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THE ROLE OF ENDOTHELIUM IN THE RESPONSES OF VASCULAR SMOOTH MUSCLE TO DRUGS

Robert F. Furchgott

Department of Pharmacology, Downtown Medical Center, State University of New York, Brooklyn, New York 11203

INTRODUCTION

Several years ago we accidentally discovered that relaxation of isolated preparations of arteries (rings, transverse strips, or helical strips) by acetylcholine (ACh) was strictly dependent on the presence of endothelial cells on the intimal surface of the preparations (1–3). This discovery helped resolve the paradox that ACh, a potent vasodilator of arteries in vivo, often produced no relaxation or even contraction of isolated preparations of arteries in vitro (4, 5). Apparently those isolated preparations that had failed to relax had had their endothelial cells unintentionally rubbed off during the course of their preparation for experiments. This discovery also led to the finding that a number of other agents, including some but not all of the most potent known endogenous vasodilators, also require endothelial cells to produce relaxation in isolated arteries. A number of laboratories are now engaged in research on various aspects of the endothelium-dependent relaxation of blood vessels by various agents. This review attempts to bring together the more important findings in this new area of research. A fuller description of early experimental results in this area obtained by the author and others is available in another recent review (6). The reader is also referred to that review for a discussion of a postulated role for endothelial cells in mediating or facilitating contractions of some blood vessels under special conditions.

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RELAXATION OF ARTERIES BY ACETYLCHOLINE

The Requirement for Endothelial Cells and the Lack of Involvement of Prostaglandins

That endothelial cells are required for relaxation by ACh was first suggested when it was found that precontracted rings or strips prepared from the descending thoracic aorta of the rabbit (henceforth referred to as rabbit aorta) would not relax in response to ACh if their intimal surfaces had been rubbed during the making of the preparation—either unintentionally, as must have been the usual case in earlier work with helically cut strips, or intentionally (1–3, 5). When care was taken not to rub the intimal surface of the aorta on foreign surfaces or on itself during the entire procedure of preparing and mounting, the rings and strips uniformly gave excellent relaxation in response to ACh. Typical records of ACh-induced relaxation of aortic preparations precontracted to moderate levels of tone by norepinephrine are shown in Figure 1. Figure 1 also illustrates the complete loss of the relaxation response in preparations after their intimal surfaces have been rubbed. That the loss of capacity to relax in response to ACh was the result of removing endothelial cells during the rubbing was clearly demonstrated using both scanning electronmicroscopy and en face microscopy after silver staining (2, 3).

The en face examination of preparations immediately after pharmacological experiments showed that those preparations giving excellent relaxation (as in

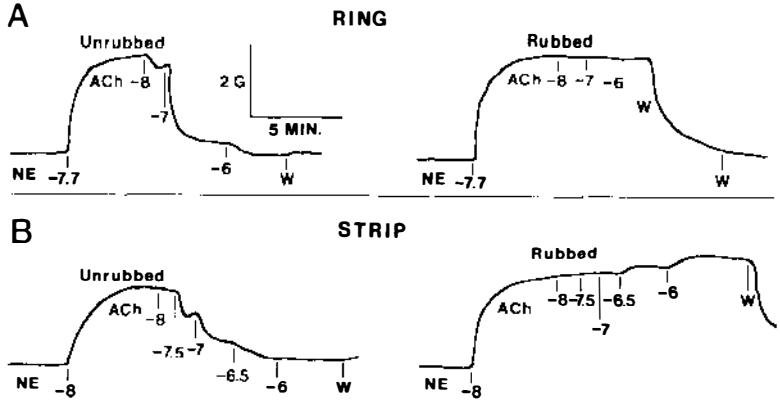

Figure 1 Relaxation by acetylcholine (ACh) of a ring (A) and a transverse strip (B) of rabbit thoracic aorta, and lack of relaxation by ACh after the intimal surfaces had been rubbed to remove endothelial cells. Concentrations of drugs added to the Krebs solution in the muscle chambers are expressed as logarithms of cumulative molar concentrations. NE is norepinephrine, and W indicates washout of the muscle chamber. Rings were mounted on L-shaped hooks, and transverse strips were mounted with specially designed clips. [From (3), used with the permission of Raven Press.]

Figure 1) usually had about 60 to 80% of their endothelial cells still present, that preparations with only a few percent of these cells remaining still gave moderate relaxation, but that preparations completely free of these cells gave no relaxation at all. It was also shown with rabbit aorta that complete removal of endothelial cells enzymatically by collagenase pretreatment also resulted in complete loss of the relaxation response to ACh (2). Although initial studies were on the rabbit aorta, we and others soon found that endothelial cells were required for relaxation by ACh in isolated arteries from all mammalian species tested, including rabbit (2), dog (2, 3, 7, 8), cat (3, 9), guinea pig (2), rat (2, 10), pig (11), cow (12), and man (13).

Although a number of other agents in addition to ACh were subsequently found to require the presence of endothelial cells for eliciting relaxation of isolated arteries (see later sections), the removal of endothelial cells did not interfere with relaxations produced by isoproterenol, sodium nitrite, glyceryl trinitrate, sodium azide (2), sodium nitroprusside (14), papaverine (8, 9), and prostaglandins such as PGE_2 and PGI_2 (8, 15). Removal of endothelial cells also did not interfere with relaxation by adenosine and adenylic acid in rabbit aorta (16) and dog femoral artery (7) but did reduce the degree of relaxation produced by those agents in pig aorta (11). Photorelaxation of rabbit aorta (17) had no endothelial-cell requirement (2), nor did relaxation of dog femoral artery by the addition of KCl after a period of exposure to K^+-free solution (7).

Very early in the work with ACh on rabbit aorta, we ruled out the possibility that an endothelium-derived prostaglandin serves as a mediator of relaxation. Not only did PGI_2, PGE_2, and a variety of other prostaglandins fail to produce relaxation of this artery, but the cyclooxygenase inhibitors indomethacin and aspirin did not interfere with ACh-induced relaxation (2). Also, in other rabbit arteries and in arteries from other species, inhibition of cyclooxygenase did not interfere with endothelium-dependent relaxation by ACh (3, 10, 11, 13, 15, 18).

Characteristics of Relaxation by Acetylcholine

The receptor on endothelial cells whose activation by ACh leads to relaxation of arterial smooth muscle is a muscarinic receptor. In arteries from all species tested, ACh-induced relaxation is very sensitive to blockade by atropine (2, 3, 7, 8, 9, 10). In the rabbit aorta, the estimated equilibrium constant (K_B) of atropine acting as a competitive antagonist was 0.35 nM, and the relative potencies of three muscarinic agonists acting in the presence of physostigmine were ACh > methacholine > carbamylcholine (3). Contractions elicited by higher concentrations of muscarinic agonists acting on rabbit aorta and some other arterial preparations are also mediated by muscarinic receptors, but these receptors are located on the smooth muscle cells (2, 5). In rabbit aorta, the degree of contraction with ACh (best studied after removal of endothelium)

varies markedly among different preparations. It is not yet established whether the greater sensitivity of the ACh-induced relaxation in comparison to the ACh-induced contraction indicates different subtypes of the muscarinic receptor in the endothelial cells and in the smooth muscle cells respectively.

It is worth noting at this point that muscarinic receptors located on prejunctional adrenergic nerve terminals have also been implicated in the vasodilating effect of ACh. If isolated vessels or perfused vascular beds are vasoconstricted by stimulation of their adrenergic nerves, then ACh acting on the prejunctional receptors can inhibit the stimulation-evoked release of norepinephrine from the nerve terminals and thus inhibit the stimulation-evoked vasoconstriction. [For reviews, see (19, 20).] In the isolated, perfused central ear artery of the rabbit [which generally has its endothelial lining sloughed off during the initial stages of perfusion (O. Steinsland, personal communication)], the prejunctional muscarinic receptor on the adrenergic nerve terminals has pharmacological characteristics similar to the receptor on the endothelial cells (e.g. similar potency ratios for ACh, methacholine, and carbachol, and sensitivity to blockade by atropine (21)). It should be emphasized, however, that the prejunctional muscarinic mechanism for vasodilation is limited to situations where vasoconstriction is the result of adrenergic nerve stimulation, whereas the endothelium-dependent muscarinic mechanism is effective whether or not the vasoconstriction is produced by nerve stimulation.

