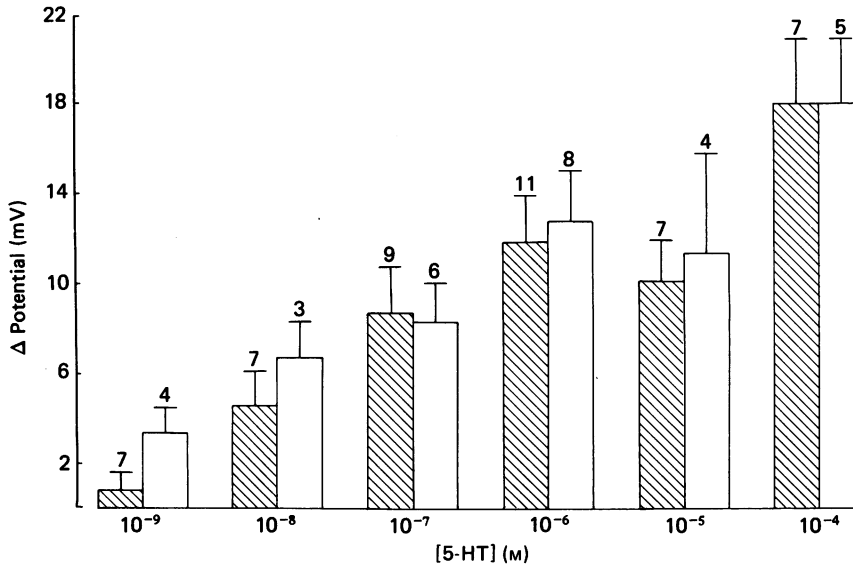

Fig. 5. Histogram showing the level of smooth muscle depolarization produced by 5-HT in basilar arteries with (hatched columns) and without a functional endothelium (open columns). Numbers of observations are indicated at the top of each column.

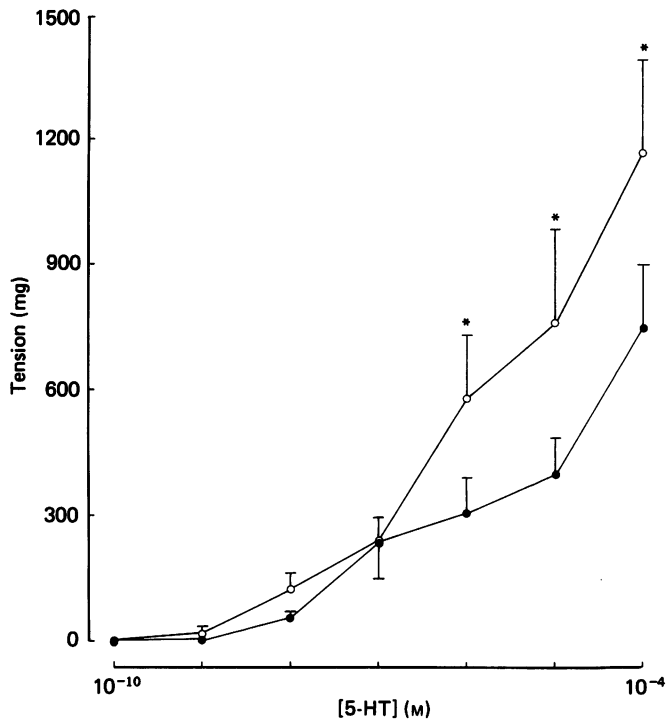

Fig. 6. Concentration–response curves showing the contraction produced by 5-HT in basilar arteries with (●; $n = 7$–16) and without (○; $n = 5$–8) a functional endothelium. Unlike depolarization, contractions were increased by the removal of the endothelium, significantly so (*) with concentrations greater than 10^{-7} M.

TABLE 1. Steady-state membrane potential, and associated contractile responses, recorded in the basilar artery with increased extracellular K^+ concentrations

Concentration of extracellular K^+	Membrane potential (mV)	Tension (mg)
0	51·0±6·3 (5)	108±39 (4)
10	58·3±5·0 (7)	49±34 (7)
20	45·5±5·2 (7)	239±65 (7)
40	30·7±4·6 (7)	1810±141 (8)
80	18·6±3·1 (8)	2058±112 (8)
100	14·9±3·7 (7)	2111±107 (8)
127	8·4±2·0 (2)	2022±215 (3)

Numbers in parentheses are numbers of observations (n).

