Brief Review

Endothelium-Derived Relaxing Factors
A Perspective From In Vivo Data

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We review below published studies of endothelium-dependent vasodilation in vivo. Endothelium-dependent vasodilation has been demonstrated in conduit arteries in vivo and in the cerebral, coronary, mesenteric, and femoral vascular beds as well as in the microcirculation of the brain and the microcirculation of cremaster muscle. The available evidence, although not complete, strongly suggests that the endothelium-derived relaxing factor generated by acetylcholine in the cerebral microcirculation is a nitrosothiol. The endothelium-derived relaxing factor generated by bradykinin in this vascular bed is an oxygen radical generated in association with enhanced arachidonate metabolism via cyclooxygenase. In the microcirculation of skeletal muscle, on the other hand, the vasodilation from bradykinin is mediated partly by prostacycline and partly by an endothelium-derived relaxing factor similar to that generated by acetylcholine. Basal secretion of endothelium-derived relaxing factor is controversial in vivo but is usually present in vitro. On the other hand, it appears that endothelium-derived relaxing factor mediates flow-dependent vasodilation in both large vessels and in the microcirculation in vivo. The generation and release of endothelium-derived relaxing factor from endothelium may be abnormal in a variety of conditions including acute and chronic hypertension, atherosclerosis, and ischemia followed by reperfusion. Several mechanisms for these abnormalities have been identified. These include inability to generate endothelium-derived relaxing factor or destruction of endothelium-derived relaxing factor by oxidants after its release in the extracellular space. These abnormalities in endothelium-dependent relaxation may contribute to the vascular abnormalities in these conditions. (Hypertension 1990;16:371–386)

The discovery of endothelium-derived relaxing factor (EDRF) generated by acetylcholine opened a new field of investigation into endothelium-derived vasoactive factors. It is now well established that a number of vasoactive agents indirectly induce vascular smooth muscle relaxation with ensuing vasodilation by acting on the endothelium to induce the generation and release of EDRFs. These substances are short-lived; they diffuse to the vascular smooth muscle where they activate soluble guanylate cyclase. This results in an increase in the intracellular concentration of cyclic guanosine monophosphate (cGMP) with resultant relaxation of vascular smooth muscle (Figure 1). Therefore, the final pathway for the vasodilation induced by EDRF is the same as that for the nitrodiators, which generate nitric oxide either spontaneously (like nitroprusside) or via interaction with tissue components (like nitroglycerin) and eventually cause activation of soluble guanylate cyclase.

There have been numerous investigations attempting to identify the chemical nature of EDRF. Several suggestions, including that EDRF is a product of lipoxygenase metabolism of arachidonate, a product of cytochrome P-450 oxygenases, or a carbonyl-containing compound, have now been disproved. Most recently, strong evidence has been gathered suggesting that the EDRF generated by acetylcholine in blood vessels and by bradykinin in cultured endothelial cells is nitric oxide. This view is supported by pharmacological similarities between nitric oxide and EDRF, by demonstration of the release of nitric oxide from the target tissues by these agonists and by inhibition of the action of both nitric oxide and EDRF by various pharmacological blocking agents, such as hemoglobin and methylene blue, and inhibition of the production of EDRF and nitric oxide from arginine by N\(^{\text{O}}\)-monomethyl-L-arginine (L-NMMA).

Despite these findings, no unanimity of opinion has resulted that nitric oxide and EDRF are indeed identical. The main reasons for this skepticism are...
that some investigators have been able to identify pharmacological differences between nitric oxide and EDRF; other investigators have shown that the amount of nitric oxide released by acetylcholine from vessels or by bradykinin from endothelial cells is not sufficient to explain the observed vasodilation; finally, other investigators have been unable to find release of nitric oxide by electron-spin resonance techniques coupled with the use of spin-traps. It has been suggested, therefore, that EDRF may be a nitric oxide–containing compound that is much more active in inducing vasodilation than nitric oxide itself. Nitrosothiols have properties that render them appropriate candidates for this purpose.

Most of the investigations into endothelium-derived relaxing factors have been done in the in vitro setting using isolated vessel segments or strips, cultured cells, perfusion cascade experiments, and bioassay techniques. These in vitro studies are well-suited for the biochemical characterization of different factors and the identification of the mechanisms of their action. It is clear, however, that the conditions of these in vitro experiments are drastically different from the conditions prevailing in intact in vivo vascular beds. Therefore, it is likely that some of the features of endothelium-dependent relaxation identified in in vitro experiments may not be applicable to in vivo conditions, or may even be artifacts of the abnormal conditions that exist in the in vitro experiments. Also, the physiological significance of each newly discovered factor may be difficult to establish exclusively on the basis of in vitro studies.

Recognition of the need for in vivo studies of endothelium-dependent relaxation to complement the results of the in vitro studies has resulted in an increasing number of in vivo studies of this mechanism. It is the purpose of this review to consider the in vivo work that supports the existence of endothelium-dependent relaxation, the properties and possible identity of the factors involved, and their physiological significance.

Evidence Supporting the Existence of Endothelium-Derived Relaxing Factor in Vivo

Several investigators reported evidence supporting the existence of EDRF in vivo. A variety of techniques have been used. These include: 1) removal of the endothelium by mechanical or chemical means; 2) selective damage to the endothelium with light-and-dye techniques; 3) inhibition of the generation or release of EDRF or its destruction after release by pharmacological means, and 4) bioassay of EDRF.

We review below, separately in conduit arteries and in the microcirculation, the evidence supporting the existence of EDRF in vivo.

**Conduit Vessels**

There is conclusive evidence for the existence of endothelium-dependent relaxation in large vessels in vivo. This evidence is based on mechanical removal of the endothelium from these vessels and demonstration that the action of certain agonists is then eliminated, whereas the effect of agents that act directly on vascular smooth muscle is either totally unaffected or affected much less. Angus and his colleagues investigated femoral artery dilation from acetylcholine and from substance P in the dog. When these agents were applied intra-arterially or topically, they induced vasodilation. This dilation was abolished when a section of the artery was subjected to destruction of the endothelium with a modified Fogarty catheter. The denudation of the endothelium at the site of injury was demonstrated histologically. Blood flow and pressure were controlled in the instrumented section of the artery with a perfusion pump and a Starling resistor to adjust blood flow and pressures. Similar results were obtained in iliac arteries of conscious dogs with acetylcholine as the endothelium-dependent agonist; denudation of the endothelium with balloon inflation eliminated the vasodilator response to acetylcholine. Pohl and his colleagues found that vasodilation induced by acetylcholine and dilation induced by increases in blood flow in the femoral artery of dogs were eliminated.
after mechanical removal of the endothelium or after destruction of the endothelium by intra-arterial infusion of hydrogen peroxide. In each case, the response to nitroglycerin, a non–endothelium-dependent nitrovasodilator, remained unaffected by the elimination of the endothelium.