On rings of rabbit aorta precontracted to moderate tone with norepinephrine, half-maximal relaxation by ACh usually occurs between 0.01 and 0.1 μM (2, 3) and maximal relaxation at 1 μM. The degree of relaxation in percent of the initial contractile tone tends to decrease as the level of initial tone is increased. Rings of rabbit ear artery, celiac artery, renal artery, and superior mesenteric artery are somewhat more sensitive to the relaxing action of ACh than are those of aorta (J. Zawadzki & R. Furchgott, unpublished observations). Preparations of various dog arteries (3, 4, 7, 8, 22, 23), cat arteries (3, 9), rat aorta (10), and pig aorta (11) also exhibit high sensitivity to the relaxing action of ACh. On the other hand, the few human arteries that we have so far tested (branches of mesenteric arteries and an ovarian artery) have shown considerably less sensitivity to the action of ACh than have arteries from experimental animals (R. Furchgott, unpublished observations).

Equal, moderate contractions of rabbit aorta produced by norepinephrine, histamine, serotonin, angiotensin II, or $PGF_{2\alpha}$ have essentially the same sensitivity to the relaxing action of ACh (3). On the other hand, contractions produced by increasing K^+ in the Krebs solution are less sensitive to relaxation by ACh than are equivalent contractions produced by norepinephrine in the case of both rabbit aorta (2, 5) and canine arteries (6, 8, 23, 24). When rabbit aorta is contracted by replacing all Na^+ in the Krebs solution by K^+ and all $C1^-$ by SO^{2}_{4}, maximal relaxation by ACh is about 20% of that obtained in the

presence of an equivalent norepinephrine-induced contraction in regular Krebs solution (2, 3, 6). The fact that there is still significant relaxation in the completely depolarizing solution indicates that hyperpolarization of the smooth muscle cells is not required for the endothelium-dependent relaxation by ACh.

Release of an Endothelium-Derived Relaxing Factor (EDRF)

One hypothesis to explain the obligatory role of endothelial cells in the relaxation of arteries by ACh is that activation of the muscarinic receptor in these cells stimulates them to release a factor (or factors) that, on diffusing to the subjacent smooth muscle cells, activates a mechanism for relaxation in the latter cells. First tests of this hypothesis involved superfusion experiments in which an endothelium-free transverse strip of rabbit aorta was superfused with fluid that was first passed over the endothelium-containing intimal surface of a segment of aorta. Occasionally such experiments clearly showed that ACh caused release of an endothelium-derived relaxing factor (EDRF) but results were not consistent (P. Cherry and R. Furchgott, unpublished observations). A second procedure was therefore developed that consistently gave direct evidence for an EDRF (2, 3).

In this procedure, a transverse strip of rabbit aorta freed of endothelial cells (the recipient strip) was tested when mounted separately and also when mounted, intimal surface against intimal surface, with a longitudinal strip with endothelial cells present (the donor strip) in a so-called sandwich mount. The right-angle orientation of the muscle cells of the donor strip prevented them from exerting significant tension changes on the strain gauge during contraction. ACh, which failed to relax contraction of the recipient strip when it was mounted separately, always gave good relaxation of contraction of the same strip when it was mounted as a sandwich with the donor strip. Thus, release of an EDRF by ACh from the endothelial cells of rabbit aorta was demonstrated.

The sandwich procedure has also been used successfully to demonstrate release of an EDRF by the ionophore A23187 in rabbit aorta and by ACh in the dog renal artery (R. Furchgott et al, unpublished observations). Modifications of the procedure have also been used to demonstrate release of an EDRF by ACh and by histamine in rat aorta (10), by ACh in dog femoral artery (J. De Mey, personal communication), and by ACh and bradykinin in dog intrapulmonary arteries (6). In the modification used with the intrapulmonary arteries, the donor strip with endothelium, like the recipient strip without endothelium, was cut transversely, but it was pretreated with the "irreversible" α-adrenergic receptor blocking agent dibenamine prior to being placed in the sandwich mount with the recipient strip. Because of the pretreatment, only the recipient strip was able to contract when norepinephrine was added to produce tone prior to testing ACh or bradykinin for relaxing effects (6).

OTHER AGENTS THAT PRODUCE ENDOTHELIUM-DEPENDENT RELAXATION OF ARTERIES

A number of agents in addition to ACh have now been found to require endothelial cells for producing all or part of their relaxing effects on isolated arteries. It is noteworthy that in most cases to be discussed here, just as in the case of muscarinic agonists, prostaglandins do not appear to have any significant role as mediators of these relaxing effects. It should also be noted that with some of these agents the non-prostaglandin, endothelium-dependent relaxation of isolated arteries is not found in the case of all species tested but may be limited to specific species; in some cases, it may even be limited to specific arteries in specific species.

The Calcium Ionophore A23187

Relaxation of isolated arteries by the ionophore A23187 has been shown to be completely endothelium-dependent in the case of all mammalian species so far tested, including rabbit (4, 25, 26), dog (13), rat (14), pig (11), and man (13). In rabbit aorta, A23187 is 10–30 times more potent than ACh as a relaxing agent (6, 13). It is also more powerful than ACh, so that against high levels of contraction the maximal relaxation by A23187 (0.1 μM) is always greater than that by ACh (1–3 μM). Relaxation by A23187, like that by ACh, is not inhibited by cyclooxygenase inhibitors (13, 25, 26). When A23187 is added to a ring or strip of rabbit aorta at a concentration giving maximal relaxation, and tone is then restored by increasing the concentration of norepinephrine present, additions of ACh now fail to produce any relaxation (6, 13). We have proposed that this interference by A23187 with relaxation by ACh is the result of A23187 activating the production and release of EDRF so fully that any additional activation by ACh is precluded. When a high dose of A23187 (1 μM) is added to a preparation of rabbit aorta for several minutes and then is washed out, its relaxing action persists for long periods after the washout (6, 18). Ionomycin, another calcium ionophore, has about the same potency as A23187 in producing endothelium-dependent relaxation of rabbit aorta (P. Cherry, unpublished observations). On the other hand, monensin, a monovalent cation ionophore selective for Na^+, produces no relaxation (W. Martin, unpublished observations).

ATP and ADP

In isolated preparations of rabbit aorta (4, 13, 16), dog femoral artery (7), and pig aorta (11), the graded, concentration-dependent relaxations by ATP or ADP (1–100 μM) are markedly reduced after removal of endothelial cells. Thus, in all of these species, ATP and ADP exert most of their relaxant effects through an action on endothelial cells. Cyclooxygenase inhibitors such as

indomethacin do not inhibit these effects. In the case of rabbit aorta and dog femoral artery, concentration-dependent relaxations by AMP and adenosine (10–3000 μM) are not affected by removal of endothelial cells (7, 13, 16). It is probable that in these arteries the residual relaxation produced by ATP and ADP after removal of endothelial cells is due to the formation of their metabolic products, AMP and/or adenosine, which then act directly on the vascular smooth muscle (6). In pig aorta, a significant part of the relaxation by AMP and adenosine is reported to be endothelium-dependent (11).