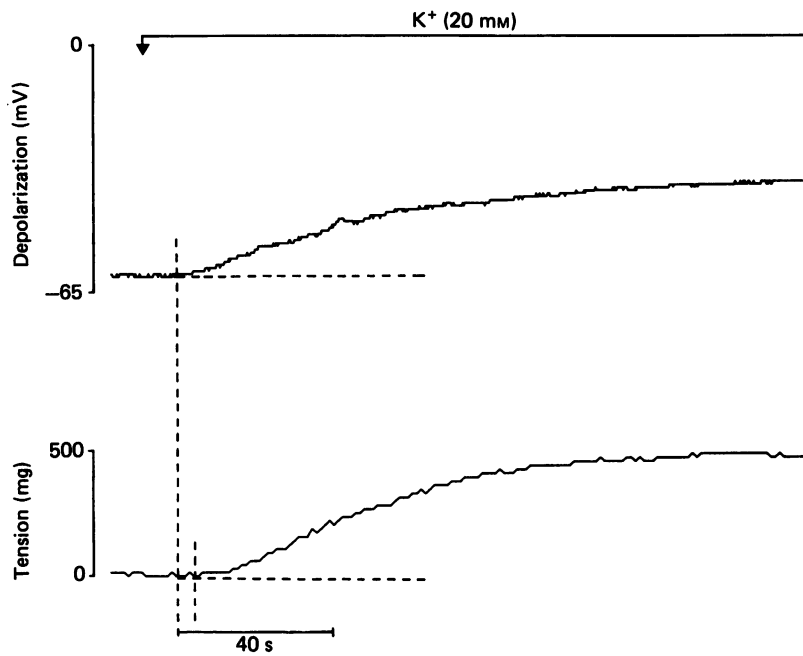

Fig. 7. Depolarization and contraction produced by 20 mM-K^+-PSS. Contraction always followed the depolarization with increased extracellular K^+. Digitized data.

action of noradrenaline released from perivascular nerves by the increase in extracellular K^+. The mean resting membrane potential and respective values for smooth muscle contraction with different concentrations of K^+ are shown in Table 1. Unlike the membrane depolarization produced with 5-HT, depolarization which followed increases in the extracellular K^+ concentration always preceded the onset of contraction by a mean of 8·8±3·2 s (n = 9). This observation is illustrated in Fig. 7. Furthermore, the change in membrane potential which followed an increase in extracellular K^+ was associated with much smaller contractions than similar-sized depolarization produced with 5-HT (Fig. 8). For example, 20 mM-K^+ depolarized the

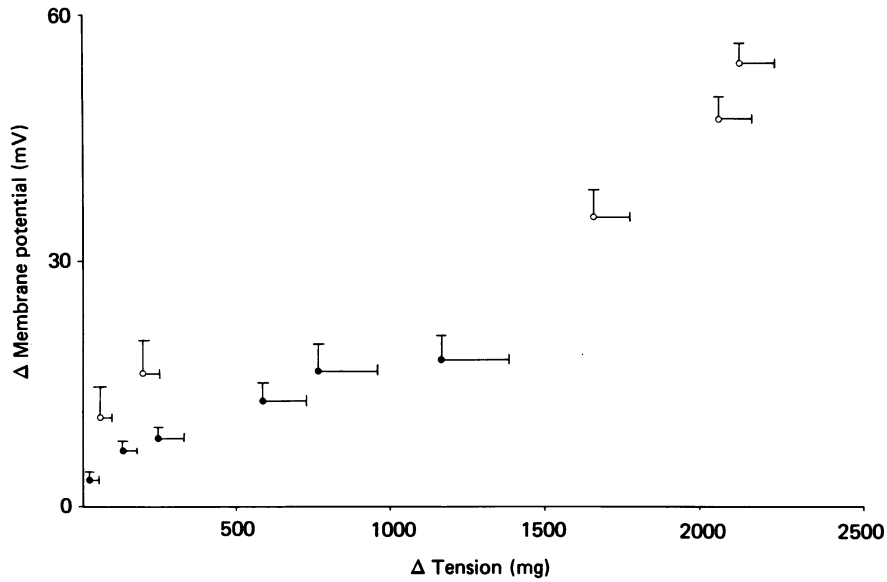

Fig. 8. Relationship between changes in membrane potential and tension with 5-HT (●), 10^{-9}–10^{-4} M, and K^+ (○), 10–100 mM. With similar changes in membrane depolarization, 5-HT produced much larger contractions than K^+.

