Relaxing and Contracting Factors

The Endothelium

Relaxing and Contracting Factors

Edited by ***Paul M. Vanhoutte,*** 1988

Relaxing and Contracting Factors

Biological and Clinical Research

Edited by

Paul M. Vanhoutte

Library of Congress Cataloging in Publication Data

Main entry under title:

Relaxing and Contracting Factors.

(Contemporary biomedicine) (The Endothelium)
Includes bibliographies and index.
1. Endothelium. 2. Blood—Circulation—Deregulation.
3. Vasodilators. 4. Blood vessels—Dilatation. 4. Blood
vessels—Contraction. I. Vanhoutte, Paul M. II. Series.
III. Series: Endothelium. [DNLM: 1. Endothelium—physiology. 2. Vasoconstrictor Agents—secretion. 3. Vasodilator Agents—secretion. QV 150 R382]
QP88.45.R45 1988 612'.18 87-22541

ISBN-13: 978-1-4612-8939-5 e-ISBN-13: 978-1-4612-4588-9
DOI: 10.1007/978-1-4612-4588-9

Softcover reprint of the hardcover 1st edition 1988

Crescent Manor
PO Box 2148
Clifton, NJ 07015

Preface

It is an exciting task to be the editor of the first monograph covering a new area of the biomedical sciences. Since the first report in 1980 by Robert Furchgott and colleagues (*see* Chapter 1) of the evidence of endothelium-dependent relaxation in isolated arteries, there are ever-increasing numbers of vascular physiologists and pharmacologists who are scraping away the endothelium to look into its role in cardiovascular control. And the more one looks, the more one discovers. Not only is the list of substances that can induce endothelium-dependent relaxations impressively long, but these intriguing cells can also secrete vasoconstrictor substances. The ability of the endothelium to modulate the degree of contraction of the underlying smooth muscle is an ancestral property of the blood vessel wall, illustrating the logic of nature, since the endothelial cells are located in the best possible strategic location to continuously monitor the properties (chemical or physical) of the blood. And more and more data emerge suggesting that in several cardiovascular diseases perturbations in endothelium-dependent responses are one of the early signs of the abnormal process. Thus, the importance of endothelium-dependent responses, triggered by the intellectual curiosity of one of the pioneers of vascular physiology and pharmacology, is now recognized not only by basic scientists, but also by all concerned with the cardiovascular diseases. The purpose of this monograph is to provide them with a reference work, so that they know where to start.

As editor, I recognized the overlapping of information among the authors. Because it is crucial, however, at such an early stage of this particular quest for knowledge to confirm, reconfirm, and extend the original observations, and with each author having a different approach, the outcome is a richer knowledge of this growing field.

I would like to thank the authors for their exciting chapters. My secretaries, Mrs. J. Beckman and Mrs. C. Camrud, deserve my gratitude for helping me to coach the effort. And my special thanks goes to Mr. Lanigan and his staff, at Humana Press, who masterly converted the edited chapters into a publication of the highest technical quality.

Paul M. Vanhoutte

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Chapter 18

Endothelium-Dependent Responses in Large Arteries and in the Microcirculation

J. A. Angus, T. M. Cocks, C. E. Wright, K. Satoh, and G. R. Campbell

1. Introduction

Studies focusing on specific components of the blood vessel wall have yielded an important secret of how the "microenvironment" can control large and small artery diameter. As a result of the now classic discovery of endothelium-derived relaxing factor (EDRF) by Furchgott and Zawadzki (1980), any discussion of the physiology or pathology of vascular reactivity must consider the integrity and function of the cells lining the intimal surface.

This chapter reviews our experiments concerned with the reactivity of large and small blood vessels. It deals with the identification of specific receptors for serotonin and norepinephrine on the endothelium. The reactivity of large arteries to endothelium-dependent dilator agents is compared over five arteries in the dog and in the coronary arteries of cow and pig. The reactivity of large arteries to EDRF is assessed in vitro and in vivo after the removal of the endothelium and the subsequent growth of a neointima. At the level of the microcirculation, we assessed whether resistance vessel responsiveness to endothelium-dependent vasodilator agents and to reactive hyperemia were altered by hypertension or hypercholesterolemia in the hindquarter vasculature of conscious rabbits.

P. M. Vanhoutte (ed.), *Relaxing and Contracting Factors*

These studies emphasize the regional and species-dependent differences in population of receptors on endothelial cells and smooth muscle and demonstrate that marked alterations in reactivity of larger arteries can occur when the architecture of the blood vessel wall is altered.

2. EDRF and Reactivity of Large Arteries In Vivo

2.1. Sonomicrometry in Femoral and Coronary Arteries under Conditions of Controlled Flow and Pressure

To test whether endothelial cells were obligatory for the relaxation caused by acetylcholine in blood-perfused arteries, femoral arteries and left anterior descending coronary arteries of the greyhound were perfused with heparinized blood under conditions of controlled flow and perfusion pressure (by means of a distal Starling resistor). These experimental conditions were considered necessary to allow for precise measurement of the changes in diameter of the large arteries caused by acetylcholine-induced release of EDRF and not by changes in blood flow or confounded by the fall in perfusion pressure (Fig. 1). These latter events occur as a result of acetylcholine acting in the distal resistance vessels.

The external diameter of the arteries was measured at two points 1–2 cm apart by pairs of sonomicrometer crystals attached to the upper and lower surfaces of the artery (Angus et al., 1983; Angus and Cocks, 1984). Acetylcholine (0.1–1 μM) infused directly into the arteries proximal to the crystals caused increase in diameter (5–15%) at both sites that was sustained as long as the drug infusion was maintained (Fig. 2). Removal of the endothelium by intraarterial balloon inflation at the distal crystal site abolished the relaxation in response to acetylcholine, substance P, bradykinin, or adenosine triphosphate, but not to nitroglycerin. Thus the proximal endothelial cells could not apparently transfer the EDRF that they produce via the blood to relax the artery just 1 cm downstream. This suggests that (1) EDRF was diluted in the blood to below threshold concentration, or (2) EDRF was released only from the abluminal surface onto the underlying smooth muscle cells, or (3) hemoglobin or other proteins in the blood had reduced the concentration of free EDRF. In any event these experiments suggest that it is unlikely that EDRF is a circulating hormone. Sodium nitroprusside also dilated the distal vasculature, but appeared to have little effect on the diameter of large arteries in either the uncontrolled (Fig. 1) or controlled preparations, regardless of the presence of the endothelium (Fig. 3). The concentration of sodium nitroprusside infused intraarterially was very high, as shown by the delayed depressor

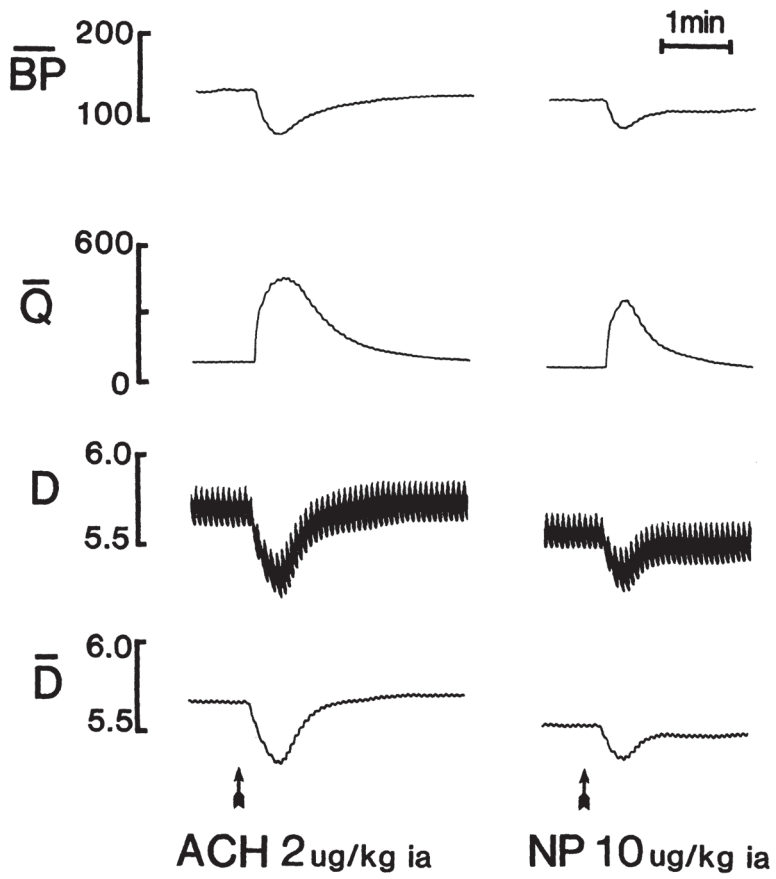

Fig. 1. Recording of femoral hemodynamics in an anesthetized dog. Acetylcholine (Ach, left) and sodium nitroprusside (NP, right) were given as bolus intraarterial (ia) injections into the femoral artery. Traces from top are BP, systemic blood pressure, mm Hg; Q, femoral blood flow (electromagnetic flowmeter, mL/min); D, $\overline{D}$ phasic and mean femoral diameter, mm. (Angus J. A., unpublished data).

action as the drug reached the systemic circulation in the controlled preparation (Fig. 3). In contrast, acetylcholine had no effect on the systemic circulation presumably because it was destroyed rapidly in the blood (Fig. 2).