Substance P and Some Related Peptides

Relaxation of isolated arteries from rabbits, dogs, and cats by substance P is strictly dependent on the presence of endothelial cells (13, 27). This peptide is the most potent endothelium-dependent relaxing agent studied in our laboratory, with threshold concentrations ranging from about 30 pM in rabbit aorta to 1 pM in dog celiac and superior mesenteric arteries. Desensitization to substance P occurs in all arteries on which it has been tested. Depending on the artery used, desensitization to a maximally effective dose (about 100–1000× threshold) is usually complete within 3–10 minutes. After full development of desensitization to substance P, there is no loss of sensitivity to the relaxing actions of ACh, A23187, and bradykinin. Thus, desensitization appears to be at the level of the receptor for substance P. Full sensitivity to substance P returns readily after washout of a desensitizing dose. Cyclooxygenase inhibitors do not interfere with relaxation by substance P. Recently, we have found that kassanin, physalaemin, and eledoisin also require endothelial cells to produce relaxations of isolated rabbit and dog arteries (28; R. Furchgott, unpublished observations) and that desensitization also develops to these agents. These peptides, which have some structural similarities to substance P near the C-terminal, fail to produce relaxation in the presence of a desensitizing dose of substance P and vice versa. This cross-desensitization strongly suggests that they cause relaxation by acting on the same set of receptors as substance P. Physalaemin is about the same potency as substance P, whereas kassanin and eledoisin are about 1/10th–1/30th as potent. Another peptide, octacholecystokinin, also requires endothelial cells to produce relaxation of rabbit and dog arteries. It is about 1/30000th as potent as substance P (28), and even though its structure has little in common with that of substance P, relaxation to it is lost in preparations desensitized to substance P.

Bradykinin

Bradykinin is a vasodilator that relaxes isolated arteries by one of two different indirect mechanisms depending on the species in question. We found that in rings of superior mesenteric and celiac arteries from rabbit and cat, mechanical removal of endothelial cells did not usually lead to a loss of sensitivity to the relaxing effects of bradykinin (15, 29). However, in these preparations relaxa-

tion by bradykinin was completely blocked by cyclooxygenase inhibitors such as indomethacin and flurbiprofen. Thus, in these arteries from rabbit and cat, relaxation by bradykinin appears to be mediated by prostaglandins whose synthesis is stimulated by the peptide, probably in both endothelial and other types of cells. This finding with rabbit arteries was not unexpected in view of the previous results of others indicating that relaxation of certain vascular beds and isolated arteries of the rabbit by bradykinin is mediated by released prostaglandins (30–32). It should be noted that neither bradykinin nor prostaglandins, including PGI_2, relax isolated preparations of rabit aorta and renal artery. On the other hand, PGI_2 is a potent relaxant of rabbit superior mesenteric and celiac arteries and is the prostaglandin that mediates bradykinin-induced relaxation of these arteries (J. W. Aiken, personal communication).

In contrast to arteries from the cat and rabbit, all arteries that we have tested from the dog (splenic, gastric, celiac, femoral, renal, coronary, intrapulmonary, and superior mesenteric) have shown a strict requirement for endothelial cells in the relaxation by bradykinin, and, in addition, their relaxing response to bradykinin has not been interfered with by cyclooxygenase inhibition by either indomethacin or flurbiprofen (15, 29). In dog arteries, bradykinin was usually about ten times more potent than ACh, with a threshold concentration ranging from 0.1–1.0 nM. Similar results with dog intrapulmonary and renal arteries have been reported by others (8, 22). Our results on dog coronary arteries differ somewhat from those of Toda (33), who found that indomethacin reduced to a small extent the relaxation of these arteries by bradykinin. We found that treatment of canine arteries with inhibitors of cyclooxygenase almost always gave considerable potentiation of the contracting activity of the agents used to produce initial tone (usually NE or $PGF_{2\alpha}$). This potentiation presumably occurred because prior to treatment with the inhibitor the isolated arteries were synthesizing prostaglandins with relaxing activity (e.g. PGI_2 and PGE_2) that opposes the contracting activity of added NE or $PGF_{2\alpha}$ (15). Our studies do not exclude the possibility that bradykinin stimulates the release of some prostaglandins (as well as EDRF) in canine arteries, but they do suggest that prostaglandins make little or no contribution to the relaxing effect of bradykinin. In a few experiments on rings of isolated human arteries (branches of mesenteric arteries and ovarian artery), relaxation by bradykinin was found to be dependent on the presence of endothelial cells and was not reduced by inhibition of cyclooxygenase (13, 15). Recently, Gordon & Martin (11) have reported that relaxation of the pig aorta by bradykinin is also endothelium dependent and prostaglandin independent.

Histamine on Rat Aorta

Recently, Van de Voorde & Leusen (10) reported that rings of rat thoracic aorta precontracted with NE exhibit dose-dependent relaxation to histamine (10–

1000 μM). According to them, this relaxation, like that produced by ACh (0.01–10 μM) in rat aorta, is dependent on the presence of endothelial cells and is not blocked by indomethacin. The relaxation by histamine was inhibited by mepyramine but not by cimetidine, indicating that histamine acts on an H_1 receptor of the endothelial cells. Rapoport & Murad (14) have also reported similar results with histamine on rat aorta. Endothelium-dependent relaxation by histamine has not yet been clearly demonstrated in any artery other than the rat aorta.

Serotonin on Canine Coronary Artery

Cohen et al (34) recently made the interesting finding that aggregating human platelets produce relaxation of rings of canine coronary arteries precontracted with $PGF_{2\alpha}$ if endothelial cells are present but produce only contraction if these cells have been previously removed. They propose that part of the relaxation response is the result of serotonin, released from the platelets on aggregation, acting on endothelial cells to produce a signal for relaxation of the coronary smooth muscle cells. Consistent with this proposal is their further finding that serotonin itself causes relaxation of precontracted rings of canine coronary arteries only if endothelial cells are present (35). In these arteries, as in many others, the direct effect of serotonin on the smooth muscle is contraction. The endothelium-dependent relaxing effect of serotonin was not inhibited by indomethacin, was not significantly inhibited by the serotonin antagonist ketanserin, but was largely inhibited by methysergide. Endothelium-dependent relaxation by serotonin has not yet been reported for any arteries other than canine coronary arteries.

Thrombin

De Mey and co-workers (18, 23) demonstrated that bovine thrombin (0.1–10 μ/ml) elicits dose-dependent relaxation of precontracted rings of dog arteries (femoral, splenic, pulmonary, spahenous). Relaxation, like that by ACh, was lost when the rings had been denuded of endothelial cells. Relaxation was not inhibited by inhibitors of cyclooxygenase and prostacyclin synthetase but was inhibited by heparin. Similar findings have been made on thrombin-induced endothelium-dependent relaxation of isolated canine coronary arteries (36). In these arteries, the relaxation response to each addition of thrombin was transient, giving way in time to a contractile response.

Arachidonic Acid

On the basis of our early findings on some conditions and agents that inhibit endothelium-dependent relaxation by ACh, we proposed that ACh acting on the endothelial cells "activates a reaction sequence in which arachidonic (or some other unsaturated fatty acid) is liberated and then oxidized by lipoxy-

genase to a product that is responsible for the relaxation of the smooth muscle cells" (2). In view of this proposal, a number of laboratories have investigated whether arachidonic acid itself can produce an endothelium-dependent relaxation of isolated arteries. Recently, Singer & Peach (38) reported that this agent, in the concentration range of 10–100 μM, produced transient endothelium-dependent relaxation of rings of rabbit aorta. Indomethacin, which inhibited the acute contractions produced by arachidonic acid in aortic rings freed of endothelium, potentiated both the degree and duration of relaxation produced by this agent in intact rings. After indomethacin pretreatment, these rings, when precontracted to modrate tone by phenylephrine, exhibited a maximum relaxation of about 40% in response to 100 μM arachidonic acid (38). Very similar results have been obtained in our laboratory in experiments with arachidonic acid on rabbit aortic strips precontracted with norepinephrine (6, 39).