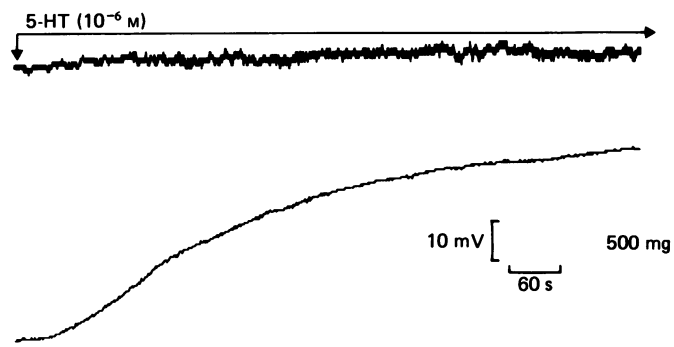

Fig. 9. Response of the basilar artery to 5-HT, recorded in the absence of extracellular Na^+. Digitized data. Reduced depolarization (upper trace) and normal-amplitude contraction (lower trace) produced by 10^{-6} M-5-HT at pH 8.

membrane by 18·9±4·6 mV and stimulated a contraction of 239±66 mg ($n = 7$), whereas 10^{-4} M-5-HT depolarized the smooth muscle membrane to a similar extent, 18·0±3·0 mV ($n = 5$), but produced a mean increase in tension of 1172±231 mg ($n = 7$) in de-endothelialized arteries, five times greater than the contraction with K^+. Rhythmic contraction and depolarization was not observed during increases in the extracellular K^+ concentration.

Effect of removing extracellular Na^+ on depolarization and contraction with 5-HT

Superfusion of artery segments with modified PSS in which Na^+ had been totally replaced isosmotically with either sucrose or Tris, was followed by an increase in

smooth muscle tension which spontaneously returned to the resting pre-load over a period of 5–15 min. The resting potential was not significantly altered ($< \pm 5$ mV) during superfusion with these modified solutions.

Superfusion with sucrose-containing PSS for 15–30 min was associated with $98{\cdot}3 \pm 1{\cdot}0$ and $91{\cdot}0 \pm 6{\cdot}3\%$ ($n = 5$) reduction in the level of contraction and depolarization, respectively, produced with 10^{-7}, 10^{-6} or 10^{-4} M-5-HT. Similarly, superfusion with Tris-containing PSS for 20–60 min was associated with a mean reduction of $78{\cdot}5 \pm 6{\cdot}6$ and $97{\cdot}2 \pm 2{\cdot}7\%$ ($n = 4$) in the contraction and depolarization produced with 10^{-6} or 10^{-4} M-5-HT. In one of the experiments with Tris–PSS, 10^{-6} and 10^{-4} M-5-HT induced rhythmic depolarization and contraction (14·5–18·5 mV: 75–100 mg amplitude) in normal PSS, which was abolished in the Tris-containing PSS. In both modified solutions the contraction produced with 10^{-6} M-histamine was only slightly (25–35%) reduced.

Normal-sized contraction, but not depolarization, could be produced with 5-HT, and in the absence of extracellular Na^+, if the segments of basilar artery were superfused with Tris-containing PSS at pH 8·0. Depolarization produced with 10^{-7} or 10^{-6} M-5-HT was reduced by $95{\cdot}6 \pm 4{\cdot}4\%$ compared with control, whereas contractions, although slower to develop, were only reduced by a mean of $10{\cdot}4 \pm 15{\cdot}9\%$ ($n = 6$) (Fig. 9). In these experiments, a second application of 5-HT, in concentrations up to 10^{-4} M, failed to produce any depolarization or a second contraction.