Partial dependence on the endothelium has been demonstrated in the coronary circulation with respect to the action of serotonin. In anesthetized dogs, serotonin given by constant intracoronary infusion caused epicardial vessel constriction. When a segment of the coronary artery was denuded of its endothelium by using balloon inflation, the constriction due to serotonin was potentiated, suggesting that this agent causes, in part, endothelium-dependent relaxation whose elimination potentiates the constriction due to other actions of the drug. In conscious dogs, the administration of serotonin into the left atrium resulted in an initial transient dilation of epicardial arteries followed by a sustained vasoconstrictor response. The initial dilation was abolished after the endothelium was removed, and the sustained constrictor effect was potentiated. Recently, vasodilation due to intra-arterial administration of acetylcholine was demonstrated in apparently healthy epicardial coronary arteries in humans. It was suggested that this was due to the release of EDRF, as indicated by the fact that intracoronary methylene blue blocked the acetylcholine-mediated dilation.

**Microcirculation**

Unlike the situation with large conduit vessels in vivo, the demonstration of endothelium-dependent relaxation in the microcirculation has been much more difficult because it is not possible to mechanically remove the endothelium from these vessels without damaging the vascular smooth muscle. Attempts by Dacey and Bassett to remove the endothelium from small cerebral arterioles from the rat by either collagenase or by using detergents failed, because either the removal of the endothelium was not complete or, when complete removal was achieved, the responsiveness of the vascular smooth muscle was also affected severely.

Selective damage to the endothelium without injuring the vascular smooth muscle was achieved in vivo by Rosenblum and his colleagues using two light-and-dye techniques. Both methods involved the intravascular administration of a photosensitizing dye to absorb radiation followed by selective radiation of individual cerebral vessels with light containing wavelengths that were absorbed by the dye. Two techniques were used: ultraviolet (UV) radiation from a mercury lamp coupled with the intravascular administration of fluorescein and the use of a helium-neon laser coupled with the intravenous administration of Evans blue dye. Selected vessels were then subjected to radiation over a limited portion of their length between 18 and 36 μm. It was shown by electron microscopy that such vessels usually displayed minor morphological evidence of damage, mostly consisting of blebbing or vacuolization. After exposure to this form of radiation, these vessels lost their ability to respond with vasodilation to acetylcholine, bradykinin, and the calcium ionophore A23187. The loss of endothelium-dependent dilation occurred in the area exposed to radiation and the damage became progressively less pronounced as one moved away from the area of radiation either upstream or downstream (Figure 2). In the mouse, responses to sodium nitroprusside or papaverine were preserved, indicating that the injury was selectively limited to the endothelium and that vascular smooth muscle was still capable of responding normally with vasodilation to direct acting agents.

Rosenblum believes that the mechanism of the injury is due to absorption of the radiation by the dye and the generation of heat with a resultant local thermal injury to the endothelium. This, in turn, results in inability of the endothelium to generate and release EDRF. It is noteworthy that endothelial denudation is not necessary to eliminate endothelium-dependent relaxation and that the inability of the endothelium to produce EDRF under these conditions is transient. Over a period of about 4 hours, the dilator response returned in most vessels.

Most of the experiments using this type of selective damage to the endothelium were done in the cerebral microcirculation of the mouse. Similar results were obtained in the cerebral microcirculation of the cat and, more recently, in the microcirculation of the rat cremaster muscle. In this preparation, Koller and his colleagues found that the vasodilation in response to topical application of acetylcholine, ara-

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**Figure 2.** Line graph showing effects of acetylcholine (80 μg/ml) on mouse pial arteriolar diameter at different sites along length of a vessel before and after light-and-dye treatment with laser irradiation and Evans blue dye intravenously. Figure shows mean ± SD of vessel diameter as a percent of baseline induced by topical application of acetylcholine. "Lesion" is site on pial arteriole where laser was focused. Responses to acetylcholine were also tested up to 80 μm upstream and downstream from site of endothelial lesion. Each pair of points represents cumulative data from five mice.

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chidonic acid, and the calcium ionophore A23187 were eliminated or were actually converted to a small vasoconstriction after damage to the endothelium of the vessels with the light-and-dye technique. The vasodilation in response to topical application of adenosine and prostaglandin E\(_2\) two non-endothelium-dependent dilators, was unaffected by the injury to the endothelium.

There are two aspects of the light-and-dye techniques to injure the endothelium selectively that are not fully explained and thus merit further consideration. First, these techniques in some species cause vasodilation of the irradiated vessels that may be sustained for long periods of time, although in some species it abates to a variable degree. The extent of the dilation differs depending on the type of technique used and on the species: In the mouse, with the laser technique, it is minimal or entirely absent; in the cat, with fluorescein plus UV radiation of cerebral vessels, the dilation is pronounced and sustained; in the cremaster muscle of the rat, with fluorescein plus UV radiation, the dilation is pronounced, but it subsides to a considerable extent over time.43,44 This dilation in response to the light and dye raises questions about the mechanism of the injury that this technique produces. The dilation, as well as the morphological lesions produced, are remarkably similar to what is seen from exposure of the vessels to oxygen radical–generating agents or interventions.45,46

Irrespective of its cause, the dilation induced by the light-and-dye techniques may affect the interpretation of the findings. Because dose–response curves of all the agents used are not usually performed, it is possible for the effect of different dilators to be affected to a different extent. It is possible for the inhibition of the dilation to occur in such a way that the response to one agent is affected more than the other simply by virtue of the fact that one is on a different part of the dose–response curve for one dilator than for the other. Also, acetylcholine, which is frequently used as the endothelium-dependent dilator, has a vasoconstrictor effect that is mediated through direct action on the vascular smooth muscle.47 It is, therefore, generally easier to eliminate the vasodilation from acetylcholine because of the presence of this direct vasoconstricting effect than to eliminate the vasodilation due to another agent, such as a nitrodilator, that does not have vasoconstrictor effects. This may then give the false impression of selectivity because the response to acetylcholine is affected to a disproportionately greater extent.