De Mey and co-workers (18, 23), using rings of various canine arteries (femoral, saphenous, pulmonary, and splenic), found that arachidonic acid produced graded relaxation over a range extending from about 0.1–10 μM, and that the relaxation was either eliminated or markedly reduced in preparations denuded of endothelial cells. These workers also found that pretreatment of the femoral artery with idomethacin completely inhibited relaxation by arachidonic acid at all concentrations up to 30 μM (18). Using ^{14}C-arachidonic acid as a tracer, they found that a major product formed from the fatty acid was 6-keto-$PGF_{1\alpha}$, the prostaglandin to which prostacyclin (PGI_2) is spontaneously converted. Since this product was almost completely eliminated by either removing the endothelial cells or by pretreatment with indomethacin, they concluded that relaxation of dog arteries by arachidonic acid is mainly mediated by PGI_2 produced by endothelial cells from the fatty acid. In my laboratory, results with arachidonic acid on isolated canine coronary and superior mesenteric arteries (6, 39) have differed somewhat from those of De Mey et al (18) on femoral arteries: although relaxation produced by low concentrations of arachidonic acid (0.1–1 μM) was blocked by either indomethacin or flurbiprofen, endothelium-dependent relaxation produced by higher concentrations (10–100 μM) still persisted, even after pretreatment with these cyclooxygenase inhibitors.

Recently, Cherry and co-workers (39) reported that other unsaturated fatty acids besides arachidonic (e.g. cis-4, 7, 10, 13, 16, 19-docosohexaenoic, oleic, elaidic, and cis-vaccenic) produce endothelium-dependent relaxations that are not inhibited by cyclooxygenase inhibitors in both rabbit aorta and dog arteries. Since there is some possibility that these fatty acids may facilitate enzymatic reactions in the endothelial cell membranes by increasing "fluidity" of the membranes, Cherry (unpublished results) has recently tested the "membrane mobility agent" A_2C on isolated arteries. This agent, which is an ester of an 18-carbon fatty acid with a cyclopropane ring rather than a double bond

in the carbon chain (structure: $CH_3(CH_2)_7CH\overset{CH_2}{\frown}CH(CH_2)_7COOCH_2$-$CH_2OCH_2CH_2OCH_3$), was developed by Kosower and colleagues as an agent to increase the fluidity of cell membranes (40). Cherry has found that A_2C, like arachidonic acid, produces endothelium-dependent relaxations in concentrations around 100 μM in both rabbit aorta and dog superior mesenteric artery and that the relaxations are not blocked by cyclooxygenase inhibitors.

Other Agents

Recently, Spokas et al (41) reported that the antihypertensive drug hydralazine, particularly at lower concentrations (0.1–1 μM), produced the greater part of its relaxant effect on isolated rings of rabbit aorta by an indirect action mediated by endothelial cells. In contrast, the relaxant effects of nitroglycerin, nitroprusside, minoxidil, and diazoxide were reported to be independent of endothelial cells.

Another agent found to give moderate, transient, endothelium-dependent relaxation of rabbit aorta is nordihydroguiaretic acid. Paradoxically, this agent is a potent inhibitor of endothelium-dependent relaxations by ACh and A23187 (13, 42). It will be further discussed in a later section.

AGENTS AND CONDITIONS THAT INHIBIT ENDOTHELIUM-DEPENDENT RELAXATIONS

Before considering the agents and conditions that inhibit endothelium-dependent relaxations, it should again be emphasized that such relaxations, except in the case of those produced by arachidonic acid in canine arteries (18) and occasionally by bradykinin in certain arteries of rabbits and cats (15), are not inhibited by inhibitors of cyclooxygenase (i.e. by blockade of prostaglandin synthesis). Also, the agents and conditions discussed in this section are for the most part selective inhibitors of endothelium-dependent relaxations, and unless otherwise indicated they have not been found to inhibit endothelium-independent relaxations evoked by direct actions of relaxing agents on vascular smooth muscle.

Anoxia

Without being aware of the role of endothelial cells in the relaxation of arteries by ACh, De Mey & Vanhoutte (24) found that anoxia inhibited this relaxation in rings of canine femoral artery. We subsequently found that anoxia inhibits endothelium-dependent relaxation of rabbit aorta by ACh (2) and by A23187 (25), and of dog arteries (intrapulmonary and renal) by ACh and bradykinin (6). To achieve complete inhibition, special precautions had to be taken to minimize traces of O_2 in the muscle chambers.

5, 8, 11, 14-Eicosatetraynoic Acid (ETYA)

Inhibition of endothelium-dependent ACh-induced relaxation following pretreatment with ETYA, which is an inhibitor of lipoxygenase as well as of cyclooxygenase (43), was first reported for rabbit aorta (2, 3) and later for dog arteries (15, 18) and rat aorta (10). In rabbit aorta, the inhibition by ETYA was found to be irreversible if the pretreatment used 100 μM ETYA (added in an ethanol solution) for 30–60 minutes (2). Inhibition by pretreatment with ETYA has also been reported for the following endothelium-dependent relaxations: those by A23187, ATP, and substance P in rabbit aorta (16, 25, 27); by histamine and A23187 in rat aorta (10, 14); by bradykinin in dog arteries (13, 15); and by arachidonic acid in rabbit aorta (38). Also, ETYA pretreatment completely blocked relaxation by arachidonic acid in the dog femoral artery (18), but in this case its effectiveness was attributable to its inhibition of cyclooxygenase rather than of lipoxygenase. In the dog femoral artery, De Mey et al (18) found no inhibition by ETYA of endothelium-dependent relaxations by either ATP or thrombin.

If ETYA (100 μM) was added during the course of relaxation of rabbit aorta by ACh, the pre-relaxation level of contractile tone was restored almost completely within one minute (2, 3, 6). This type of acute inhibition or antagonism of relaxation by ETYA has also been found in the case of relaxation of rabbit aorta by other endothelium-dependent relaxing agents, such as A23187, ATP, and substance P (4, 6, 16, 25, 27). Acute inhibition, but with only partial restoration of contractile tone, was also found when ETYA was added to canine superior mesenteric artery during relaxation by ACh or bradykinin (15).

Quinacrine

Quinacrine (mepacrine), which is known to be an inhibitor of phospholipase A_2 (43a), effectively inhibits relaxation of rabbit aorta by ACh or methacholine (2, 3, 44). Inhibition develops quickly and is essentially complete at concentrations of 10–30 μM. Quinacrine has also been found to inhibit the following relaxations: that by ACh in dog arteries (3, 18) and rat aorta (14), by substance P in rabbit aorta (27), by bradykinin in dog arteries (15), and by histamine in rat aorta (14). Although quinacrine does inhibit ATP-induced relaxation in rabbit aorta (4), it is reported not to do so in dog femoral artery (18). Relaxation by A23187, unlike that by ACh, is not inhibited by quinacrine in either rabbit aorta or dog arteries (4, 25, 44). It has been proposed that this difference may be accounted for if the inhibition of ACh-induced relaxation is the result of some block by quinacrine of an ion channel coupled to activation of the muscarinic receptor (3, 44).

Nordihydroguiaretic Acid (NDGA)

NDGA, which is an inhibitor of lipoxygenases as well as an anti-oxidant, is a very effective inhibitor of ACh-induced relaxation in rabbit aorta (13, 42, 44), dog renal artery (45), and human mesenteric artery (13). In rabbit aorta, complete inhibition of ACh- or methacholine-induced relaxation is achieved at NDGA concentrations of 30–100 μM (42, 44). The onset of inhibition is rapid and is reversible if the aorta is not exposed to too much NDGA for too long a time (13, 42). NDGA has also been shown to inhibit endothelium-dependent relaxation by A23187 in rabbit aorta (13, 44) and human mesenteric arteries (13), by bradykinin in dog arteries (42, 45), and by arachidonic acid in rabbit aorta (38).