DISCUSSION

The contractile action of various agonists which act on vascular smooth muscle is in part a result of membrane depolarization, and in part a result of mechanisms independent of this depolarization (see Keatinge & Harman, 1980). Studies investigating the mechanism by which 5-HT produces contraction in vascular smooth muscle are limited. However, evidence has been presented which suggests that membrane depolarization induced by 5-HT is closely coupled to contraction in cerebrovascular smooth muscle (Harder & Waters, 1983). This is in contrast to the constrictor action of noradrenaline, adrenaline and histamine in the sheep carotid, an extracerebral artery (Keatinge, 1964). These agents produced much the same total contraction in K^+-rich solutions at 20 °C – and therefore in the absence of a muscle membrane potential – as they did in Na^+-based solutions. Studies were performed at 20 °C to limit the direct constrictor action of the raised extracellular K^+. As the contraction in K^+-rich solutions was slower to develop, it was suggested that membrane electrical events served to accelerate the onset of contraction with high concentrations of these agonists. The present study provides evidence that the contraction produced by 5-HT in the rabbit basilar artery occurred mainly as a result of mechanisms other than depolarization of the smooth muscle membrane. If membrane depolarization produced by 5-HT was essential for muscle contraction, it would be expected to always precede the onset of contraction. This was not the case. Further evidence against an involvement of membrane potential changes in the responses with 5-HT was the onset of smooth muscle relaxation, which followed the wash-out of 5-HT. With each concentration of 5-HT, relaxation always started before membrane repolarization.

The failure of membrane depolarization to precede the onset of contraction did not appear to reflect variation in the diffusion of exogenous 5-HT within the artery wall. The intracellular records were made from a large number of cells in different positions along the artery segments, and at different depths in the media. This would increase rather than decrease any variability due to diffusion. In addition, depolarization which did precede the onset of contraction was always observed in experiments with a raised extracellular K^+ concentration. Finally, the consistent temporal relationship between muscle relaxation and membrane repolarization is also strong evidence against the diffusion of 5-HT being of significance in the interpretation of these experiments. These observations with 5-HT are qualitatively very similar to the action of noradrenaline on smooth muscle cells in the rat mesenteric artery (Mulvany, Nilsson & Flatman, 1982), but contrast with the action of noradrenaline on the saphenous vein, where depolarization clearly precedes the onset of contraction (Cheung, 1985). The response to noradrenaline in the saphenous vein is somewhat unusual, however, in that it is mediated mainly by α_2- , rather than α_1-adrenoceptors.

The relative unimportance of a change in smooth muscle membrane potential as a necessary prerequisite for contraction is further exemplified with experiments on de-endothelialized segments of the basilar artery. When the attenuating action of the endothelium is removed, contraction but not depolarization with 5-HT is increased. Reduction in the contractile effect of 5-HT may be explained by the release of an endothelial-derived smooth muscle relaxing factor (EDRF), either spontaneously or stimulated by 5-HT. The spontaneous release of EDRF is responsible for reducing the contractile action of 5-HT in the rat aorta (Martin, Furchgott, Villani & Jothianandan, 1986), whereas the ability of 5-HT to release EDRF has been demonstrated in canine and porcine coronary arteries (Cocks & Angus, 1983; Cohen, Shepherd & Vanhoutte, 1983). The chemical structure of EDRF is not known, although its mechanism of action has a number of characteristics in common with the non-adrenergic, non-cholinergic transmitter released from inhibitory nerves supplying the bovine retractor penis muscle. For example, both stimulate an increase in the intracellular levels of cyclic GMP, which is an essential step in the subsequent smooth muscle relaxation (Rapoport, Draznin & Murad, 1983; Bowman & Drummond, 1984; Martin, Villani, Jothianandan & Furchgott, 1985). If changes in the intracellular levels of cyclic GMP are responsible for attenuating the contraction with 5-HT when the endothelium is functional, this could well explain why there is no change in the level of membrane depolarization attained with 5-HT after the endothelium has been destroyed. A similar attenuating effect of the endothelium on contractions produced with 5-HT, which is independent of a change in membrane potential, occurs in the rabbit coronary artery (Garland, 1985). At least part of the endothelial-dependent relaxation produced by acetylcholine is also brought about by means other than a change in membrane potential (Furchgott & Zawadzki, 1980), although endothelial-dependent smooth muscle hyperpolarization could sometimes be stimulated with carbachol, in the guinea-pig mesenteric artery (Bolton, Lang & Takewaki, 1984). Alternatively, the ability of endothelial cells to reduce the contractile effect of 5-HT may not be entirely due to the release of a smooth muscle relaxing factor. It might be explained, at least in part, by the ability of these cells