Acetylcholine and substance P applied to the adventitial surface of the coronary and femoral arteries also increased the diameter if the endothelium was intact. The concentrations required for topical application of both agonists were 50–100 times higher than those required for the intraarterial route (Angus et al., 1983). This illustrates that substances released

from nerve endings within the vessel wall could play a role in vasodilatation by causing the release of EDRF.

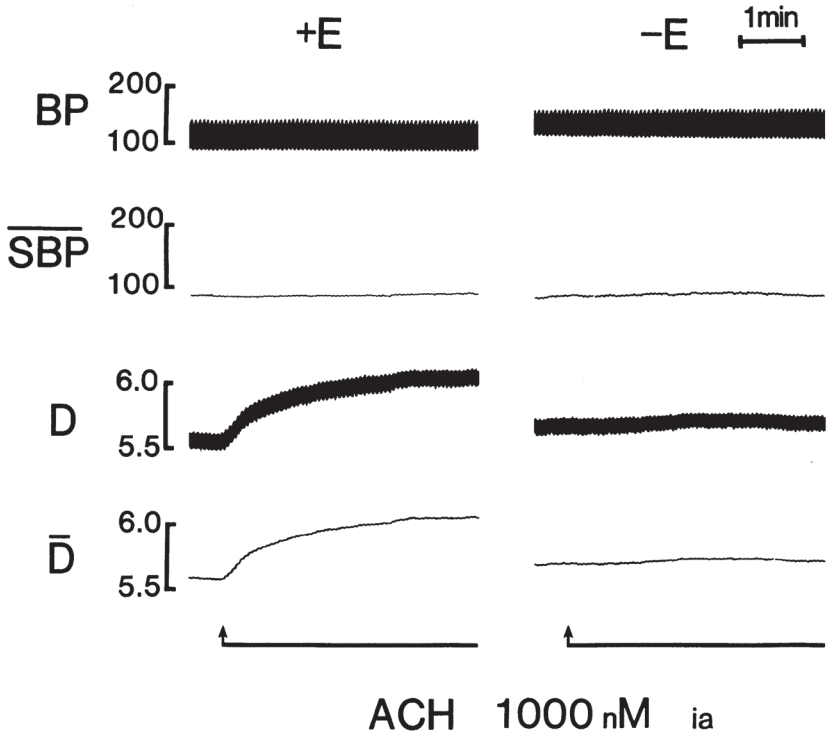

Fig. 2. Records from the femoral artery from the same dog as in Fig. 1 after controlling blood flow and distal resistance. Traces are: BP, systemic blood pressure, mm Hg; SBP, side branch pressure in the blood perfused artery, mm Hg; D and $\overline{D}$, phasic and mean femoral diameter (mm). Acetylcholine (1000 n*M*) was infused into the artery before (+E, left) and after (−E, right) endothelium removal (Angus J. A., unpublished data).

2.2. Chronic Measurement of the Diameter of the Carotid Artery after Removal of the Endothelium

2.2.1. Effect of Acetylcholine

To examine the long-term effects of the removal of the endothelium and subsequent intimal repair on vascular reactivity, greyhound dogs were surgically prepared with flow transducers (Doppler), sonomicrometer crystals, and intraarterial catheters (Herd and Barger, 1964) on both common carotid arteries. During the surgery, the endothelium was removed from one carotid artery with a balloon catheter (Fogarty). Three days later, as

the dogs rested quietly unanesthetized on a padded table, acetylcholine was infused into the carotid artery with intact endothelium. This infusion caused a small increase in carotid diameter, after an initial transient fall that coincided with the fall in carotid pressure and the cranial vasodilatation (Fig. 4).

By contrast, on the same day the left carotid artery in which the endothelium had been removed at surgery only showed the passive fall in diameter as the pressure fell and the flow increased. Over the next 10 d, the responses in the artery without endothelium gradually returned to be similar to those in the control artery indicative of a recovery of the intima by regrowth of the endothelial cells from adjoining areas. At autopsy 4 wk later, the arteries from 10 dogs showed intimal thickening of 2–20 smooth muscle cells (whose cytoplasm contained many synthetic organelles) ar-

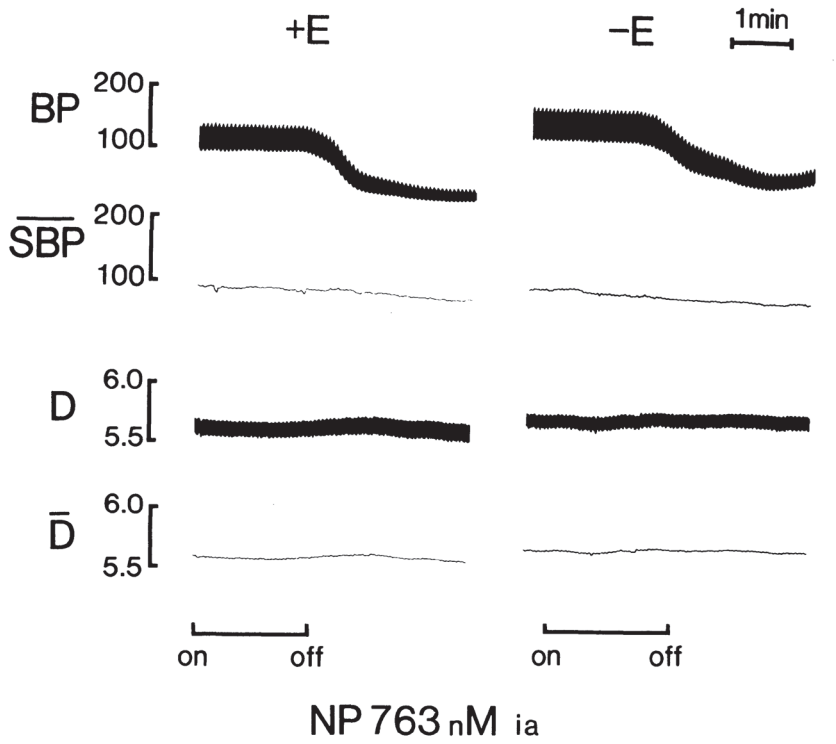

Fig. 3. Traces from the same experiment as for Fig. 2 to show the absence of effect of sodium nitroprusside (NP) on the diameter of a large artery. Note the dramatic depressor response in the systemic circulation after the drug left the controlled femoral artery circuit (Angus J. A., unpublished data). Symbols same as in Fig. 2.

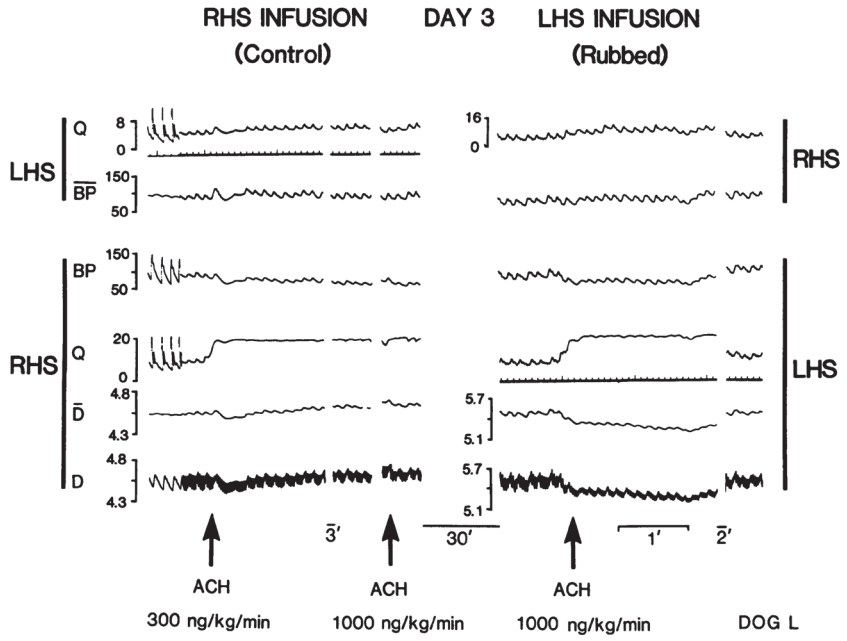

Fig. 4. Recording of intracarotid infusion of acetylcholine in a conscious greyhound 3 d after surgery at which the endothelium on the common carotid was left intact (control, right carotid, RHS) or removed by balloon (rubbed, left carotid, LHS). Measurements are from top, left panels: left carotid flow (Q, kHz Doppler shift, phasic and mean) mean left carotid blood pressure ($\overline{BP}$, mm Hg); right carotid blood pressure (BP), flow (Q) mean, and phasic carotid diameter (D, mm). Acetylcholine was infused into the right carotid (left panel) and 30 min later into the left carotid (right panel) (Angus J. A., unpublished data).

ranged in eccentric or concentric patterns, but generally covered by endothelium (Angus et al., 1986c). These arteries with thickened intima release EDRF in response to acetylcholine (*see* section 3.5).