NDGA itself (30 μM), when added to precontracted rings of rabbit aorta, produces moderate relaxation, usually transient, which is strictly dependent on the presence of endothelial cells (13). This endothelium-dependent relaxation by NDGA is more pronounced in some other vessels, such as rabbit superior mesenteric artery and especially cat celiac and superior mesenteric arteries. In the latter vessels, the marked relaxing effects of NDGA itself (both endothelium-dependent and independent) make it difficult to demonstrate the inhibitory action of NDGA against the relaxing effects of ACh or A23187 (R. Furchgott, unpublished observations).

p-Bromophenacylbromide (BPB)

BPB, an alkylating agent that has been shown to be a potent irreversible inhibitor of phospholipase A_2 in cell-free systems (46), is a potent inhibitor of ACh-induced relaxations in arteries of rabbit, dog, cat, and man (13, 42). In rabbit aorta and in other vessels, exposure to 3 μM for 20 minutes or 10 μM for 5 minutes usually sufficed to inhibit relaxation by ACh completely and irreversibly. Such an exposure also inhibited irreversibly the endothelium-dependent relaxations by other agents, such as A23187, substance P, and bradykinin (canine and human arteries) (13). It should be noted that we have recently found that exposure of rings of rabbit aorta to BPB always caused a significant, and sometimes almost total, loss of endothelial cells (6). Although in some experiments complete loss of relaxing response to ACh and A23187 occurred when only about half of the endothelial cells were lost, the possibility of general damage to endothelial cells by BPB must be considered in evaluating its inhibitory action.

Alterations of Extracellular Cations

Partly because of the potent endothelium-dependent relaxing action of A23187, we hypothesized that an increase of calcium ions in the region of some key Ca^{2+}-activated enzyme (perhaps a phospholipase) may be a primary step in the

reaction sequence leading to formation and release of an endothelium-derived relaxing substance (now called EDRF) by ACh, A23187, and other agents (3). The results of Singer & Peach (26) on rings of rabbit aorta have provided strong support for a critical role for Ca^{2+}. They found that eliminating Ca^{2+} from the incubation medium inhibited maximum relaxation by A23187 by 92%, and by methacholine (used instead of ACh) by 67%. They proposed that the lesser degree of inhibition of the methacholine-induced relaxations occurred because the muscarinic agonist, but not the ionophore, was able to have access to a separate intracellular pool of Ca^{2+} in the endothelial cells. They also found that the calcium-channel blockers verapamil and nifedipine partially inhibited relaxation by both methacholine and A23187 (26).

De Mey & Vanhoutte (24) found in the case of dog femoral artery that the degree of relaxation by ACh decreased markedly when they replaced most of the Na^+ in Krebs solution with Li^+ or sucrose and was completely inhibited after one hour of exposure of the artery to a K^+-free solution. Since they also obtained inhibition of relaxation on exposure of the artery to ouabain (2–10 μM) or to cooling (22°C), they felt that their results suggested a role for Na^+-K^+-ATPase in ACh-induced relaxation (24). Since their results were obtained before the endothelium-dependency of this relaxation was recognized, it would be well to repeat their experiments giving attention to the possibility that the ionic changes and high ouabain concentrations used may have damaged or even caused a loss of endothelial cells.

Hydroquinone

We tested hydroquinone, a potential free radical scavenger, soon after our early speculation that EDRF released by ACh may be a free radical (3). When hydroquinone (100–300 μM) was added during the course of relaxation of rings of rabbit aorta by ACh or A23187, it very rapidly antagonized the relaxation (4). This inhibitory action was reversible if the exposure to hydroquinone was limited to a few minutes, but prolonged exposure (20 minutes) resulted in complete and irreversible
A23187 (13). This irreversible loss was accompanied by a severe loss of endothelial cells during the exposure to hydroquinone (R. Furchgott, unpublished observations). Recently, Van de Voorde & Leusen (10) reported that pretreatment with hydroquinone (100 μM, time unspecified) also significantly inhibits relaxation by ACh and histamine in rat aorta.

Methylene Blue

Several studies (47–50) have shown that methylene blue can inhibit significantly the endothelium-independent relaxations of isolated preparations of bovine coronary arteries evoked by $NaNO_2$, glyceryl trinitrate, and nitroprusside, so-called nitrovasodilators that give rise to nitric oxide as the active principle

that acts on the smooth muscle (49). Recently, Holtzmann (12) has reported that methylene blue is also a potent inhibitor of the endothelium-dependent relaxation of bovine coronary arteries by ACh. Methylene blue has now been shown to rapidly and completely inhibit relaxation by ACh and by A23187 in rings of rabbit aorta (R. Furchgott, unpublished observations). At a concentration of 10 μM, its inhibition of relaxation by ACh (1 μM) or A23187 (.01 μM) is very much faster in onset and greater in degree than is its inhibition of comparable relaxation by glyceryl trinitrate. This difference probably indicates that inhibition by methylene blue of ACh- and A23187-induced relaxation is the result of an action of the redox dye either in the endothelial cells or on the released EDRF itself.

Hemoglobin

According to Bowman & Gillespie (51), hemolysates of erythrocytes exerted an antagonistic action against relaxation of the bovine retractor penis muscle elicited by an unidentified inhibitory factor extracted from the muscle or by nitroprusside. They obtained similar effects using rabbit aorta. They considered that hemoglobin might be the active antagonistic principle of the hemolysates. We have recently tested hemoglobin on rings of rabbit aorta and have found that at concentrations of 1–10 μM it is an effective inhibitor of ACh- and A23187-induced relaxation (W. Martin & R. Furchgott, unpublished observations). By contrast, methemoglobin is ineffective or only slightly effective as an inhibitor of these endothelium-dependent relaxations.

ENDOTHELIAL CELLS IN THE RELAXATION OF RESISTANCE VESSELS AND VEINS

Resistance Vessels

In our first paper on the endothelium-dependent relaxation of isolated arteries by ACh, we proposed that this mechanism is also responsible for the vasodilation of resistance vessels (arterioles) by ACh (2). Although the validity of this proposal still remains to be proven, preliminary studies are in accord with it. In my laboratory, Carvalho (52) used the isolated arterial vascular bed of the rabbit mesentery perfused at constant flow rate and vasoconstricted with norepinephrine and found that vasodilation produced by ACh was eliminated after perfusing the vascular bed with collagenase to remove endothelial cells. Unfortunately, few experiments with collagenase could be completed successfully because treatment with this enzyme preparation tended to produce marked increases in resistance to flow, probably because of developing edema formation.

Another finding in support of the proposal that vasodilation of resistance vessels by ACh is endothelium-dependent comes from recent work by M.

Owen (personal communication). She has been able to mount rings of secondary branches of the rabbit ear artery as small as 150 μ O. D. for tension recording and has shown that these branches, like the central ear artery itself, require endothelial cells for ACh-induced relaxation.

Veins

De Mey & Vanhoutte (23) have investigated the responses of rings of several canine veins (femoral, saphenous, splenic, pulmonary) to ACh and other agents that require endothelial cells for relaxing arteries. Results varied somewhat, depending on the specific vein, but in general the veins gave much less relaxation in response to ACh than did corresponding arteries. For example, in the saphenous and femoral veins, where relaxation was endothelium-dependent, ACh gave only a small degree of relaxation (20–25% maximum) over the range of 30–300 nM and caused contractions at higher concentrations. Similar results have been obtained in our laboratory with the rabbit portal vein (3, 5). De Mey & Vanhoutte (23) also found that ATP, thrombin, and arachidonic acid, all of which elicited good-to-excellent endothelium-dependent relaxations in canine femoral, saphenous, splenic, and pulmonary arteries, elicited either no relaxations or transient relaxations of small degree in the corresponding veins. In experiments in our laboratory on the canine femoral vein, bradykinin and A23187, like ACh, gave only very small endothelium-dependent relaxations (R. Furchgott, unpublished observations). The reason for the much smaller relaxations by these vasodilators in veins compared to arteries is not yet clear.