A second feature of the light-and-dye techniques that merits further exploration is the fact that the effect on endothelium-dependent dilation is very localized. Presumably, the inhibition of the generation and release of EDRF occurs in only the small area of the vessel that has been irradiated. It remains unexplained why EDRF from adjacent portions of the vessel, which have not been exposed to radiation, does not diffuse to the injured portion to induce dilation. It is well-known that EDRF is very diffusible.48 This suggests that, in addition to the inability of the endothelium to produce and release EDRF, there may be local generation of an agent that actively destroys EDRF. Rosenblum explored this possibility by retesting the responses of these injured vessels in mice after topical application of superoxide dismutase (SOD), catalase, or a combination of the two and found no restoration of the endothelium-dependent relaxation, showing that oxygen radicals are not involved in destroying EDRF (W.I. Rosenblum, personal communication). Another possibility is that light-and-dye treatment impairs the ability of the smooth muscle to respond to EDRF specifically. Although no direct evidence is available to exclude this possibility, it seems unlikely because:

1. no histological evidence of smooth muscle damage is noted by electron microscopy;37,38
2. the light-and-dye method inhibits the dilation from bradykinin, acetylcholine, and A23187 that dilate by different endothelium-dependent mechanisms.49 Thus, it is less likely that the light-and-dye treatment would selectively inhibit three separate EDRF-mediated processes and leave nitrodilator-induced dilation intact.37,50

Forstermann et al51 found that the intra-arterial administration of gossypol, a polyphenolic antioxidant, eliminated endothelium-dependent dilation in response to acetylcholine and substance P in the rabbit hind limb without affecting the dilation in response to prostaglandin E\(_2\) and nitroglycerin, which act directly on the vascular smooth muscle to induce dilation. There was no endothelial denudation by light microscopy. Therefore, it appears that, in this respect, gossypol behaves like the light-and-dye technique. The mechanism of its action has not been identified, and it is not known whether the abolition of endothelium-dependent dilation that it causes is due to damage to the endothelium with resultant inability to generate and release EDRF or whether it is due to the local production of an injurious agent that destroys EDRF.

The use of bioassay techniques is a powerful approach to the investigation of EDRF.25 This technique has been applied recently to the demonstration of EDRF in vivo in the cerebral microcirculation of the cat.48 The experimental preparation makes use of two symmetrically placed cranial windows, one of which is used as the donor and the other as the assay window. The assay window is subjected to muscarnic blockade with topical atropine to inhibit the direct effects of acetylcholine. EDRF production is induced by superfusion of acetylcholine-containing solution first through the donor window and in sequence through the assay window. The superfusion of the acetylcholine-containing fluid from the donor window is carried out either through a short route with a transit time of 6 seconds or through a long route with a transit time of 2 minutes. Under these conditions, it was shown that acetylcholine induces the generation

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and release from the donor window of a short-lived agent that does not survive a transit time of 2 minutes and that induces vasodilation in the assay window when cross-perfusion is carried out with a 6-second delay. The properties of this vasodilator are listed in Table 1. They are virtually identical to those of EDRF in vitro. The only difference is that SOD has no effect on EDRF in vivo, whereas it prolongs the half-life of EDRF in vitro. The reason for this difference is that there is no basal production of superoxide and other oxygen radicals in the in vivo preparation. The absence of an effect on SOD is, therefore, expected. For these reasons, this agent is believed to be the EDRF generated by acetylcholine in the cerebral microvessels.

It has recently been shown that an EDRF-like material can be released from cerebellar neurons by stimulation of glutaminergic receptors. This raises the possibility that one of the sources of the EDRF-like material released by acetylcholine in the bioassay experiments may be the neuronal elements. This is unlikely because the surface of the hemispheres contains glutaminergic neurons, which are not stimulated by acetylcholine. In fact, the administration of acetylcholine in unanesthetized animals equipped with cranial windows elicits no motor response and no detectable change in the behavior of the animal, showing that there is no neuronal activation (H.A. Kontos, unpublished observation). The vascular origin of the vasodilator material is also shown by the fact that interventions that have purely vascular effects, like acute hypertension, eliminate endothelium-dependent relaxation and inhibit the generation or release of the vasodilator identified in bioassay experiments.

A popular mechanism for inhibiting endothelium-dependent relaxation in vitro as well as in vivo is the generation of oxygen radicals. A variety of techniques have been used to generate the radicals: these include the use of xanthine oxidase, which generates both superoxide and hydrogen peroxide; arachidonate in high concentration, which generates superoxide via cyclooxygenase; methylene blue or hemoglobin, which generate superoxide by autoxidation; electric current, which generates superoxide; and various interventions such as acute hypertension, fluid-perfusion brain injury, and ischemia/reperfusion. Most of the studies have been done in the cerebral microcirculation. Two different mechanisms have been shown to be operative. The first involves direct injury to the endothelium with inability to generate and release EDRF. The second mechanism involves the destruction of EDRF by oxygen radicals after its release in the extracellular environment. The distinction between injury to the endothelium with inability to generate EDRF and the active destruction of EDRF in the extracellular environment is made based on the ability to rapidly restore endothelium-dependent relaxation by elimination of the radicals using specific scavengers such as SOD and catalase. It is reasoned that because these substances have high molecular weight and thus are not able to enter the intracellular environment of cells rapidly, if it can be shown that they can restore endothelium-dependent relaxation very rapidly after their application one must be dealing with an extracellular action. If, on the other hand, these agents are unable to restore endothelium-dependent relaxation, then one is more likely to be dealing with the inability to produce and release EDRF. Another possibility is that SOD and catalase might be unable to penetrate the subendothelial extracellular space.

We think this is less likely as SOD and catalase are probably working at the subendothelial extracellular space where EDRF is released in the cases where these agents do restore endothelium-dependent relaxation; also, there is no reason to think that H2O2 or arachidonate should decrease the ability of SOD and catalase to penetrate the subendothelial extracellular space.