2.2.2. Effect of Change in Blood Flow

In the coronary circulation, a change in blood flow *per se* is sufficient to cause an endothelium-dependent relaxation of large arteries (Holtz et al., 1984). This phenomenon was confirmed in the chronically instrumented carotid artery. At 4 wk, dogs were anesthetized, and a shunt was placed between the distal common carotid artery and the external jugular vein. Opening of the shunt increased the blood flow velocity (measured by Doppler flowmeter) in the carotid artery and always resulted in an increase in the diameter (Fig. 5). The time course and pattern of the diameter changes

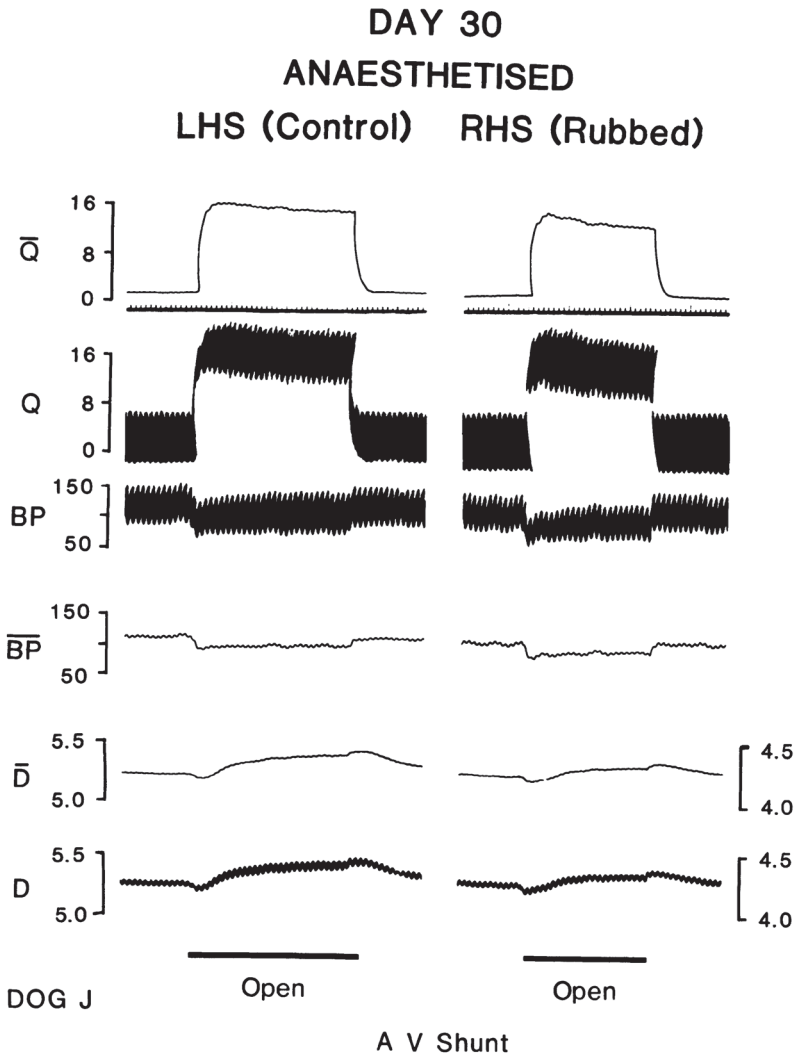

Fig. 5. Polygraph records of carotid artery blood flow (Q, kHz Doppler shift), carotid artery pressure (BP, $\overline{BP}$ mm Hg), and carotid artery diameter (D, $\overline{D}$, mm) in an anesthetized dog 4 wk after instrumentation and removal of endothelium from the right carotid artery (RHS, rubbed) or left undisturbed (LHS, control). Opening the distal carotid to jugular vein shunt for about 2 min (A-V shunt) caused a marked increase in blood flow velocity and increase in diameter, even in the presence of reduced pressure (Angus J. A., unpublished data).

did not follow the pressure trace, except when the flow was abruptly increased or decreased. The artery with previously damaged intima, responded in a similar pattern as the control artery, indicative of the presence of endothelial cells at this time.

These chronic experiments illustrate that the diameter of large arteries can be altered in vivo (1) by passive changes in transmural pressure, (2) by the release of EDRF evoked by agonists such as acetylcholine, or (3) by changes in velocity of blood flow if the endothelium is present.

3. Reactivity of Large Arteries In Vitro

3.1. EDRF Is Released by Norepinephrine and Serotonin

Loss of endothelial cells may have at least two important effects on the reactivity of large arteries. First, a denuded intimal surface would present an attractive site for platelet adhesion and release reaction leading to high local concentrations of autacoids such as serotonin. Second, if vasoconstrictor substances such as serotonin also stimulate the release of EDRF in normal arteries, then removal of endothelial cells would result in a greater direct constrictor action. This may occur in coronary artery spasm when focal loss of endothelial cells has occurred. With this in mind, the effect of the removal of endothelial cells was examined on cumulative concentration–response curves to four coronary constrictor substances in isolated rings of large coronary arteries (Cocks and Angus, 1983). In coronary arteries of the greyhound, mongrel dogs, and the pig, the maximal contractile response to serotonin was more than doubled in rings denuded of endothelium, and the EC_{50} concentration moved to the left compared to that obtained in arteries with intact endothelium (Fig. 6). This has been confirmed in canine coronary arteries in vivo and in vitro (Cohen et al., 1983; Lamping et al., 1985). Similarly, in the presence of propranolol, the concentration–response curves to norepinephrine were amplified and slightly shifted to the left by the removal of the endothelium (Fig. 6). This did not occur with two other coronary constrictor agents, K^+, or the thromboxane mimetic U46619, ruling out a nonspecific effect of intimal damage.

The amplified response for norepinephrine and serotonin are consistent with at least two hypotheses. First, the loss of endothelium could remove a barrier and a site of uptake or metabolism for these two amines across the artery wall allowing for higher concentrations of the amines at the level of the medial smooth muscle. Second, when the endothelium is intact, the amines stimulate the endothelial cells to release EDRF that coincidentally attenuates the direct constrictor response of the medial smooth muscle to the amine. The loss of the endothelium thus uncovers the full

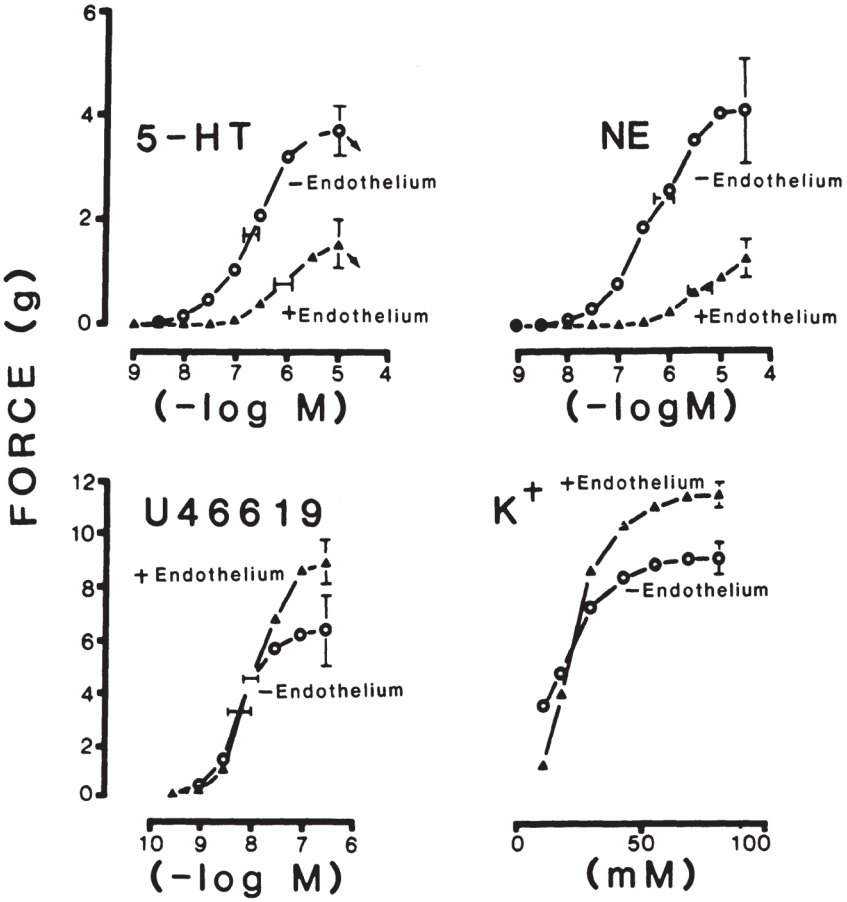

Fig. 6. Concentration–contraction curves to serotonin (5-HT), norepinephrine (NE) (in the presence of propranolol 1 μM), thromboxane mimetic (U46619), and K^+ in ring segments of pig coronary artery in the absence or presence of endothelium (data redrawn from Cocks and Angus, 1983).

direct constrictor response. More direct evidence for this latter hypothesis was obtained by contracting porcine arteries and observing a concentration-dependent relaxation to norepinephrine in the presence of high concentrations of propranolol (Cocks and Angus, 1983). In the coronary artery of the greyhound, the alpha$_1$-adrenoceptor blocker, prazosin was required to directly observe the relaxation to norepinephrine, whereas serotonin only relaxed coronary arteries of the dogs and pigs when the 5-HT$_2$-serotonergic blocker ketanserin was present. Thus a unifying hypothesis is that the endothelial cells on dog and pig large coronary arteries have receptors for norepinephrine and serotonin that cause the release of EDRF.