THE ENDOTHELIUM-DERIVED RELAXING FACTOR(S) (EDRF)

The chemical identity of EDRF is not yet known. Early work on the relaxation of rabbit aorta by ACh ruled out adenosine, AMP, or any prostaglandin (2). The finding that the calcium ionophore A23187 was a potent endothelium-dependent relaxing agent and that relaxation by ACh could be inhibited by anoxia, ETYA (a potential lipoxygenase inhibitor), quinacrine (a potential inhibitor or arachidonic-acid release from phosphatides), and hydroquinone (a potential free-radical scavenger) led to the following speculation: (*a*) activation of the endothelial cell muscarinic receptor leads to mobilization of Ca^{++} in the region of a Ca^{++}-activated lipase(s) that releases arachidonic acid from phosphatides; (*b*) oxidation of the liberated arachidonic acid (or some other unsaturated fatty acid) by a lipoxygenase leads to the formation and release of an active, short-lived intermediate, possibly a free radical, which is the relaxing factor (3). As discussed in the next section, one reason for favoring the idea of a

free radical as the ACh-evoked EDRF was that certain free radicals were already known to stimulate guanylate cyclase and to cause relaxation of certain smooth muscle preparations (49). Consistent with the speculation that the EDRF released by ACh is a labile product of a lipase-lipoxygenase pathway were the later findings that NDGA (a potential lipoxygenase inhibitor) and BPB (a potential phospholipase A_2 inhibitor) inhibited ACh-induced relaxation. Also, our recent results on rapid acute inhibition of ACh-induced relaxation by methylene blue and by hemoglobin could conceivably indicate reactions of these agents with an EDRF that is a free radical. (For details and references related to the inhibitory agents, see earlier section.)

Because the inhibitory agents cited above may well lack the desired specificity, the results obtained with them are not sufficient for a firm conclusion about the source and nature of EDRF. Indeed, there are other findings that appear inconsistent with the speculative scheme. For example, the lipoxygenase inhibitor BW755C (53) does not inhibit ACh-induced relaxation of rabbit aorta. This lack of inhibition by BW755C could possibly be accounted for in a modified scheme recently proposed by Singer & Peach (35, 44). These workers did find that the endothelium-dependent relaxation of rabbit aorta by arachidonic acid during cyclooxygenase inhibition could be prevented by preincubation with ETYA or NDGA (38), but they proposed that these agents may be inhibiting the oxidation of the fatty acid by cytochrome P450 rather than by a lipoxygenase. If arachidonic acid is actually the precursor of EDRF no matter what oxidizing enzyme system is involved, ETYA or NDGA added during the course of relaxation of cyclooxygenase-inhibited rabbit aorta by arachidonic acid could be expected to acutely antagonize this relaxation just as it does relaxation by ACh and A23187 (13, 42). But neither ETYA nor NDGA acutely antagonizes arachidonic acid-induced relaxation (6). The meaning of this discrepancy is not clear, but a possibility is that the non-prostaglandin EDRF released by arachidonic acid is different from that released by ACh.

The speculation that EDRF released by ACh is a labile oxidation product of arachidonic acid is also difficult to reconcile with the recent findings that endothelium-dependent relaxations in rabbit and dog arteries can be evoked by other unsaturated fatty acids (e.g. cis-4, 7, 10, 13, 16, 19-docosohexaenoic, oleic, elaidic, and cis-vaccenic) and even by a saturated membrane mobility agent, A_2C (39; also see above). Since these agents would be expected to increase membrane fluidity, it has been proposed that such an increase may be a primary step in a reaction sequence in endothelial cells leading to formation of an EDRF (P. Cherry, personal communication). It is conceivable that not only these agents, acting to increase membrane fluidity, but also ACh, stimulating membrane changes via its receptor, alter the membrane to allow the activation of some common ordinarily restrained reaction sequence that gives rise to an EDRF not itself an oxidation product of an unsaturated fatty acid (6).

Regardless of its chemical nature, the EDRF released by A23187 and substance P in rabbit, canine, and feline arteries, and by bradykinin in canine and human arteries, is most likely the same substance (or substances) as the EDRF released by ACh in these arteries. This conclusion is based on the common susceptibilities to inhibitory agents (e.g. anoxia, ETYA, NDGA, BPB) of the relaxation produced by each of these agents and that produced by ACh in any given artery (13; also see above). On the same basis, it seems likely that EDRF released by ATP and ADP in rabbit aorta is similar to that released by ACh (13). However, De Mey et al (18) concluded that in the canine femoral artery relaxation by ATP is mediated by a different "signal" (EDRF?) than is relaxation by ACh, since relaxation by ATP, unlike that by ACh, was not inhibited by pretreatment with ETYA or quinacrine. They also found no inhibition by ETYA or quinacrine of the endothelium-dependent relaxation by thrombin.

STUDIES ON THE MECHANISM OF RELAXATION BY EDRF

Prior to our findings on endothelium-dependent relaxation by ACh, a number of reports indicated that in certain smooth muscles there was a positive relationship between an increase in cyclic GMP and relaxation (49, 54–56), and that guanylate cyclase was markedly stimulated by hydroperoxides of arachidonic acid (57, 58) and by free radicals, particularly nitric oxide and the hydroxyl radical (49). Murad and his colleagues proposed that many potent vasodilators, such as nitroprusside, organic nitrates, azide, and inorganic nitrite, activate guanylate cyclase indirectly via nitric oxide, which they release as a reaction product (49). These findings of others were partly responsible for our speculation that EDRF may be a short-lived hydroperoxide or free radical resulting from the oxidation of arachidonic acid by a lipoxygenase pathway, and that it stimulates the guanylate cyclase of the arterial smooth muscle, causing an increase in cyclic GMP that then somehow activates relaxation (3). Although a causal relationship between increases in cyclic GMP levels and relaxation in vascular smooth muscle has not yet been proven, evidence consistent with such a relationship in the case of relaxation of isolated bovine coronary arteries by nitric oxide and nitric oxide-yielding vasodilators (nitroglycerin, nitroprusside, inorganic nitrite) has recently been presented in several reports (48, 50, 59).

Our speculation that EDRF would stimulate an increase in cyclic GMP of the arterial muscle has been proven correct in recent experiments on rat aorta by Rapoport & Murad (14), and on rabbit aorta by Jothianandan and myself (60). In the former experiments, strips of rat aorta with and without endothelium were exposed to norepinephrine, followed by ACh (10 μM), histamine (100 μM), or A23187 (3 μM), all at concentrations optimal for endothelium-

dependent relaxation, for varying times prior to freezing in liquid N_2 (14). Each of these agents produced a marked increase (20- to 40-fold) in cyclic GMP above the control level (about 1 pmol/mg protein) in intact strips. Peak increases occurred in about 30 seconds, followed by a considerable decline over the next 2 minutes. None of the agents produced increases in cyclic GMP in endothelium-free strips. In contrast to these three relaxing agents, nitroprusside (1 μM), which does not depend on endothelium for its relaxant action, produced marked increases in cyclic GMP in both endothelium-free and intact strips. In our laboratory, rings of rabbit aorta were mounted to allow both recording of tension and rapid freezing at any desired stage of contraction or relaxation (60). Norepinephrine produced no change in cyclic GMP from the basal level (about 0.2 and 0.06 pmol/mg protein in intact and endothelium-free rings respectively). Relaxations of norepinephrine-induced contraction by ACh (1 μM) and by A23187 (0.1 μM) in intact rings were accompanied by 5-fold and 7-fold increases in cyclic GMP respectively. The cyclic GMP level peaked between 15 and 30 seconds after ACh addition and then remained at essentially the peak level out to 3 minutes. In endothelium-free rings, ACh and A23187 produced neither relaxation nor any change in cyclic GMP. Glyceryl trinitrate, which relaxed endothelium-free rings and intact rings equally well, produced marked increases in cyclic GMP in both types. In the case of ACh, histamine, and A23187 on rat aorta (14), as well as ACh on rabbit aorta (60), the onset of rise in cyclic GMP occurred before the onset of relaxation. Also, in aortas of both species, relaxation by ACh was not associated with any change in cyclic AMP (14, 60). Evidence that the observed increase of cyclic GMP produced by ACh in strips of aortas of both species was in the vascular smooth muscle rather than in the endothelial cells was obtained in experiments in which rapid removal of endothelial cells in a short interval between incubation with ACh and freezing still left elevated levels of cyclic GMP in the tissue. Removal of the cells was accomplished in rat aorta in 5 seconds by scraping the intimal surface with a scalpel (37), and in rabbit aorta in 3 seconds by dragging that surface over filter paper (R. Furchgott, unpublished observations). Very recently, Holzmann (12) has reported that endothelium-dependent relaxation of bovine coronary arteries by ACh is also accompanied by an increase in cyclic GMP.