Table 2 shows the various interventions that interfere with endothelium-dependent vasodilation in the cerebral circulation and identifies the mechanisms involved. Topical application of arachidonate in high concentration generates oxygen radicals via cyclooxygenase. After application of arachidonate, endothelium-dependent vasodilation from acetylcholine is absent, and bioassay shows the absence of transferable EDRF, although the tissue responds normally to EDRF from another source. In the presence of SOD plus catalase, EDRF continues to be absent, showing that the mechanism involved is inability to produce EDRF due to endothelial injury. Histologically demonstrable injury is present under these conditions in the form of blebs or craters in the endothelium. These are due to the effects of oxygen radicals because they are prevented if the tissue is...
pretreated with oxygen radical scavengers. In the case of methylene blue and hemoglobin, which produce oxygen radicals at a low rate by autoxidation, the vasodilator response to acetylcholine is absent, but it can readily be restored in the presence of SOD or catalase or deferoxamine. In some cases either mechanism may be involved depending on the concentration of the agent used. This is the case with hydrogen peroxide. After a short application of hydrogen peroxide at a concentration of 1 μM, the vasodilator response to acetylcholine is eliminated and no EDRF can be demonstrated in bioassay experiments. EDRF release at normal concentration is reestablished in the presence of SOD plus catalase. On the other hand, after application of 3 μM hydrogen peroxide, the vessels respond to acetylcholine with vasoconstriction, and EDRF is absent in bioassay experiments both in the absence as well as in the presence of SOD plus catalase.

There are several important considerations concerning the effect of oxygen radicals on endothelium-dependent relaxation. The first involves the nature of the radical that interacts with EDRF. This is important because it may disclose features that may help identify the chemical identity of EDRF. In vitro experiments, it is believed that superoxide interacts directly with EDRF. This is based on the demonstration that SOD protects EDRF, whereas catalase has little effect and direct scavengers of hydroxyl radical have none at all. On the other hand, in in vivo experiments, especially when agents such as methylene blue or hemoglobin that produce oxygen radicals at a low rate are used, EDRF is protected either by SOD, by catalase, or by deferoxamine, showing that the agent that destroys EDRF is probably the hydroxyl radical generated from the precursor agents, superoxide and hydrogen peroxide, in the presence of catalytic iron via the iron-catalyzed Haber–Weiss reaction. The hydroxyl radical, a powerful oxidizing agent, probably inactivates EDRF by direct chemical oxidation.

The reasons for these differences between in vivo and in vitro experiments have not been fully identified. However, the fact that catalase protects EDRF in in vitro experiments and the fact that iron destroys EDRF from cultured endothelial cells suggests that a simple interaction between superoxide and EDRF may not be a complete explanation. In addition, it should be borne in mind that superoxide in aqueous solution at physiological pH prefers to interact with itself in the dismutation reaction rather than react with other agents.

It is important to recognize that the mechanism of action of some of the agents that inhibit endothelium-dependent relaxation by generating radicals depends on some properties of the preparation used. Of particular importance is cellular permeability, which determines whether these agents are able to enter into vascular smooth muscle. In the cerebral microcirculation of the cat, under in vivo conditions, methylene blue and hemoglobin do not enter vascular smooth muscle. Therefore, it is possible in this preparation to show their effect on EDRF via the generation of oxygen radicals in the extracellular environment. If one increases the permeability of the vascular smooth muscle cell membrane by topical application of the detergent sodium dodecyl sulfate, then methylene blue and hemoglobin enter the intracellular environment of these cells and induce more widespread inhibition of responses by blocking guanylate cyclase (H.A. Kontos, unpublished observations). Under these conditions, the effect of the nitroagents is also inhibited, but the effect of adenosine, which acts via adenylyl cyclase, is unaffected. The action of methylene blue in vitro is different than in vivo in several studies. In vitro studies, methylene blue inhibits the dilation from both acetylcholine and nitroagents, although not with equivalent efficacy. Ignarro et al and Martin et al showed that higher concentrations or prolonged incubation times were necessary to partially inhibit the dilation from nitroglycerin or sodium nitroprusside, whereas acetylcholine-mediated dilation was easily abolished with lower doses of methylene blue and substantially less preincubation time. So, although the inhibition of vasodilation by methylene blue has usually been ascribed to the inhibition of the guanylate cyclase enzyme in in vitro studies, these data indicate that the actions of methylene blue in vitro are more complex. Indeed, Martin and Furchgott (Martin et al) suggested that this might be due, in part, to a direct action of methylene blue on EDRF. Also, Read and Dusting, using cultured endothelial cells as a source of EDRF, found that methylene blue and hemoglobin blocked the EDRF-mediated dilation from bradykinin but did not affect nitroglycerin-induced dilation. The simplest explanation for this differential action of methylene blue in vivo versus its effect in vitro is that smooth muscle permeability is high in some in vitro preparations and allows access by methylene blue to the cytosolic guanylate cyclase enzyme, whereas in vivo methylene blue is denied that access.

Finally, it should be borne in mind that because oxygen radicals interact not only with EDRF but also with other tissue components and are capable of inducing not only endothelial but also vascular smooth muscle damage, it is not uncommon, especially when the concentration of radicals is high, to see a variety of responses affected in addition to endothelium-dependent relaxation. For example, after acute hypertension or topical application of arachidonic acid in the cerebral microcirculation, the endothelium-dependent vasodilation due to acetylcholine is eliminated or converted to a small vasoconstriction. In addition, the vasodilation in response to topical nitroprusside, which acts directly on vascular smooth muscle, is also diminished, and the vasoconstrictor response to arterial hypoxia, which is not endothelium-dependent, is depressed. Also, oxygen radicals dilate cerebral microvessels, and the ensuing change in baseline caliber may affect
a number of responses in a nonspecific fashion. In such cases, the use of bioassay is much more informative than the study of pharmacological responses to the various vasoactive agents alone because bioassay experiments clearly identify whether there is any change in the amounts of EDRF produced.

**Chemical Nature of Endothelium-Derived Relaxing Factor In Vivo**

There have been very few in vivo studies investigating the chemical identity of EDRF. The major difficulty has been in obtaining cell-free fluid that contains EDRF from an in vivo vascular bed. This difficulty has been obviated recently by the demonstration that superoxide from the brain surface of cats can be obtained that has these characteristics; that is, it does not contain cells and has EDRF.48 Most of the studies discussed below come from a relatively small number of laboratories and thus some of these data await confirmation by other laboratories.