3.2. Classification of Endothelial Alpha$_2$-Adrenoceptors

In contracted coronary arteries of dogs and pigs the endothelium-dependent relaxation response to norepinephrine was mimicked by epinephrine in the presence of propranolol and prazosin. The alpha$_1$-adrenoceptor agonist methoxamine was without effect, and the preferential alpha$_2$-adrenoceptor agonist clonidine partially relaxed the rings when the endothelium was present. The nonselective alpha-adrenoceptor blocker phentolamine caused a parallel shift to the right of the relaxation–response curve to norepinephrine (Cocks and Angus, 1983). Direct evidence that the alpha-adrenoceptor on endothelial cells was of the alpha$_2$-subtype was that the selective alpha$_2$-adrenergic agonist UK 14,304 could relax contracted canine coronary rings (in the presence of propranolol and prazosin) provided the endothelium was present (Angus et al., 1986a). The concentration–relaxation curves to UK 14,304 were displaced in an apparently competitive fashion by idazoxan, the relatively selective alpha$_2$-adrenoceptor antagonist (Fig. 7).

3.3. Distribution of Alpha$_2$-Adrenoceptors on Endothelium

The distribution of alpha$_2$-adrenoceptors on endothelial cells was examined in five large arteries of the dog. To ensure that the most favorable conditions were present to observe relaxations mediated by alpha$_2$-adrenoceptors, all arteries were contracted with U46619 at a concentration that caused about 80% of its maximal effect. These conditions would avoid relaxation by EDRF being masked by an excessive contraction to U46619. Each ring segment of the coronary, carotid, renal, mesenteric, and femoral artery was stretched passively (before applying U46619) to a level of resting force, determined from the Laplace relationship to be equivalent to a transmural pressure of approximately 70 mm Hg (Angus et al., 1986a). The alpha$_2$-mediated maximal relaxation to norepinephrine in the presence of propranolol and prazosin was measured as a percentage of the maximal relaxation to the endothelium-independent vasodilator drug, nitroglycerin, and were in the order of 70% in the coronary; 34% in the carotid, 19% in the femoral; 7% in the renal; and 2% in the mesenteric arteries (Fig. 8). To determine whether the same rank order applied for another endothelium-dependent agonist, the maximal response to substance P was compared across the five blood vessels. Substance P showed a higher efficacy than norepinephrine, but a similar order in the degree of maximum relaxation (i.e., coronary, 100%; carotid, 80%; femoral, 71%; renal, 49%; and mesenteric, 41%). The same rank order for two endothelium-dependent vasodilator agents would suggest that the release of EDRF is site-dependent,

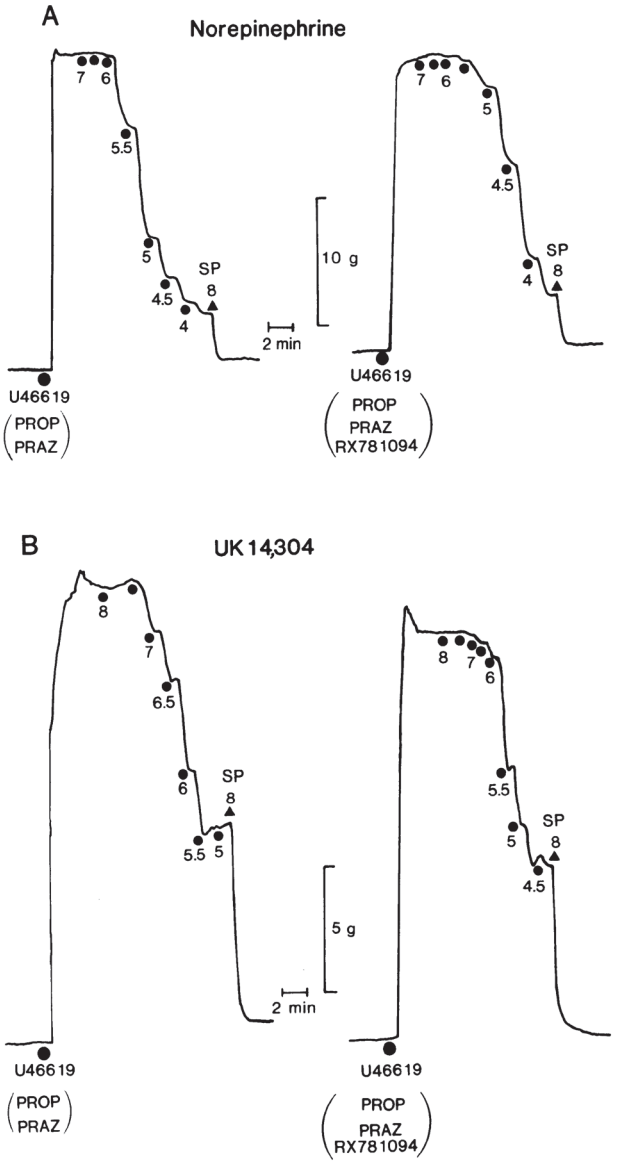

Fig. 7. Recordings of relaxations to norepinephrine (A) and UK14304. (B) in four coronary artery rings from the dog, contracted with U46619 (30 n*M*) in the presence of propranolol (prop, 3 μM) and prazosin (praz, 1 μM). Right traces were in the presence of idazoxan (RX781094, 1 μM). Substance P (SP) was applied where indicated (reproduced from Angus et al., 1986a, with permission of Macmillan Publishers Ltd.).

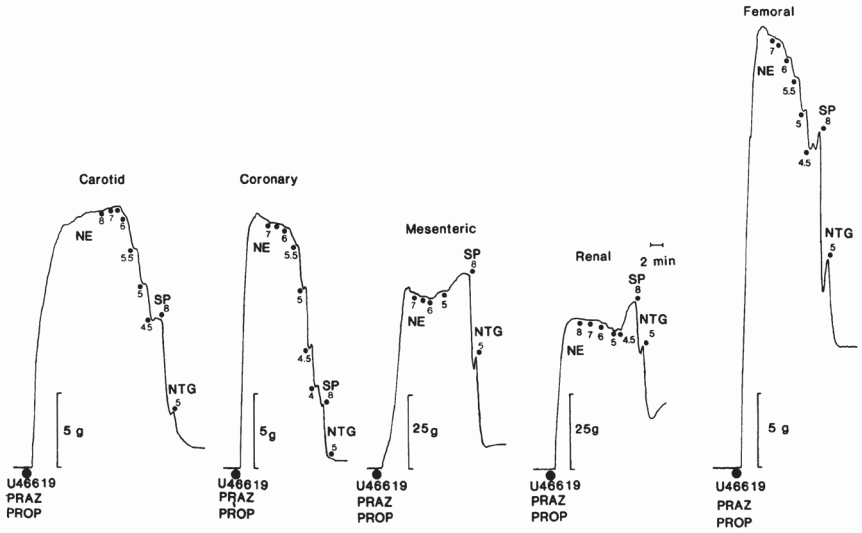

Fig. 8. Traces of relaxations to norepinephrine (NE) in five large arterial rings of the greyhound. Each ring was contracted with U46619 (EC_{80}) in the presence of prazosin (praz, 1 μM) and propranolol (prop, 1 μM). Substance P (SP) and nitroglycerin (NTG) were given where indicated. Numbers refer to $-\log M$ concentration (Angus J. A., Cocks T. M., and Satoh K., unpublished data).

or that the efficacy of EDRF on smooth muscle varies, being greatest in the coronary and weakest in the renal and mesenteric arteries in the dog.

This correlation does not appear to hold for other species. In the bovine coronary artery, no alpha$_2$-adrenoceptor mediated relaxation was observed, but this artery was readily relaxed by bradykinin, a response that was endothelium-dependent (Angus et al., 1986b). In the pig, the coronary and mesenteric arteries readily responded to norepinephrine in the presence of prazosin and propranolol (Table 1). In small branches (200–400 μm in diameter) of the coronary artery from the dog and pig contracted with K^+, norepinephrine could also relax the vessels in the presence of prazosin and propranolol (Angus et al., 1986b). This suggested that endothelial cells in the microvasculature (at least in that of the heart of these species) also possess alpha$_2$-adrenoceptors.