to take up and metabolize 5-HT, as they do in the aorta and blood vessels of the lung (Bakhle & Vane, 1974; Small, Macarak & Fisher, 1977). The uptake of 5-HT by endothelial cells may well explain why intra-carotid injections of 5-HT in the baboon did not produce any significant decrease in cerebral blood flow. A significant decrease in cerebral blood flow only occurred after osmotic disruption of the blood–brain barrier, a procedure which will disrupt the intimal–endothelial cells (Harper & MacKenzie, 1977).

The responses obtained in the presence of raised extracellular K^+ are also consistent with the idea that membrane potential changes are of little importance in mediating the contraction produced by 5-HT. Comparing similar levels of smooth muscle depolarization produced by either K^+ or 5-HT, the contraction produced with K^+ – as a direct result of smooth muscle depolarization – was always much less than that with 5-HT.

Contraction produced by 5-HT could also be recorded when membrane depolarization had been markedly reduced by the removal of extracellular Na^+. In Na^+-free solutions at pH 7·4, the depolarization and contraction with 5-HT was reduced or abolished. This indicates that the depolarization can be explained mainly by movement of Na^+ ions. Chloride is unlikely to make a significant contribution, as depolarization was markedly depressed in Tris–PSS which contained 70–100 mM-Cl^-. There is likely to be some contribution from the movement of Ca^{2+}, probably through receptor-operated channels. However, the apparent effect on membrane potential will be limited by the opening of Ca^{2+}-dependent K^+ channels (Bolton, 1986). Reduced smooth muscle contraction in Na^+-substituted, sucrose-containing solutions at physiological pH has also been recorded in the rabbit pulmonary artery with noradrenaline. However, in this study the depolarization produced by noradrenaline changed to a small hyperpolarization (Casteels, Kitamura, Kuriyama & Suzuki, 1977). In the present study, the contraction produced by 5-HT was not significantly reduced at pH 8, although the depolarization was. Why smooth muscle contraction with 5-HT should be maintained by raising the pH of the Na^+-free solution is not clear, but may be related to changes in intracellular pH. The removal of extracellular Na^+ is followed by a decrease in intracellular pH in a number of cell types, including skeletal muscle (Aickin & Thomas, 1977) and sheep Purkinje fibres (Ellis & MacLeod, 1985). A similar decrease in intracellular pH may explain the depressed smooth muscle contraction in the present study at pH 7·4, as it does in both cardiac and skeletal muscle fibres (Fabiato & Fabiato, 1978). A high external pH will increase the diffusion gradient for intracellular H^+, which will accumulate at an increased rate due to the absence of extracellular Na^+. This may limit the drop in intracellular pH and preserve the ability of the smooth muscle cells to contract. In any event, whatever the precise explanation, the results show that normal-sized contraction with 5-HT can occur without depolarization.

Although nearly all the contraction produced by 5-HT in the basilar artery was mediated by non-electrical means, additional contraction sometimes followed membrane responses stimulated in the presence of high concentrations of 5-HT. Rhythmic oscillations in membrane potential, with concentrations of 5-HT above 10^{-6} M, always preceded rhythmic smooth muscle contraction. The rhythmic nature of these depolarizations, and the contractions which followed, is a strong indication that the

contraction was a direct result of the depolarization. The magnitude of the contraction was, however, small compared to the contraction produced by non-electrical means. Similar rhythmic smooth muscle contraction was recorded with high concentrations of 5-HT ($> 10^{-5}$ M) in the guinea-pig basilar artery. The rhythmic, but not the normal tonic contraction, was abolished by nicardipine, so it may well have reflected the activation of voltage-dependent Ca^{2+} channels (Fujiwara & Kuriyama, 1983). An interesting possibility is that rhythmic depolarizations induced by localized exposure of the inner layers of the arterial media to high concentrations of platelet-released 5-HT, may spread round the artery wall. The spread of rhythmic depolarization round the artery wall will be aided by the space constant, which is much longer than in the direction of the vessel's longitudinal axis (Graham & Keatinge, 1975). The resulting smooth muscle contraction will amplify the response to 5-HT, and may serve to occlude an artery already partially narrowed by an eccentric stenosis.