Recently, Rapoport and co-workers (61) have reported that the endothelium-dependent increase in cyclic GMP in rat aorta produced by ACh is accompanied by a change in the pattern of phosphorylated proteins. They determined this pattern using two-dimensional gel electrophoresis and autoradiography to ascertain incorporation of ^{32}P-phosphate into tissue proteins. They found that the phosphorylation of nine proteins was increased and that of two proteins (probably myosin light chains) was decreased. This finding is particularly exciting because this change of pattern of phosphorylated proteins is the same

as they had previously found in rat aorta after exposure to the endothelium-independent vasodilator sodium nitroprusside and to 8-bromo cyclic GMP (62). They hypothesize that endothelium-dependent relaxation by ACh, as well as endothelium-independent relaxation by nitrovasodilators, is mediated through cyclic GMP-dependent protein phosphorylation and dephosphorylation of myosin light chains.

CONCLUDING REMARKS

It is now well established that a major mechanism for the relaxation of certain isolated arteries by ACh and a number of other potent endogenous vasoactive agents (including ATP and ADP, substance P, bradykinin, histamine, serotonin, and thrombin) is an indirect one in which the agent first acts on endothelial cells to stimulate the production and release of a non-prostaglandin relaxing factor (EDRF), which then in turn acts on the smooth muscle cells of the artery. The chemical identity of EDRF—or perhaps EDRF's, since there is some evidence for more than one factor—is still not known. The original proposal that EDRF is an intermediate product of the oxidation of arachidonic (or some other unsaturated fatty acid) via a lipoxygenase pathway (2, 3) is supported by some but not all recent findings. Regardless of its chemical identity, EDRF does appear to be very labile, and the possibility that it is a free radical is still attractive. The speculation that EDRF would stimulate an increase in cyclic GMP in the arterial smooth muscle (3), just as nitrovasodilators do, has now been proven correct (14, 60). Recent findings suggest that cyclic GMP-dependent protein phosphorylation and dephosphorylation of myosin light chains in arterial muscle may mediate relaxation by ACh and certain other endothelium-dependent relaxing agents (61, 62). The role of endothelial cells in mediating relaxation of blood vessels by a number of endogenous agents must now be a factor in our consideration of the physiological control and pharmacological modifications of regional circulation and probably of certain pathological disorders of circulation.

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Literature Cited

1. Furchgott, R. F., Zawadzki, J. V. 1980. Acetylcholine relaxes arterial smooth muscle by releasing a relaxing substance from endothelial cells. *Fed. Proc.* 39:581 (Abstr.)
2. Furchgott, R. F., Zawadzki, J. V. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373–76
3. Furchgott, R. F., Zawadzki, J. V., Cherry, P. D. 1981. Role of endothelium in the vasodilator response to acetylcholine. In *Vasodilation,* ed. P. Vanhoutte, I. Leusen, pp. 49–66. New York: Raven. 536 pp.
4. Furchgott, R. F. 1981. The requirement for endothelial cells in the relaxation of arteries by acetylcholine and some other vasodilators. *Trends Pharmacol. Sci.* 2:173–76
5. Furchgott, R. F. 1982. Acetylcholine and blood vessel relaxation: complications and clarifications. In *Trends in Autonomic Pharmacology,* ed. S. Kalsner, 2:497–510. Baltimore: Urban & Schwarzenberg. 363 pp.
6. Furchgott, R. F. 1983. Role of endothelium in responses of vascular smooth muscle. *Circ. Res.* 53:557–73
7. De Mey, J. G., Vanhoutte, P. M. 1981. Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. *J. Physiol.* 316:347–55
8. Chand, N., Altura, B. M. 1981. Acetylcholine and bradykinin relax intrapulmonary arteries by acting on endothelial cells: Role in lung vascular diseases. *Science* 213:1376–79
9. Lee, T. J. -F. 1982. Cholinergic mechanism in the large cat cerebral artery. *Circ. Res.* 50:870–79
10. Van de Voorde, J., Leusen, I. 1983. Role of endothelium in the vasodilator response of rat thoracic aorta to histamine. *Eur. J. Pharmacol.* 87:113–20
11. Gordon, J. L., Martin, W. 1983. Endothelium-dependent relaxation of the pig aorta: Relationship to stimulation of Rb efflux from isolated endothelial cells. *Br. J. Pharmacol.* 79:531–41
12. Holzmann, S. 1982. Endothelium-induced relaxation by acetylcholine associated with larger rises in cyclic GMP in coronary arterial strips. *J. Cyclic Nucleotide Res.* 8:409–19
13. Furchgott, R. F., Cherry, P. D., Zawadzki, J. V. 1983. Endothelium dependent relaxation of arteries by acetylcholine, bradykinin and other agents. In *Vascular Neuroeffector Mechanisms: 4th International Symposium,* ed. J. Bevan, et al, pp. 37–43. New York: Raven
14. Rapoport, R. M., Murad, F. 1983. Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ. Res.* 52:352–57
15. Cherry, P. D., Furchgott, R. F., Zawadzki, J. V., Jothianandan, D. 1982. The role of endothelial cells in the relaxation of isolated arteries by bradykinin. *Proc. Natl. Acad. Sci. USA* 79:2106–10
16. Furchgott, R. F., Zawadzki, J. V. 1980. ATP relaxes rabbit aortic smooth muscle by both an indirect action via endothelial cells and a direct action. *Pharmacologist* 22:271 (Abstr.)
17. Furchgott, R. F., Erreich, S. J. Greenblatt, E. 1961. The photoactivated relaxation of smooth muscle of rabbit aorta. *J. Gen. Physiol.* 44:499–519
18. De Mey, J. G., Claeys, M., Vanhoutte, P. M. 1982. Endothelial-dependent inhibitory effects of acetylcholine, adenosine triphosphate, thrombin and arachidonic acid in the canine femoral artery. *J. Pharmacol. Exp. Ther.* 222:166–73
19. Vanhoutte, P. M. 1977. Cholinergic inhibition of adrenergic transmission. *Fed. Proc.* 36:2444–49
20. Fozard, J. 1979. Cholinergic mechanisms in adrenergic function. See Ref. 5, 1:145–95
21. Steinsland, O. S., Furchgott, R. F., Kirpekar, S. M. 1973. Inhibition of adrenergic neurotransmission by parasympathomimetics in the rabbit ear artery. *J. Pharmacol. Exp. Ther.* 184: 346–56
22. Altura, B. M., Chand, N. 1981. Bradykinin-induced relaxation of renal and pulmonary arteries is dependent upon intact endothelial cells. *Br. J. Pharmacol.* 74:10–11
23. De Mey, J. G., Vanhoutte, P. M. 1982. Heterogeneous behavior of the canine arterial and venous wall. *Circ. Res.* 51:439–47
24. De Mey, J. G., Vanhoutte, P. M. 1980. Interaction between Na^+, K^+ exchanges and the direct inhibitory effect of acetylcholine on canine femoral arteries. *Circ. Res.* 46:826–36
25. Zawadzki, J. V., Cherry, P. D., Furchgott, R. F. 1980. Comparison of endothelium-dependent relaxation of rabbit aorta by A23187 and by acetylcholine. *Pharmacologist* 22:271 (Abstr.)
26. Singer, H. A., Peach, M. H. 1982. Calcium-and endothelial-mediated vascular