The resistance of some arteries with intact and functional endothelium to relax to norepinephrine in the presence of prazosin clearly could be explained by (1) the absence of alpha$_2$-adrenoceptors on endothelial cells, or (2) the poor efficacy of norepinephrine at alpha$_2$-adrenoceptors releasing EDRF, or (3) the presence of alpha$_2$-adrenoceptors on the medial smooth muscle cells that cause contraction and thus counter the relaxation to EDRF. Evidence that this latter event may be operative is that renal

Table 1
Comparison of Relaxation Responses to Norepinephrine in Large Arteries Contracted In Vitro by U46619 in the Presence of Propranolol and Prazosin[a]

Species	Artery				
	Coronary	Carotid	Mesenteric	Renal	Femoral
Pig	+++++[b]	++	+++++	+++	++
Cattle	0	—	—	—	—
Dog					
Mongrel	+++	++	+	+	++
Greyhound	++++	+++	+	+	++

[a]Relaxation scale: +++++, 70–100%; ++++, 40–70%; +++, 20–40%; ++, 10–20%; +, 0–10%; —, no experiment.
[b]No prazosin.

and femoral arteries of the pig contract further to norepinephrine in the absence of endothelium and the presence of prazosin (Angus et al., 1986b).

These data suggest that the integrated response of the artery wall to norepinephrine will depend upon at least four populations of adrenoceptors; alpha$_2$- on the endothelium; and alpha$_1$-, alpha$_2$- and beta- on the smooth muscle (Fig. 9). Responses to norepinephrine should be interpreted with caution when the integrity of the endothelium has not been considered.

3.4. Comparison of Endothelium-Dependent Agonists in Five Large Arteries

Full concentration–response curves were obtained for acetylcholine, substance P, bradykinin, ATP, substance P, and the endothelium-indeendent dilator nitroglycerin in rings of five large arteries contracted with U46619 (EC_{80}). The logistic-fitted EC_{50} values for relaxation to acetylholine ranged from $-7.09 + 0.18$ (log $M \pm 1$ SEM) in the carotid to -7.59 ± 0.18 in the mesenteric artery, a 3.2-fold difference (Fig. 10). Similarly, the EC_{50} values for substance P differed by less than twofold and that for ATP by fivefold over the five arteries; the carotid artery was the least sensitive. In contrast the sensitivity to bradykinin differed by 20-fold (from the EC_{50} of -8.64 ± 0.09 in the renal to -7.33 ± 0.16 in the carotid artery). The arterial rank order for the maximal relaxation as a percentage of the contraction to U46619 did not correlate with the sensitivity (EC_{50}). For example, substance P relaxed the coronary to a maximum of $88.6 \pm 2.9\%$, but the mesenteric artery, only to a maximum of $32.3 \pm 4.1\%$ (Table 2). In general the coronary and carotid arteries relaxed to a greater extent than the other blood vessels. This may reflect the favorable functional antagonism between EDRF (or nitroglycerin) and

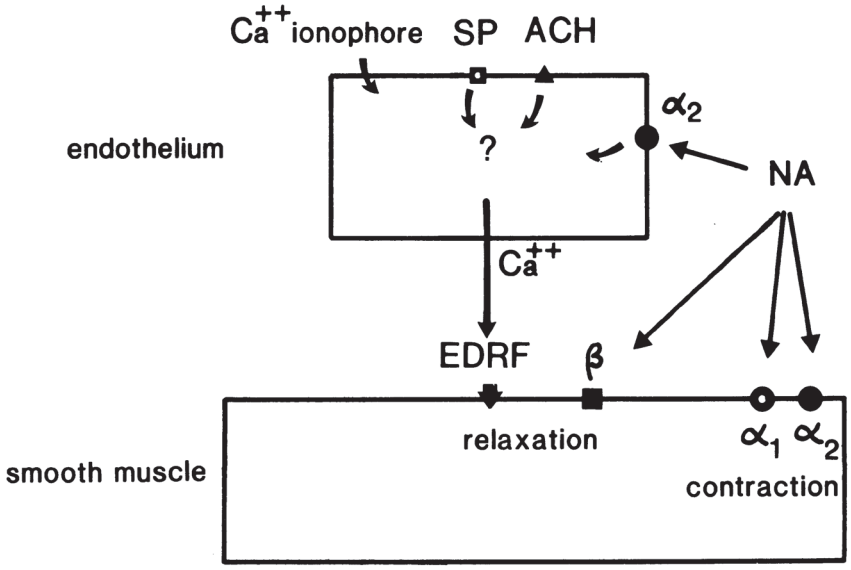

Fig. 9. Proposed receptors for norepinephrine (NA) and other vasoactive agonists on endothelial cells and smooth muscle cells. Abbreviations: α, alpha-adrenoceptor; β, beta-adrenoceptor; ACH, acetylcholine; SP, substance P; EDRF, endothelium-dependent relaxing factor.

the tone generated by U46619 in these particular arteries. Importantly, the sensitivity to substance P and acetylcholine was quite similar in the different blood vessels, indicating similar densities of receptors and/or similar efficacy of these agonists at the different locations on endothelial cells. This was not the case with bradykinin and, to a lesser extent, ATP.

The sensitivity to nitroglycerin varied by 15-fold from the most sensitive (coronary; $EC_{50} = -7.28$) to the least sensitive (mesenteric, -6.11) artery. This may be the consequence of a difference in activity of guanylate cyclase in the smooth muscle or to a difference in distribution of the nitroglycerin receptors. EDRF and nitroglycerin probably relax smooth muscle through the generation of cyclic GMP (Rapoport and Murad, 1983). Clearly if there was a variation in the production of cyclic GMP among arteries, the pattern of curves for the different blood vessels should have been similar for substance P and nitroglycerin.

3.5. Carotid Artery Reactivity 4 wk after Endothelial Denudation

Removal of endothelial cells experimentally by balloon rubbing of the intimal surface initiates a repair reaction from migrating medial smooth

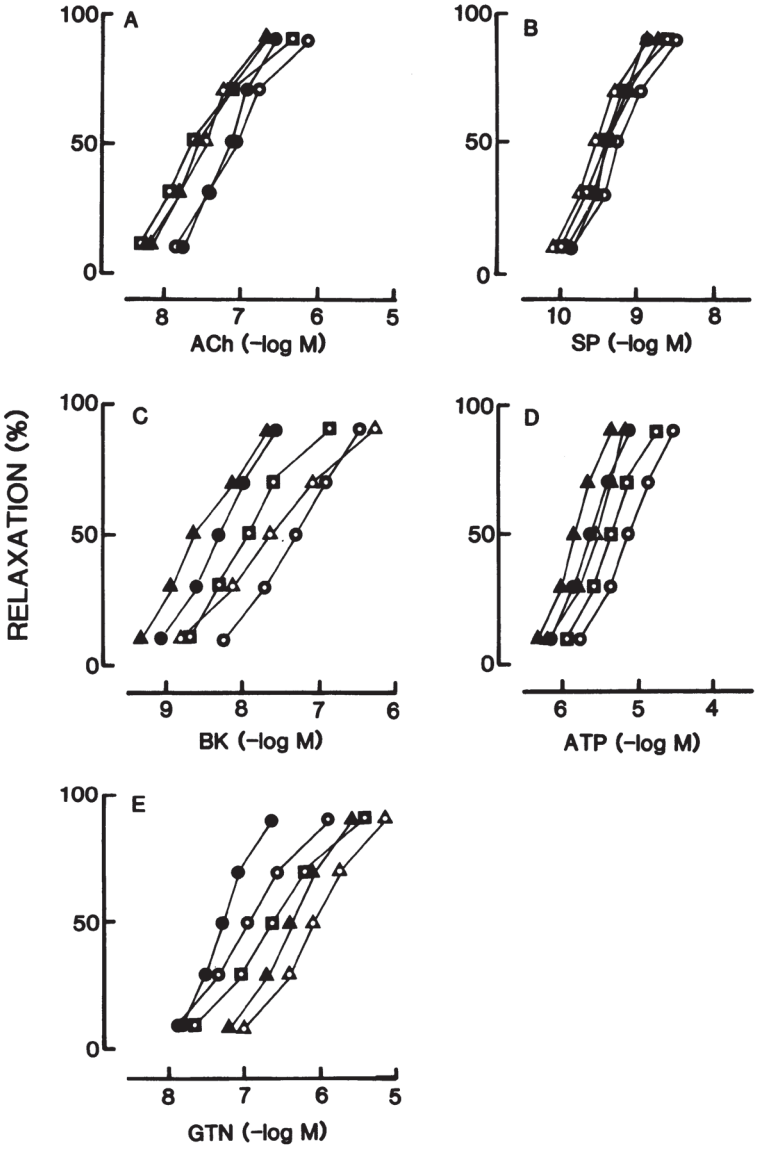

Fig. 10. Concentration–relaxation curves for acetylcholine (ACh), substance P (SP), bradykinin (BK), ATP, and nitroglycerin (GTN) in the coronary (●), carotid (○), femoral (□), renal (▲), and mesenteric arteries (△) of the dog. There were 6–8 arteries in each group (error bars at EC_{50} are within the symbols). Relaxations are expressed as % of maximal responses to each vasodilator agent (Satoh K. and Angus J., unpublished data).