In conclusion, the present study demonstrates that in the rabbit basilar artery, smooth muscle contraction with 5-HT is mainly due to mechanisms other than a change in muscle membrane potential. In the presence of high concentrations of 5-HT, oscillations in smooth muscle membrane potential can produce a small, additional contraction, which might be relevant in some cerebrovascular disorders.

I should like to thank John Robertson for his assistance during the course of this work, Dr P. A. T. Kelly for his comments on the manuscript and the Wellcome Trust for financial support.

REFERENCES

AICKEN, C. C. & THOMAS, R. C. (1977). An investigation of the ionic mechanism of intracellular pH regulation in mouse soleus muscle fibres. *Journal of Physiology* **273**, 295–316.

BAKHLE, Y. S. & VANE, J. R. (1974). Pharmacokinetic function of the pulmonary circulation. *Physiological Reviews* **54**, 1007–1045.

BOLTON, T. B. (1986). Calcium metabolism in vascular smooth muscle. *British Medical Bulletin* **42**, 421–429.

BOLTON, T. B., LANG, R. J. & TAKEWAKI, T. (1984). Mechanisms of action of noradrenaline and carbachol on smooth muscle of guinea-pig mesenteric artery. *Journal of Physiology* **351**, 549–572.

BOWMAN, A. & DRUMMOND, A. H. (1984). Cyclic GMP mediates neurogenic relaxation in the bovine retractor penis muscle. *British Journal of Pharmacology* **81**, 665–674.

CASTEELS, R., KITAMURA, K., KURIYAMA, H. & SUZUKI, H. (1977). Excitation–contraction coupling in the smooth muscle cells of the rabbit main pulmonary artery. *Journal of Physiology* **271**, 63–79.

CHEUNG, D. W. (1985). An electrophysiological study of α-adrenoceptor mediated excitation–contraction coupling in the smooth muscle cells of the rat saphenous vein. *British Journal of Pharmacology* **84**, 265–271.

COBURN, R. F. & YAMAGUCHI, T. (1977). Membrane potential-dependent and independent tension in the Canine tracheal muscle. *Journal of Pharmacology and Experimental Therapeutics* **201**, 276–284.

COCKS, T. M. & ANGUS, J. A. (1983). Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. *Nature* **305**, 627–630.

COHEN, R. A., SHEPHERD, J. T. & VANHOUTTE, P. M. (1983). 5-Hydroxytryptamine can mediate endothelium-dependent relaxation of coronary arteries. *American Journal of Physiology* **245**, H1077–1080.

CORBETT, M. C., GARLAND, C. J., GREENIDGE, E., KEATINGE, W. R., ORTEU, C., SALVAGE, D. & TATE, J. (1985). Pattern of response of smooth muscle of isolated bovine coronary arteries to a thromboxane A_2 analogue. *Journal of Physiology* **369**, 140*P*.

EDVINSSON, L., DEGUEURCE, A., DUVERGER, D., MACKENZIE, E. T. & SCATTON, B. (1983). Central serotonergic nerves project to the pial vessels of the brain. *Nature* **306**, 55–57.

ELLIS, D. & MACLEOD, K. T. (1985). Sodium-dependent control of intracellular pH in Purkinje fibres of sheep heart. *Journal of Physiology* **359**, 81–105.

EVANS, D. H. L., SCHILD, H. O. & THESLEFF, S. (1958). Effects of drugs on depolarized plain muscle. *Journal of Physiology* **143**, 474–485.

FABIATO, A. & FABIATO, F. (1978). Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *Journal of Physiology* **276**, 233–255.

FUJIWARA, S. & KURIYAMA, H. (1983). Nicardipine actions on smooth muscle cells and neuromuscular transmission in the guinea-pig basilar artery. *Journal of Pharmacology and Experimental Therapeutics* **225**, 447–455.