There are certain distinct advantages to studying the chemical identity of EDRF in vivo: 1) Although endothelial cells in culture are a popular source of EDRF, it should be kept in mind that these cells display evidence of dedifferentiation with alteration in enzymatic and receptor processes.75 For example, endothelial cells in culture typically do not have muscarinic receptors and do not respond to acetylcholine.12-20 In addition, these cells are phagocytic76 and have been shown to produce superoxide even under basal conditions.77-78 2) In most in vitro studies the prevailing oxygen tensions are much higher than those occurring under physiological conditions in vivo.76 This is probably an important reason why most in vitro vascular preparations used in studies of endothelium-dependent relaxation generate oxygen radicals.2,14,18,79 This is not the case in vivo.46,52 As noted above, oxygen radicals destroy EDRF and may also affect basal vascular tone.80 Oxygen radicals may interact with tissues and generate other vasoconstrictor substances that may confuse interpretation. Finally, exposure of endothelial cells to enzymatically generated superoxide induces the production of a stable EDRF that is not a prostaglandin.81 3) The permeability of endothelial82 and smooth muscle cells in vitro may be altered by the surgical preparation, by the use of bathing media devoid of serum proteins,82 or by oxygen radicals generated as a result of the conditions of the experiment.51 This may alter the mechanisms of action of some of the inhibitors used as pharmacological probes in the investigation of the nature of EDRF. This is, for example, the case with hemoglobin and with methylene blue.

As noted above, acetylcholine induces the generation and release of an EDRF from the cerebral microcirculation of cats that has properties very similar to those of EDRF induced by acetylcholine in vessels in vitro or by bradykinin in cultured endothelial cells. For these reasons, we investigated whether this EDRF is nitric oxide or a similar substance.

The following evidence suggested that the EDRF induced by acetylcholine in the cerebral microcirculation of the cat in vivo is not nitric oxide but a nitrosothiol: 1) Methylene blue and hemoglobin, which release oxygen radicals at a low rate in the extracellular fluid, destroyed EDRF from acetylcholine and eliminated its vasodilator action but did not affect the dilation from nitric oxide, from nitroprusside, or from nitroglycerin.64 This effect of methylene blue64 and hemoglobin58 was abolished either by SOD, by catalase, or by deferoxamine, suggesting that the mechanism involved is oxidation of EDRF by hydroxyl radical generated via the iron-catalyzed Haber–Weiss reaction. 2) Hydrogen peroxide23 in low concentration (1 μM) or nitro blue tetrazolium,64 which oxidize thiols in vascular smooth muscle, inhibited the dilation from nitroprusside and from nitroglycerin but not the dilation from l-cysteine-S-nitrosothiol (cysNO) or from EDRF from another source.23 This shows that nitric oxide or agents that generate nitric oxide require interaction with thiols in vascular smooth muscle to generate the final mediator of soluble guanylate cyclase activation. On the other hand, EDRF and CysNO activate soluble guanylate cyclase directly without the need for such interaction with tissue components.23 3) Agents that oxidize thiols in endothelial cells, such as arachidonate or hydrogen peroxide in high concentration (3 μM), stop the production of EDRF by acetylcholine, as demonstrated by bioassay experiments. These findings suggest that acetylcholine causes endothelium-dependent dilation by the scheme shown in Figure 3, which also illustrates the effect of inhibitors like hydrogen peroxide or nitro blue tetrazolium. According to this scheme, the EDRF from acetylcholine is a nitrosothiol that is generated via interaction of thiols with nitric oxide produced by acetylcholine. The nitrosothiol exits into the extracellular space and diffuses to the vascular smooth muscle where it activates guanylate cyclase directly. On the other hand, nitric oxide, or nitroditators that generate nitric oxide spontaneously or by interaction with tissue must first interact with the thiols in the vascular smooth muscle to generate the nitrosothiols, which are the direct activators of guanylate cyclase. Hydrogen peroxide eliminates endothelium-dependent relaxation from acetylcholine by two mechanisms. Because it is freely diffusible, it enters endothelial cells and oxidizes thiols. This does not allow the generation of EDRF from acetylcholine. Hydrogen peroxide also enters vascular smooth muscle where it oxidizes thiols and thereby inhibits the generation of nitrosothiols from nitric oxide–releasing agents. This effect eliminates the vasodilation from nitroditators but does not affect the dilation from EDRF from another source. Nitro blue tetrazolium has the same action as that of hydrogen peroxide on vascular smooth muscle.

Bradykinin causes endothelium-dependent dilation of cerebral arterioles by a different mechanism from that mediating the action of acetylcholine. The principal differences between the action of acetylcholine and bradykinin in the cerebral microcirculation of the cat are: 1) The vasodilation from bradykinin is completely
by indomethacin. 2) The dilation from bradykinin is partially inhibited by topical application of deferoxamine,83 which scavenges iron and prevents the formation of hydroxyl radical from the iron-catalyzed Haber-Weiss reaction.84 The dilation from acetylcholine is unaffected. 3) Unlike acetylcholine, in bioassay experiments bradykinin does not generate a transferrable dilator even when the transit time is as short as 6 seconds. This may be explained if the EDRF from bradykinin is a radical oxygen species generated by cyclooxygenase-mediated metabolism of arachidonic acid.63-87 In vitro, cyclooxygenase is capable of generating superoxide by a mechanism that involves interaction of nicotinamide adenine dinucleotide phosphate (NADPH) with an intermediate radical form of this interaction is the radical form of NAD or NADP. These readily interact with oxygen to produce superoxide. Bradykinin is well-known to accelerate arachidonate metabolism by activating phospholipases and releasing endogenous arachidonate.63 Kallikrein activates the same mechanism by generating bradykinin from its precursors. Topical application of bradykinin on the brain surface of cats generates superoxide.52 The entire mechanism is shown diagrammatically in Figure 4. Notably, the proposed mechanism of hydroxyl radical production by bradykinin has not been demonstrated directly by, for example, electron spin resonance techniques. However, the inhibitor data presented above strongly support the mechanism advanced.