Table 2
Comparison by Rank Order of Maximum Relaxations to Five Vasodilator Agents in Five Large Arteries of the Dog In Vitro[a]

Agent	Endothelium-dependent	Order of relaxation
Acetylcholine	Yes	Co > Ca = F > R = M
Subtance P	Yes	Co > Ca = F > R > M
Bradykinin	Yes	Co > R > F > M > Ca
ATP	Yes	Co > Ca > R = M = F
Nitroglycerin	No	Co > Ca > M = R > F

[a]Abbreviations: Co, coronary; Ca, carotid; F, femoral; R, renal; M, mesenteric (from Satoh K. and Angus J., unpublished data).

muscle cells. By 2 wk, a neointima consisting of smooth muscle cells with few myofilaments, but large numbers of synthetic organelles surrounded by extracellular matrix forms (Clowes et al., 1983). This thickened intima is often covered by endothelium. This increased intimal thickness could offer a significant barrier to EDRF and thus prevent relaxations to acetylcholine. To investigate this possibility, greyhound dogs were anesthetized and the endothelium from a 10-cm length of one common carotid artery was removed under sterile conditions by means of a balloon catheter; the other artery served as control. Four weeks later, rings of both carotid arteries were taken and suspended in organ baths. A length–tension curve was obtained in each ring to determine the precise resting force to apply to reach a similar passive stretch (equivalent to a transmural pressure of 70 mm Hg) in arteries of different geometry (Mulvany and Halpern, 1977; Angus et al., 1986a). Cumulative contraction curves (one per ring) to norepinephrine, serotonin, U46619, and K^+ were not different as regards maximal force between control or rings with previous intimal damage (Fig. 11), but were significantly more sensitive (lower EC_{50} values) by 3.4-, 2.1.-, and 1.4-fold for norepinephrine, serotonin, and K^+, respectively. The histological examination of each ring with the light microscope [thick sections (2–4 μm) stained with methylene blue] indicated that control rings had either one or no smooth muscle cells in the intima, but more than 90% covering with endothelial cells. Arterial rings previously subjected to intimal damage showed mostly eccentric intimal thickening of 2–20 smooth muscle cells, largely covered by endothelial cells. To test the integrity of these endothelial cells, ring segments were submaximally contracted with either serotonin or with U46619. There was no difference in the sensitivity (EC_{50}) or in the maximal relaxation to acetylcholine or nitroglycerin when comparing responses from control and damaged rings

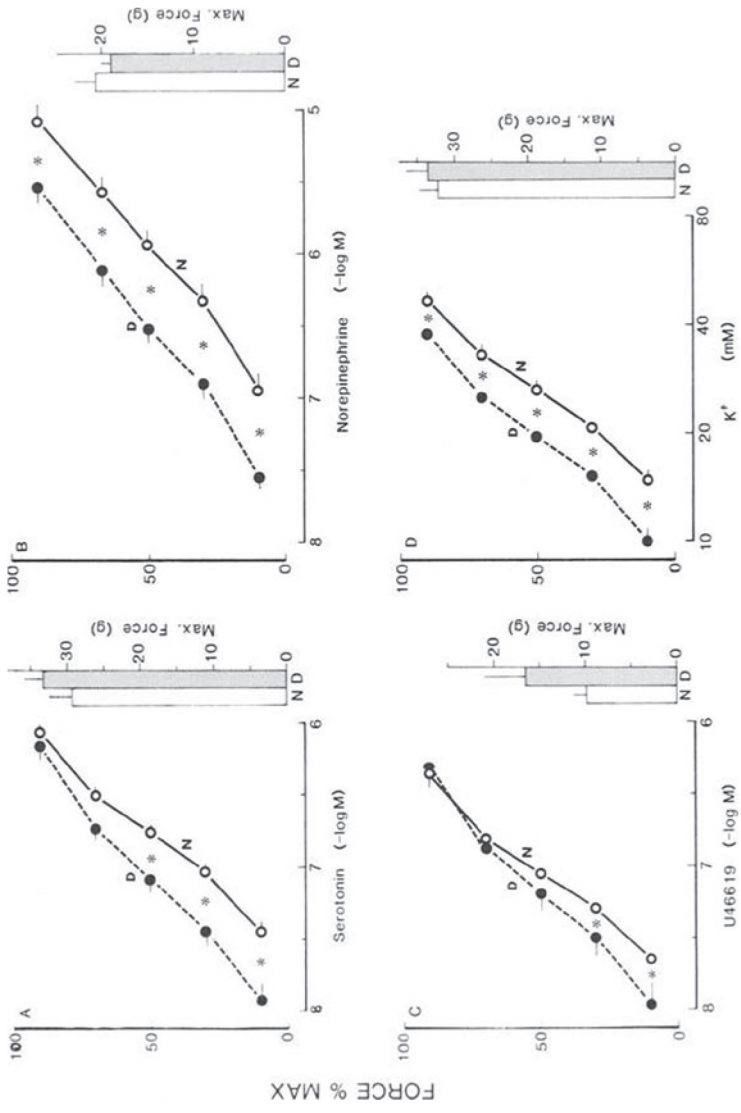

Fig. 11. Comparison of concentration–contraction curves in rings of carotid artery of the dog, 4 wk after removal of the endothelium (damaged, D, solid circles) or with endothelium left intact at surgery (normal, N, open circles). Lines are logistic fitted data to compare sensitivity with SEM bars. Asterisks indicate significant differences. Histograms are maximal responses developed by the rings in the two groups (Satoh K., Angus J., Cocks T., and Campbell G., unpublished data).

(Fig. 12). There was a small enhancement in the relaxation to adenosine in the rings contracted with U46619, but not in those contracted with serotonin.

These findings suggest that there is a small (less than fourfold) increase in sensitivity to norepinephrine and to serotonin in carotid arteries with neointima. In these studies the neointima was well covered with endothelial cells that responded normally to acetylcholine, suggesting that the neointima did not constitute an effective barrier to EDRF. The relaxation to the endothelium-independent vasodilators (nitroglycerin and adenosine) was essentially unchanged.

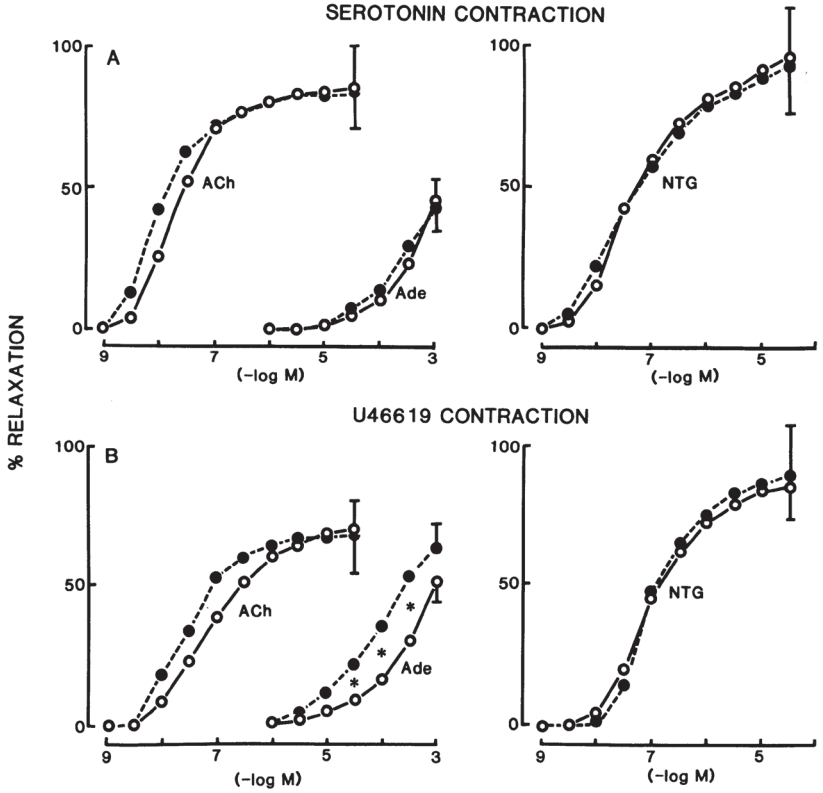

Fig. 12. Concentration–relaxation curves to acetylcholine (ACh), adenosine (Ade), and nitroglycerin (NTG) in normal (open circles) and damaged (closed circles) carotid artery rings (endothelium removed 4 wk previously). The relaxations are expressed as percentage of the resting contraction to serotonin (0.3 μM, top) or to U46619 (0.3 μM, bottom).

4. Reactivity of Microcirculation to EDRF

4.1. Effect of Hypertension on Reactivity of the Hindquarter of the Rabbit

A reduction in the production of a powerful local vasodilator substance is an attractive hypothesis to at least partly explain the development of hypertension. The identification of EDRF as a powerful dilator of large and small arteries prompted the evaluation of the reactivity of an intact vascular bed in conscious normotensive and hypertensive rabbits. First, the animals were anesthetized, and pulsed Doppler-flow transducers were implanted on the lower abdominal aorta just proximal to the iliac bifurcation. A catheter was placed in the aorta proximal to the flow probe (Herd and Barger, 1964) to allow for local intraarterial infusion of agonists. The kidneys were left undisturbed (sham) or wrapped in cellophane (wrap) to cause perinephritic hypertension over the next 5 wk. On the 35th day after surgery, the rabbits were treated with mecamylamine (10 mg/kg) and propranolol 0.5 mg/kg to prevent autonomic reflexes. Blood pressures (mm Hg measured in the ear artery) before and after the ganglion blockade were 79.4 and 67.4 for sham and 124.8 and 103.6 for kidney-wrapped rabbits, respectively. The changes in hindquarter vascular resistance (pressure in ear artery/blood flow in the hindquarter) obtained with intraarterial infusions of acetylcholine, adenosine, and serotonin were characterized by (1) the range from resting to maximal dilatation, (2) the sensitivity (ED_{50}), and (3) the average slope about ED_{50} (Wright et al., 1987). There were no significant differences in ED_{50} values for each of the three dilator drugs between normotensive and hypertensive animals. In the hypertensive rabbits, however, the range and slope of the curves for all agents were about twice that of the normotensive rabbits. These results suggest that hypertrophy of the muscle in the precapillary vessels make them a nonspecific amplifier of changes in vascular resistance induced by a variety of endothelium-dependent (acetylcholine) and endothelium-independent dilator drugs (such as adenosine). Since responses to acetylcholine mimicked those of adenosine in the two groups, an alteration in the response to EDRF in the resistance vessels seems unlikely in this experimental model of hypertension (5 wk of duration).