smooth muscle relaxation in rabbit aorta. *Hypertension* 4 (Suppl. 2): II19–25
27. Zawadzki, J. V., Furchgott, R. F., Cherry, P. D. 1981. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by substance P. *Fed. Proc.* 40:689 (Abstr.)
28. Zawadzki, J. V., Furchgott, R. F., Cherry, P. D. 1983. Endothelium-dependent relaxation of arteries by octasubstance P, kassinin and octacholecystokinin. *Fed. Proc.* 42:619 (Abstr.)
29. Cherry, P. D., Furchgott, R. F., Zawadzki, J. V. 1981. The indirect nature of bradykinin relaxation of isolated arteries: Endothelial dependent and independent components. *Fed. Proc.* 40:689 (Abstr.)
30. Aiken, J. W. 1974. Inhibitors of prostaglandin synthesis specifically antagonize bradykinin- and angiotensin-induced relaxations of the isolated celiac artery from rabbit. *Pharmacologist* 1:295 (Abstr.)
31. Blumberg, A. L., Denny, S. E., Marshall, G. R., Needleman, P. A. 1977. Blood vessel-hormone interactions: Angiotensin, bradykinin and prostaglandin. *Am. J. Physiol.* 232:H305–10
32. Needleman, P., Marshall, G., Sobel, B. E. 1975. Hormone interactions in the isolated rabbit heart: Synthesis and coronary vasomotor effects of prostaglandins, angiotensin, and bradykinin. *Circ. Res.* 37:802–8
33. Toda, N. 1977. Actions of bradykinin on isolated cerebral and peripheral arteries. *Am. J. Physiol.* 232:H267–74
34. Cohen, R. A., Shepherd, J. T., Vanhoutte, P. M. 1983. Inhibitory role of the endothelium in the response of isolated coronary arteries to platelets. *Science* 221:273–74
35. Cohen, R. A., Shepherd, J. T., Vanhoutte, P. M., 1983. 5-hydroxytryptamine released from aggregating platelets can mediate endothelium-dependent inhibition of coronary smooth muscle. *Am. J. Physiol.* In press
36. Ku, D. 1982. Coronary vascular reactivity after acute myocardial ischemia. *Science* 218:576–78
37. Deleted in proof
38. Singer, H. A., Peach, M. J. 1983. Endothelium-dependent relaxation of rabbit aorta. I. Relaxation stimulated by arachidonic acid (AA). *J. Pharmacol. Exp. Ther.* 227:790–95
39. Cherry, P. D., Furchgott, R. F., Zawadzki, J. V. 1983. The endothelium-dependent relaxation of vascular smooth muscle by unsaturated fatty acids. *Fed. Proc.* 42:619 (Abstr.)
40. Kosower, E. M., Kosower, N. S., Faltin, Z., Diver, A., Saltoun, G., Frensdorff, A. 1974. Membrane mobility agents: A new class of biologically active molecules. *Biochim. Biophys. Acta* 363:261–66
41. Spokas, E. G., Folco, G., Quilley, J., Chandler, P., McGiff, J. C. 1983. Endothelial mechanism in the vascular action of hydralazine. *Hypertension* 5 (Suppl. 1): I104–11
42. Furchgott, R. F., Zawadzki, J. V., Jothianandan, D., Cherry, P. D. 1982. Nordihydroguiaretic acid (NDGA) and α,p-dibromoacetophenone (BPB) inhibit endothelium-dependent relaxation of arteries by acetylcholine, bradykinin and A23187. *Fed. Proc.* (Abstr.)
43. Flower, R. J. 1974. Drugs which inhibit prostaglandin biosynthesis. *Pharmacol. Rev.* 26:33–67
43a. Flower, R. J., Blackwell, G. J. 1975. The importance of phospholipase-A_2 in prostaglandin biosynthesis. *Biochem. Pharmacol.* 25:285–91
44. Singer, H. A., Peach, M. J. 1983. Endothelium-dependent relaxation of rabbit aorta. II. Inhibition of relaxation stimulated by methacholine and A23187 with antagonists of arachidonic acid metabolism. *J. Pharmacol. Exp. Ther.* 227:796–801
45. Chand, N., Altura, B. M. 1981. Inhibition of endothelial cell-dependent relaxations to acetylcholine and bradykinin by lipoxygenase inhibitors in canine isolated renal arteries. *Microcirculation* 1:211–23
46. Roberts, M. F., Deems, R. A., Mincey, T. C., Dennis, E. A. 1977. Chemical modification of the histidine residue in phospholipase A_2 (Naja naja naja). *J. Biol. Chem.* 252:2405–11
47. Gruetter, C. A., Kadowitz, P. J., Ignarro, L. F. 1981. Methylene blue inhibits coronary arterial relaxation and guanylate cyclase activation by nitroglycerin, sodium nitrite and amyl nitrite. *Can. J. Physiol. Pharmacol.* 59:150–56
48. Gruetter, C. A., Gruetter, D. Y., Lyon, J. E., Kadowitz, P. J., Ignarro, L. F. 1981. Relationship between cyclic guanosine 3':5'-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: Effects of methylene blue and methemoglobin. *J. Pharmacol. Exp. Ther.* 219:181–86
49. Murad, F., Arnold, W. P., Mittal, C. K., Braughler, J. M. 1979. Properties and regulation of guanylate cyclase and some

proposed functions for cyclic GMP. *Adv. Cyclic Nucleotide Res.* 11:175–204
50. Kukovetz, W. R., Pöch, G., Holzmann, S. 1981. Cyclic nucleotides and relaxation of vascular smooth muscle. See Ref. 3, pp. 339–53
51. Bowman, A., Gillespie, J. S. 1981. An erythrocyte-associated antagonist of inhibitory mechanisms in the bovine retractor penis muscle. *Br. J. Pharmacol.* 74: P181–P82
52. Carvalho, M. H., Furchgott, R. F. 1981. Vasodilation of the rabbit mesenteric vascular bed by acetylcholine and A23187. *Pharmacologist* 23:223 (Abstr.)
53. Higgs, G. A., Copp, F. C., Denyer, C. V., Flower, R. J., Tateson, J. E., Walker, J. M. G. 1978. Reduction of leukocyte migration by a cycleoxygenase inhibitor. *Abstr. 7th Int. Congr. Pharmacol.*, p. 374. Oxford: Pergamon (Abstr.)
54. Katsuki, S., Murad, F. 1977. Regulation of adenosine cyclic 3',5'-monophosphate levels and contractility in bovine tracheal smooth muscle. *Mol. Pharmacol.* 13:330–41
55. Böhme, E., Graf, H., Schultz, G. 1978. Effects of sodium nitroprusside and other smooth muscle relaxants on cyclic GMP formation in smooth muscle and platelets. *Adv. Cyclic Nucleotide Res.* 9:131–43
56. Schultz, K. D., Bohme, E., Kreye, V. W., Schultz, G. 1979. Relaxation of hormonally stimulated smooth muscular tissues by the 8-bromo derivative of cyclic GMP. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 301:1–9
57. Hidaka, H., Asano, T. 1977. Stimulation of platelet guanylate cyclase by unsaturated fatty acid peroxides. *Proc. Natl. Acad. Sci. USA* 74:3657–61
58. Goldberg, N. D., Graff, G., Haddox, M. K., Stephenson, J. H., Glass, D. B., Moser, M. E. 1978. Redox modulation of splenic ccll solublc guanylatc cyclase activity: Activation by hydrophilic and hydrophobic antioxidants represented by ascorbic and dehydroascorbic acids, fatty acid hydroperoxides, and prostaglandin endoperoxides. *Adv. Cyclic Nucleotide Res.* 9:101–30
59. Ignarro, L. J., Lippton, H., Edwards, J. C., Baricos, W. H., Hyman, A. L., Kadowitz, P. J., Gruetter, C. A. 1981. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: Evidence for the involvement of s-nitrosothiols as active intermediates. *J. Pharmacol. Exp. Ther.* 218:739–49
60. Furchgott, R. F., Jothianandan, D. 1983. Relation of cyclic GMP levels to endothelium-dependent relaxation by acetylcholine in rabbit aorta. *Fed. Proc.* 42:619 (Abstr.)
61. Rapoport, R. M., Draznin, M. B., Murad, F. 1983. Endothelium-dependent vascular relaxation may be mediated through cyclic GMP-dependent protein phosphorylation. *Clin. Res.* 31:526A (Abstr.)
62. Rapoport, R. M., Draznin, M. B., Murad, F. 1982. Sodium nitroprusside-induced protein phosphorylation in intact rat aorta is mimicked by 8-bromo cyclic GMP. *Proc. Natl. Acad. Sci. USA* 79:6470–74