Evidence by others shows that the same mechanism underlies the dilation from bradykinin in the cerebral microcirculation in mice,49 in rats, and in rabbits.69 Rosenblum found that in mice the dilation from bradykinin was inhibited by SOD, by catalase, or by deferoxamine, whereas that of acetylcholine was not affected.49 In Rosenblum’s experiments, the inhibition from deferoxamine was more complete than in the cerebral microcirculation of the cat, showing that the mediator was hydroxyl radical,
whereas in the cat, superoxide and hydrogen peroxide were also making a contribution. In the rabbit, indomethacin, as well as SOD and catalase, inhibits the vasodilator action of topical bradykinin. Similar findings have been obtained in the rat.  

Because bradykinin acts by releasing endogenous arachidonate, it is likely that the effect of exogenous arachidonate may be mediated by a mechanism similar to that of bradykinin. This, indeed, seems to be the case in the cerebral microcirculation of the cat where topical application of arachidonate causes dilation that is inhibited by indomethacin, by SOD plus catalase, and by deferoxamine. So far the available evidence suggests that the mediation of the vasodilator action of bradykinin by oxygen radicals is a unique feature of the cerebral microcirculation. In large vessels in vitro or in cultured endothelial cells, bradykinin releases an EDRF that is similar to that released by acetylcholine. This is also the case in the isolated basilar artery of the dog in vitro. 

The only other microvascular bed where the mechanism of action of bradykinin has been investigated is the microcirculation of the rat cremaster muscle. In this vascular bed the vasodilator effect of bradykinin is partially inhibited by indomethacin, but it is unaffected by SOD plus catalase, showing that radicals do not participate. Therefore, it appears that the vasodilator effect is in part mediated by prostaglandins. 

The residual dilation after blockade with indomethacin is blocked by methylene blue, showing that bradykinin in this vascular bed releases an EDRF similar to that of acetylcholine. Thus, the mechanism of action of bradykinin seems to be both species-dependent and vascular bed-dependent with at least three mechanisms identified: the generation of oxygen radicals, production of vasodilator prostaglandins, and release of an EDRF similar to that released by acetylcholine.

**Physiological Significance of Endothelium-Derived Relaxing Factor In Vivo**

The exact physiological roles of EDRFs are not known with certainty. However, several studies have suggested roles for EDRF in specific physiological and pathophysiological circumstances. We discuss some of these possibilities below.

**Basal Vessel Tone**

For EDRF to contribute to the basal tone of a vessel it obviously must be produced and released under tonic conditions. Most in vitro studies have indicated that there is basal secretion of EDRF. These results must be interpreted cautiously in light of certain features of the experimental models used and in light of divergent in vivo findings. In vitro experiments that have demonstrated basal EDRF secretion used 95% O2 to oxygenate the buffers used. This results in partial pressures of O2 in the 400 mm Hg range. Such hyperoxia is known to generate significant quantities of oxygen radicals that can alter physical properties of EDRF, such as its biologic half-life. Under hyperoxic conditions, the half-life of EDRF is markedly reduced when compared with a system in which the P02 is kept in the physiological range. The abbreviated half-life of EDRF in the hyperoxic system is due to destruction of EDRF by free radicals. Further complicating the matter, once superoxide is being generated in a system, it will spontaneously dismutate to H2O2. The presence of H2O2 in an experimental system complicates interpretation of the mechanisms of vasodilation because H2O2 is a potent vasodilator in vitro and in vivo. In vitro, hydrogen peroxide has been shown to be an endothelium-dependent dilator; thus, dissecting any "basal" secretion of EDRF from H2O2-induced dilation in a superoxide generating system is difficult. In vivo studies demonstrate that H2O2 is an endothelium-independent dilator. Specifically, Burke and Wolin have shown that the molecular species responsible for H2O2-mediated dilation in pulmonary artery segments in vivo is compound I of catalase, an intermediate radical that is generated when catalase reacts with H2O2. Regardless of the differences between in vivo and in vitro findings, the presence of H2O2 confounds any interpretation regarding basal secretion of EDRF in superoxide-producing systems. Furthermore, it has recently been shown that superoxide stimulates the release of a stable endothelium-dependent dilator different from the EDRF produced by acetylcholine. Finally, other investigators have been unable to demonstrate basal secretion of EDRF in vitro.

Contrary to most findings in vitro, several in vivo studies have been unable to demonstrate basal EDRF secretion. If tonic EDRF secretion was occurring in situ, any maneuver that inhibited its production, secretion, or action on the vascular smooth muscle should result in vasoconstriction. Therefore, the absence of basal EDRF secretion is supported in vivo when a putative inhibitor of EDRF or endothelial denudation fails to cause vasoconstriction. Pohl et al noted that endothelial denudation did not change the baseline caliber of the dog femoral artery. A similar result was obtained by Lamping et al. In the microcirculation of the dog hind limb, treatment with gossypol (an agent that selectively blocks EDRF-mediated dilation) had no effect on basal flow or pressure across that vascular bed. Using methylene blue as a putative EDRF inhibitor in vivo, Kaley and Wolin noted that the basal caliber of rat cremaster microvessels was not altered by topical methylene blue. We have noted a similar lack of vasoconstriction after topical application of methylene blue on the cerebral microvessels of the cat in situ. Furthermore, basal EDRF secretion is absent in bioassay experiments of the cerebral microcirculation. Recent studies using methylene blue in the human coronary circulation have also demonstrated a lack of basal secretion or production of EDRF.
Other investigators suggest that basal secretion of EDRF is present in vivo. In the rat cremaster muscle microcirculation in vivo, light-and-dye treatment eventually results in a small but significant decrease in vessel caliber. This has been interpreted as a loss of basally secreted endothelium-derived vasoactive substances. Koller and his colleagues demonstrated that this vasoconstriction is mostly dependent on a loss of basally secreted vasodilator prostaglandins. These light-and-dye results must be interpreted with care because the light-and-dye treatment evokes a complex vasomotor response in these vessels. After light-and-dye treatment, these vessels dilate to nearly 60% of their baseline caliber and eventually return to a diameter 12–13% less than baseline. The mechanisms of these changes caused by the light-and-dye technique have not been fully identified.

In vitro studies of the rabbit ear microcirculation also suggest the presence of basal secretion of an EDRF. These microangiographic studies of nonpulsatile microcirculatory flow indicate that first order "feed vessels" coordinate downstream perfusion. These authors also show that the vasomotor responses of these feed vessels have proportionally more dependence on basal EDRF secretion than smaller or larger arterioles, as demonstrated by greater constriction to intravascular hemoglobin, an EDRF antagonist.