4.2. Effect of Cholesterol on EDRF

An increase in plasma cholesterol levels from dietary intake could affect production and/or release of EDRF by the endothelial cells. Experi-

ments were designed to test whether this could occur in the resistance vessels of the hindquarters of normotensive and hypertensive rabbits—areas of the vascular bed well distal from the classical atheromatous lesions seen in the aorta or other major arteries (*see* section 5).

Rabbits were fed 1% cholesterol chow or normal chow for 4 wk and had their kidneys wrapped in cellophane or left undisturbed. On the day of the experiment (5 wk postsurgery) autonomic reflexes were blocked pharmacologically and vascular resistance in the hindquarter was measured during intravenous infusions of increasing doses of acetylcholine, adenosine, or serotonin (Wright and Angus, 1986). The sensitivity (ED_{50}) to the three dilator agonists was altered less than twofold for any treatment group compared with the normotensive group on normal chow. The resting vascular resistance increased (over that of the sham-operated rabbits on normal chow, 100%) in sham-operated animals receiving cholesterol (121%), hypertensive rabbits on normal chow (223%), and hypertensive rabbits receiving cholesterol (229%). These values are consistent with the increase in viscosity of plasma during the cholesterol diet (plasma cholesterol approximated 1000 mg%) and with the presence of medial hypertrophy in the resistance vessels of hypertensive animals. The maximal vasodilator response to acetylcholine, but not that for adenosine or serotonin, was significantly reduced by hypercholesterolemia, hypertension, or a combination of both. This study suggested that the function of the intima of resistance vessels of the hindquarters of the rabbit was not altered greatly by raised plasma cholesterol or by hypercholesterolemia combined with hypertension as far as the sensitivity to the endothelium dependent agonist acetylcholine was concerned. In addition, the cholesterol diet and/or hypertension had no effect on reactive hyperemia induced by balloon inflation of the abdominal aorta for 5–80 s in the hindquarters (Wright and Angus, 1986).

5. EDRF and Coronary Atheroma

To study the effects of atheroma on responses to EDRF in coronary arteries, rings were prepared from arteries with concentric atheromatous lesions, near the origin of the left anterior descending coronary artery in rabbits fed a 1% cholesterol diet for 5 mo. A coronary resistance vessel that was obviously clear of atheroma was prepared from a distal side branch from the same heart. Both vessels were suspended on 40-μm diameter wires in the same myograph (Mulvany and Halpern, 1977). Potassium contracted the vessels to a steady level of force. Addition of acetylcholine caused the small vessel to relax, but caused the large atheromatous artery to contract (Fig. 13). At the highest concentrations, the small vessel began to

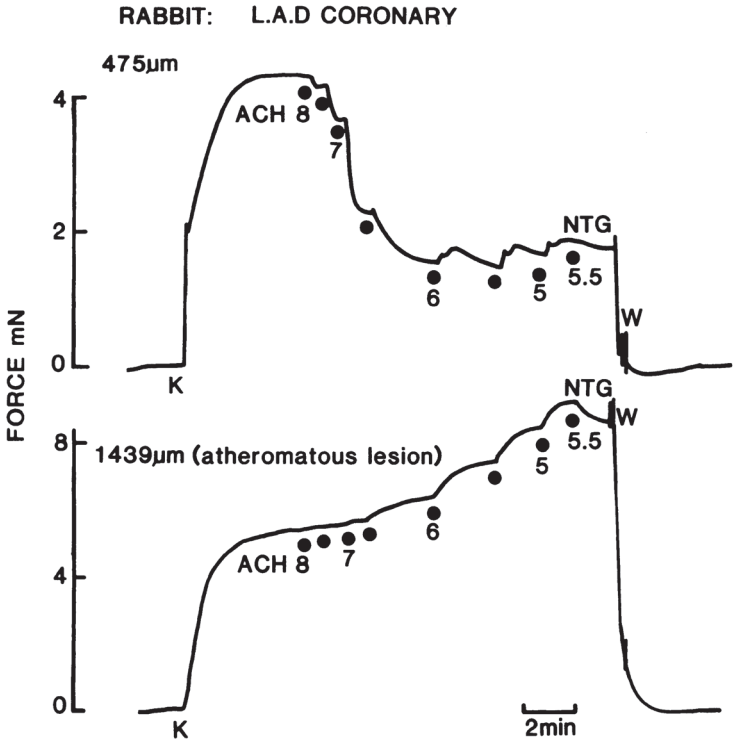

Fig. 13. Isometric force recording in a segment of microvessel (top) and a segment of large coronary artery with concentric atheromatous plaque (bottom) studied in the same myograph. Cumulative concentrations of acetylcholine (−log *M*) were applied during steady contraction obtained with K^+ 30 m*M* (histology of vessels, *see* Figs. 14 and 15).

contract weakly; only further contractions were observed in the large artery. These observations are consistent with a dual effect of acetylcholine, to cause the release of EDRF and to stimulate the smooth muscle cells to contract. If the EDRF cannot penetrate the neointima (as in the atheromatous vessel) the contraction component of the response would proceed unchecked and be amplified. Substance P relaxed the small vessel, but had no effect on the large artery (data not shown). The vessels were fixed for light microscopy, and both showed generally intact endothelium, except at the sites where the suspending wires had damaged the wall (Figs. 14 and 15). The concentric neointima that had formed in the large artery was filled with extracellular cholesterol and lipid-engorged smooth muscle cells and macrophage-derived foam cells (Fig. 15). In three other large coronary arteries taken from rabbits on a normal diet, only relaxation, similar to that shown here for the small artery, was observed.

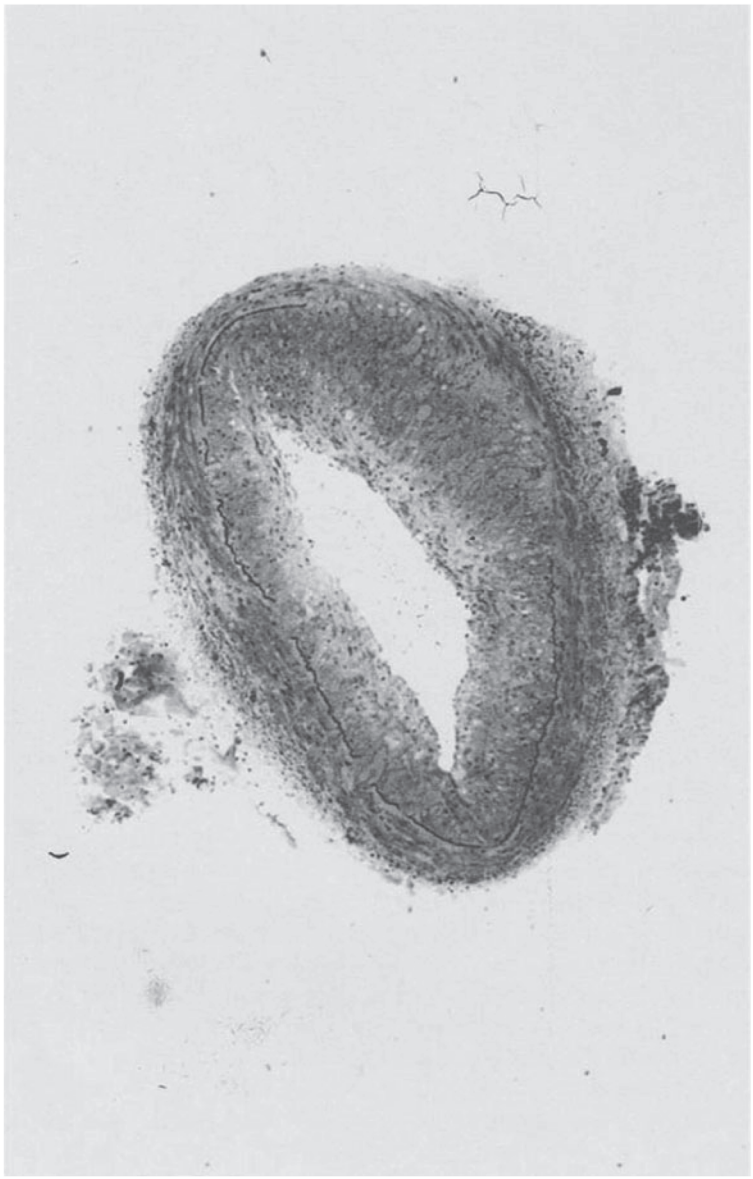

Fig. 14A. Transverse section through the left coronary artery of a rabbit fed at 1% cholesterol supplemented diet for 5 mo. Note the atheromatous plaque completely lining the lumen of the vessel (×63).