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

GARLAND C. J. (1985). Endothelial cells and the electrical and mechanical responses of the rabbit coronary artery to 5-hydroxytryptamine. *Journal of Pharmacology and Experimental Therapeutics* **233**, 158–162.

GARLAND, C. J. (1987). Membrane and contractile responses to 5-hydroxytryptamine in the isolated rabbit basilar artery. *Journal of Physiology* **386**, 52*P*.

GARLAND, C. J., ROBERTSON, J. & STENT, C. J. (1987). Programs to capture, store and analyse membrane and tension data from vascular smooth muscle. *Journal of Physiology* **388**, 4*P*.

GRAHAM, J. M. & KEATINGE, W. R. (1975). Responses of inner and outer muscle of the sheep carotid artery to injury. *Journal of Physiology* **247**, 473–482.

GRIFFITH, S. G., LINCOLN, J. & BURNSTOCK, G. (1982). Serotonin as a neuromuscular transmitter in cerebral arteries. *Brain Research* **247**, 388–392.

HAMBERG, M., SVENSSON, J. & SAMUELSSON, B. (1975). Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proceedings of the National Academy of Sciences of the U.S.A.* **72**, 2994–2998.

HARDER, D. R. & WATERS, A. (1983). Electromechanical coupling in feline basilar artery in response to serotonin. *European Journal of Pharmacology* **93**, 95–100.

HARPER, A. M. & MACKENZIE, E. T. (1977). Cerebral circulatory and metabolic effects of 5-hydroxytryptamine in anaesthetized baboons. *Journal of Physiology* **271**, 721–733.

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

KEATINGE, W. R. (1964). Mechanism of adrenergic stimulation of mammalian arteries and its failure at low temperatures. *Journal of Physiology* **174**, 184–205.

KEATINGE, W. R. & HARMAN, M. C. (1980). Local mechanisms controlling blood vessels. *Monographs of the Physiological Society*, No. 37, pp. 17–32. New York: Academic Press.

MARTIN, W., FURCHGOTT, R. F., VILLANI, G. M & JOTHIANANDAN, D. (1986). Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. *Journal of Pharmacology and Experimental Therapeutics* **237**, 529–538.

MARTIN, W., VILLANI, G. M., JOTHIANANDAN, D. & FURCHGOTT, R. F. (1985). Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *Journal of Pharmacology and Experimental Therapeutics* **232**, 708–716.

MEKATA, F. (1984). Different electrical responses of outer and inner muscle of the rabbit carotid artery to noradrenaline and nerves. *Journal of Physiology* **346**, 589–598.

MOULDS, R. F. W., IWANOV, V. & MEDCALF, R. L. (1984). The effects of platelet-derived contractile agents on human digital arteries. *Clinical Science* **66**, 443–451.

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. *Journal of Physiology* **332**, 363–373.

MULVANY, M. J., NILSSON, H., FLATMAN, J. A. & KORSGAARD, N. (1982). Potentiating and depressing effects of ouabain and potassium-free solutions on rat mesenteric resistance vessels. *Circulation Research* **51**, 514–524.

POOLE, J. C. F., SANDERS, A. G. & FLOREY, H. W. (1958). The regeneration of aortic endothelium. *Journal of Pathology and Bacteriology* **75**, 133–143.

RAND, M. & REID, G. (1951). Source of "serotonin" in serum. *Nature* **168**, 385.

RAPOPORT, R. M., DRAZNIN, M. B. & MURAD, F. (1983). Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. *Nature* **306**, 174–176.

RICHARDSON, B. P., ENGEL, G., DONATSCH, P. & STADLER, P. A. (1985). Identification of serotonin M-receptor subtypes and their specific blockage by a new class of drugs. *Nature* **316**, 126–131.

SMALL, R., MACARAK, E. & FISHER, A. B. (1977). Production of 5-hydroxyindolacetic acid from serotonin by cultured endothelial cells. *Journal of Cellular Physiology* **90**, 225–231.

WAUGH, W. H. (1962). Adrenergic stimulation of depolarized arterial muscle. *Circulation Research* **11**, 264–276.