Finally, evidence from the use of the specific inhibitor of the cellular synthesis of nitric oxide L-NMMA supports basal secretion of EDRF in vivo. In rabbits, systemic injection of L-NMMA resulted in increased mean arterial blood pressure in the resting state. Furthermore, intra-arterial infusion of L-NMMA into the forearms of healthy volunteers resulted in a 50% decrease in the basal forearm blood flow.

In summary, the evidence regarding basal EDRF secretion in vivo and in vitro preparations is conflicting. These differences may be due to the different experimental models used, and further investigation is needed to resolve these divergent findings.

Flow-Mediated Dilation

Flow-mediated dilation in conduit arteries was observed more than 50 years ago. It was believed for many years that this large vessel dilation was due to a retrograde signal from smaller downstream vessels until Lie et al disproved this theory with dissection experiments. Holtz et al determined that not only did vasodilator drugs produce femoral artery dilation through direct smooth muscle relaxation, but also via endothelium-dependent, flow-mediated dilation. Removal of endothelial cells from the femoral arteries of dogs instrumented with velocity probes and sonomicrometers abolished flow-mediated dilation and the dilation from acetylcholine. These observations were expanded when it was noted that femoral artery dilation during postocclusive hyperemia was an endothelium-dependent phenomenon. Furthermore, this endothelium-dependent, flow-mediated femoral artery dilation from either vasodilator drugs or postocclusive hyperemia could not be inhibited with cyclooxygenase inhibitors in vivo or in vitro. Also, although prostacycline synthesis is increased in response to increased flow, the inability of cyclooxygenase inhibitors to block the endothelium-dependent, flow-mediated dilation coupled with its blockade by methylene blue is compelling evidence that this phenomenon is due to EDRF and not due to prostacycline.

Similar results have been reported in the coronary circulation. Hintze and Vatner showed that the dilation after a 15-second coronary occlusion in conscious dogs could be eliminated by holding flow constant. This reactive dilation was not blocked by inhibitors of β-adrenergic and α-adrenergic receptors, ganglionic blockers, aminophylline or by cyclooxygenase inhibitors. The endothelium dependence of this flow-mediated dilation has been confirmed. It is noteworthy that flow-mediated dilation has also been demonstrated in the human coronary circulation in situ.

More recent studies have also demonstrated flow-mediated, endothelium-dependent dilation in individual microvessels of the rat cremaster muscle in vivo. After light-and-dye treatment of these microvessels, flow-mediated dilation was abolished. Others have also shown that flow-mediated dilation in rabbit ear arteries is partly endothelium dependent. However, in these myograph studies removal of the endothelium did not abolish the flow-mediated dilation. This suggests that a portion of the flow-mediated dilation is dependent on the media of the rabbit ear artery in vitro.

Flow-mediated, endothelium-dependent dilation may be important in the regulation of blood flow in response to physiological demands for increased blood flow by bringing about dilation and increased flow in those channels that perfuse tissue with the highest need for blood flow. It is important to realize that EDRF control or modulation of blood flow might well vary between different vascular beds. Differential responses of vascular beds to a single endothelium-dependent dilator may also serve to preferentially regulate blood flow to vital organs in stressful situations. For example, Vanhoutte et al suggested that vasopressin release during hypotension could mediate such a redistribution of blood flow. Because vasopressin constricts systemic arteries and dilates coronary and cerebral conduit arteries, this would serve to elevate systemic blood pressure and increase blood flow to the heart and brain.

Pathophysiological Significance of Endothelium-Derived Relaxing Factor In Vivo

An understanding of the possible physiological roles of EDRF and the interactions between EDRF and free radicals suggests a number of instances where an attenuation or lack of EDRF could cause or contribute to the vascular abnormalities in certain pathophysiological states. We discuss below three
such states: hypertension, both acute and chronic; atherosclerosis; and ischemia/reperfusion injury.

**Hypertension**

Chronic\(^{112}\) and acute\(^{87}\) hypertension are known to cause morphological changes in endothelial cells. Severe acute hypertension due to drug infusion\(^{46}\) or fluid-percussion brain injury\(^{60}\) damages the endothelium of cerebral microvessels with only minimal damage to the vascular smooth muscle.\(^{87}\) The vasoconstriction response of the pial arterioles to severe hypertension is one of prolonged vasodilation. This vasodilation is minimized by topical application of free radical scavengers. The mechanism of the free radical-mediated damage to cerebral microvessels has been well described.\(^{45}\) Briefly, physical perturbations like hypertension or topically applied chemicals that mimic the injury provoked by hypertension damage endothelial cells by inducing accelerated arachidonate metabolism that generates superoxide. Superoxide radicals then escape into the extracellular fluid through the anion channel.\(^{45}\) In addition to direct damage to cerebral microvessels, the free radicals produced by acute hypertension also have deleterious indirect effects on cerebral vasomotor tone, mediated by destruction of EDRF. Cerebral arteriolar dilation from acetylcholine was converted to constriction 30 minutes after the onset of severe hypertension (mean arterial blood pressure 190–200 mm Hg).\(^{46}\) Subsequent treatment with SOD and catalase partially restored the acetylcholine-mediated dilation suggesting that the free radical production from acute hypertension was destroying EDRF and unmasking the direct vasoconstrictor effect of acetylcholine on the vascular smooth muscle.\(^{46}\) Other experiments have confirmed that the hydroxyl radical destroys EDRF from acetylcholine in the cerebral microcirculation of the cat\(^{63,64,83}\) in situ.