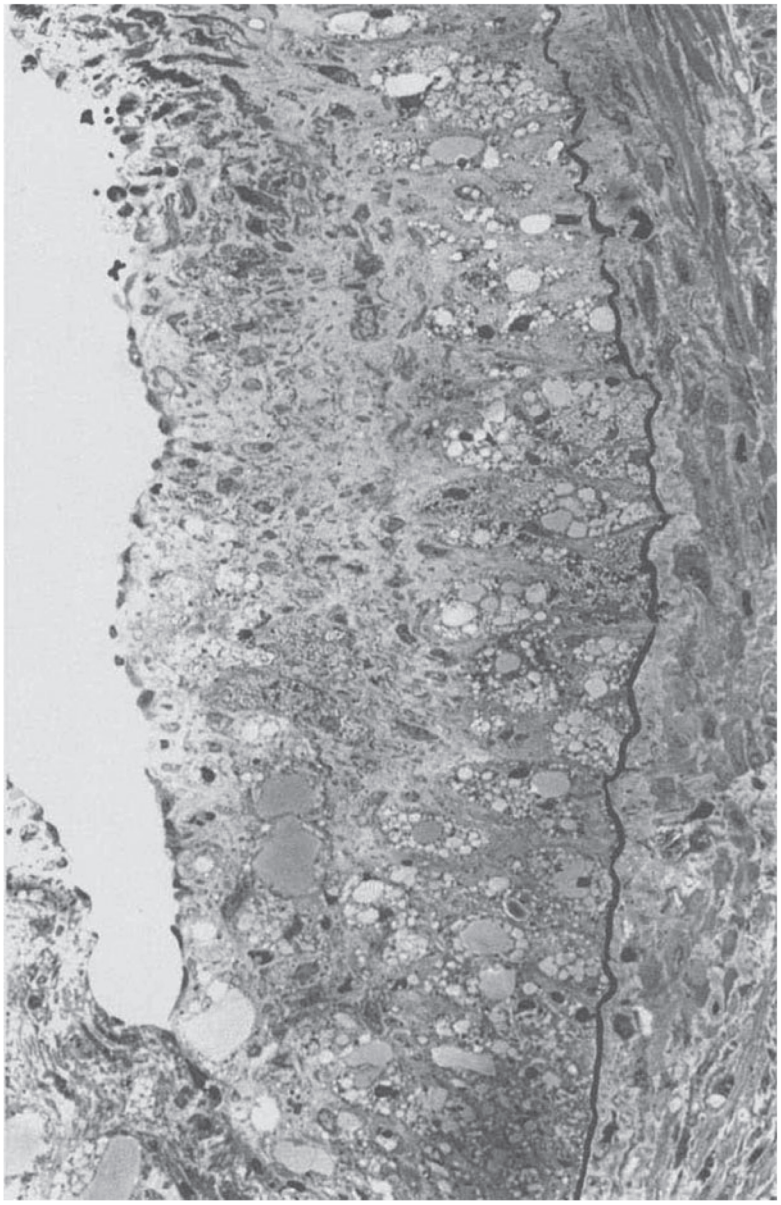

Fig. 14B. Enlargement of intima from Fig. 14A. Note that the vessel is lined by an endothelium and contains extracellular cholesterol and a large number of lipid-engorged cells (×350).

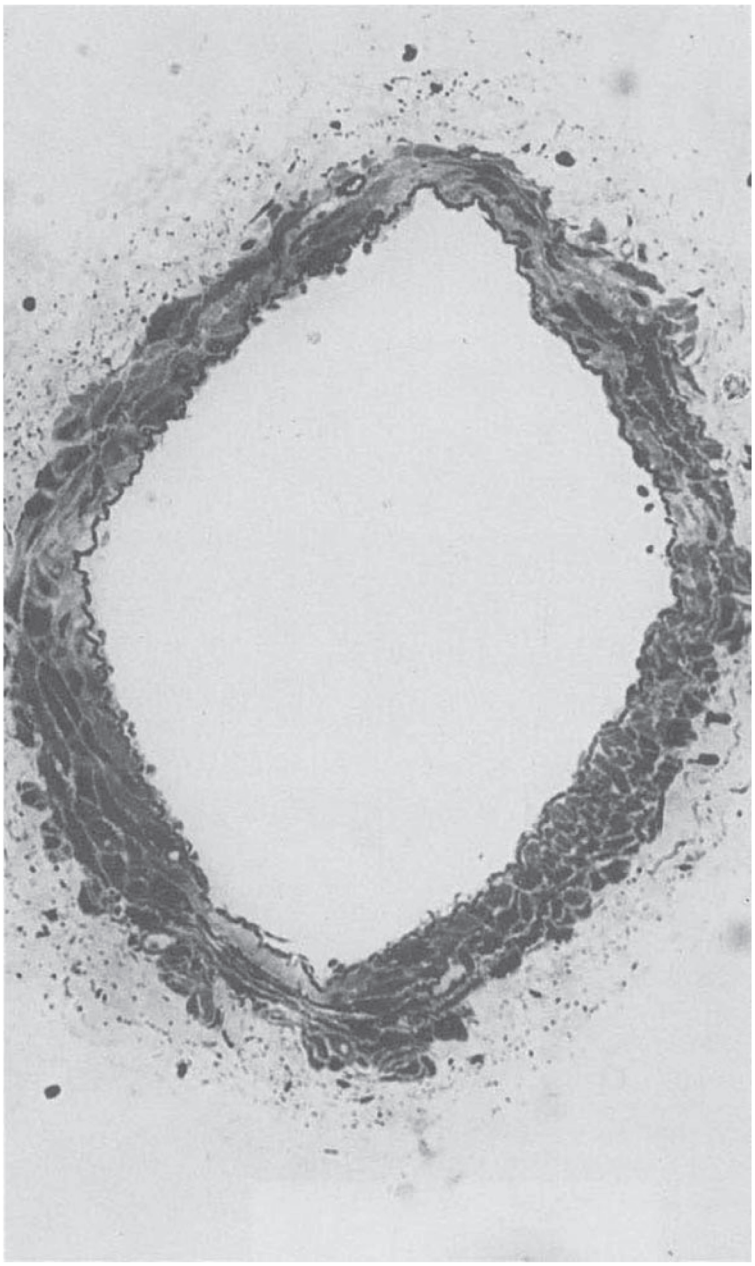

Fig. 15. Transverse section through a coronary microvessel from the same heart as in Fig. 14. Note the vessel is lined by an endothelium, and there is no evidence of atheromatous plaque formation or intimal thickening (×430).

We concluded that an increase in plasma cholesterol *per se* does not interfere with the release of and/or the response to EDRF, but that atheromatous lesions of large coronary arteries may be a sufficient barrier to trap or destroy EDRF released from the obviously intact endothelium. Similar conclusions have been reported for the aorta from rabbits fed 0.3% cholesterol for 16 wk (Coene et al., 1985).

6. Conclusions

EDRF is released from large arteries in vivo to relax the underlying artery wall. It does not appear to circulate in blood even for short distances. When blood flow and perfusion pressure are controlled, the diameter of the artery increases in response to EDRF released by acetylcholine, but not to nitroglycerin or sodium nitroprusside. In the carotid artery of chronically instrumented greyhounds, acetylcholine can increase diameter only if endothelium is present.

Coronary endothelial cells release EDRF in response to serotonin and norepinephrine. The response to serotonin is not caused by activation of 5-HT_2-serotonergic receptors; that to norepinephrine is mediated by alpha$_2$-adrenoceptors. Endothelial alpha$_2$-adrenoceptors are present in other large arteries of the dog and coronary resistance vessels of dog and pig. The distribution of bradykinin receptors on endothelial cells appears to vary widely across five large arteries of the dog, a phenomenon not shared with substance P or acetylcholine. Hypertension and hypercholesterolemia do not appear to alter the release of or the response to EDRF in the resistance vessels of the hindquarter of the conscious rabbit. Neo-intimal thickening in large arteries *per se* does not appear to constitute an important barrier to EDRF. When neo-intimal thickening involves foam cell formation and the deposition of extracellular cholesterol, however, the response to EDRF is absent, suggesting that the lipid involvement has trapped the factor preventing it from reaching the medial smooth muscle.

Taken together, these studies suggest that the environment of an artery in which there is neo-intimal thickening, with lipid involvement, would reflect (1) some enhanced sensitivity of the smooth muscle to constrictor stimuli; (2) prevention of EDRF from reaching the media; and (3) encroachment of the atheroma in the lumen, which perhaps results in a hyperresponse, especially if the perfusion pressure is low. Is this the scenario for large artery vasospasm?

Acknowledgments

This work was supported by an Institute Grant from the National Health and Medical Research Council of Australia. We thank Matthew Le Duc, Peter Coles, Eileen Wright, David Prior, and Juliana Wong for excellent technical assistance.

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