In chronic hypertension, a number of in vitro studies have shown altered endothelium-dependent responses from conduit arteries and microvessels dissected from hypertensive animals. The endothelium-dependent responses of vessels from chronically hypertensive animals were attenuated, whereas endothelium-independent responses remained intact.\(^{114-117}\) Recently, Mayhan and his colleagues\(^{118}\) have shown that in situ endothelium-dependent dilation of pial arterioles by acetylcholine or methacholine in stroke-prone spontaneously hypertensive rats (SHRSP) is absent. In contrast, normotensive Wistar-Kyoto (WKY) rat pial arterioles dilated to acetylcholine and methacholine in a dose-dependent fashion. The responses to adenosine and nitroglycerin, two endothelium-independent dilators, were not different in SHRSP and WKY rats. This suggests that the endothelium-dependent ability of pial arterioles to modulate vasomotor tone is impaired by chronic hypertension. The nature of this impairment is not known with confidence, but Mayhan et al\(^{119}\) have shown that microvessels in SHRSP produce a prostanoid vasoconstrictor substance in response to adenine diphosphate and serotonin. Similar results by Luscher and Vanhoutte\(^{117}\) suggested that the generation of an endothelium-dependent constricting factor by endothelial cells from hypertensive rats diminishes the endothelium-dependent dilator responses.\(^{117}\) Still others have suggested that this impairment is due to the increased distance that EDRF must travel from the endothelial cells through thickened subendothelium and hypertrophied media in the hypertensive vessels as compared with a lesser distance in normotensive rats.\(^{116}\) Whether the free radicals generated in severe acute hypertension have any role in the loss of endothelium-dependent responses in chronic hypertension is not known. It is possible, for example, for acute transient alterations in blood pressure, which are known to occur in hypertensive animals and humans, to generate radicals and eliminate EDRF. It is notable that vessels from hypertensive animals are more prone to vasoconstriction under oxidant stress.\(^{120}\) Importantly, reduction in blood pressure with drugs in rats with salt-induced hypertension resulted in return of endothelium-dependent dilation in an ex vivo study.\(^{121}\) Further study is needed to determine the mechanism of impairment of endothelium-dependent dilation in chronic hypertension; such studies need to be extended to hypertensive patients.

**Atherosclerosis**

Atherosclerosis causes severe alterations in endothelial cells.\(^{116}\) It is also well-known that patients with atherosclerosis are prone to the acute ischemic syndromes of stroke, myocardial ischemia, and occlusive peripheral vascular disease. Vasospasm may play a major role in these types of clinical syndromes.\(^{123}\) Because the interaction between platelets and their by-products with the endothelium may cause vasoconstriction or vasodilation depending on the ability of the endothelium to generate and release EDRF, it is not surprising that the study of endothelium-dependent dilation in atherosclerosis has gained close attention.

Demonstration of altered endothelium-dependent responses in atherosclerotic animals has been reported by many investigators.\(^{114,124-126}\) Heistad and his colleagues\(^{125}\) studied the effects of hypercholesterolemia and atherosclerosis on the vasomotor responses of the in situ monkey hind limb to serotonin and norepinephrine. They found that serotonin dilated both resistance and conduit vessels in the normal and hypercholesterolemic monkey hind limbs. In contrast, serotonin constricted the resistance and the conduit vessels in the atherosclerotic monkey hind limb. The serotonin-induced constrictor responses were so pronounced and sustained in the large vessels that they could be classified as spasm. The constrictor responses to norepinephrine were not augmented in the atherosclerotic monkeys, but in the hypercholesterolemic monkeys, the constrictor response was augmented in the resistance vessels. Thus, atherosclerosis converted serotonin-induced
dilation to constriction and possibly spasm. Although a number of explanations for this response are possible, atherosclerosis could have damaged the endothelial cells rendering them incapable of generating EDRF. Serotonin dilates other arteries in an endothelium-dependent fashion. Expansion and clarification of these findings have been possible through ex vivo studies in which experimental changes were made in living animals, but the effects of those changes on vascular responses were studied in vitro. Ex vivo studies in primates confirmed that atherosclerosis but not hypercholesterolemia impaired endothelium-dependent dilation. Acetylcholine- and thrombin-mediated dilation in atherosclerotic iliac arteries was markedly impaired, but the endothelium-independent dilation to nitroglycerin was not different from that seen in control animals. Monkey jugular veins exposed to hypercholesterolemia but not afflicted with atherosclerosis had normal responses to acetylcholine. In a similar fashion, dog coronary arteries exposed to hypercholesterolemia, but studied before atherosclerosis had developed, demonstrated normal endothelium-dependent responses. Thus, altered vasomotor responses by atherosclerotic vessels might be attributed to the inability of the damaged endothelium to produce or secrete EDRF. Others, studying human coronary arteries in vitro, suggested another possible mechanism for the lack of activity of some endothelium-dependent agonists on atherosclerotic vessels. Henry (Bossaller et al) showed that the receptor-mediated dilation to acetylcholine was abolished but that non-receptor-mediated, endothelium-dependent dilation to the calcium ionophore A23187 remained intact in atherosclerotic vessels. They concluded it was a loss of muscarinic receptors on the atherosclerotic endothelial cells that caused the lack of acetylcholine-mediated, endothelium-dependent dilation. Harrison et al on the other hand, showed that ex vivo atherosclerotic monkey iliac arteries had reduced, but not absent, receptor-mediated, endothelium-dependent dilation from acetylcholine. Additionally, the endothelium-dependent dilation to the non–receptor-mediated agonist A23187 was impaired in the atherosclerotic vessels. Thus, there was impairment of both receptor-mediated and non–receptor-mediated, endothelium-dependent dilation. Harrison et al also found that the lack of endothelium-dependent dilation to acetylcholine was not due to endothelium-dependent constricting factor secretion or due to a direct constrictor effect of acetylcholine on the vascular smooth muscle. Forstmann’s findings were more compatible with the findings of Harrison. In vitro human coronary arteries he showed that both receptor and non–receptor-mediated, endothelium-dependent dilation was attenuated in atherosclerotic arteries. These differences could be reconciled if the EDRF from acetylcholine and other receptor agonists is different from the EDRF from the calcium ionophore, which does not use a specific receptor. Rosenblum et al have recently shown that this is the case in the cerebral microcirculation.

Interestingly, this endothelial defect from atherosclerosis has also been demonstrated in the microcirculation in vivo. Yamamoto et al studying the cremaster muscle of atherosclerotic and normal rabbits, found pronounced attenuation of acetylcholine-mediated responses in atherosclerotic rabbits, whereas responses to nitroprusside were unaffected. Thus, although atherosclerosis impairs endothelium-dependent responses, the nature of that impairment needs further investigation.

Ischemia/Reperfusion Injury

From in vitro studies we know that some of the products of platelet aggregation (e.g., serotonin and adenosine diphosphate) are endothelium-dependent dilators. When platelets aggregate and release their by-products adjacent to normal endothelium, EDRF-mediated dilation ensues and may flush the platelet nidus downstream where it is fragmented and destroyed. Thus, the intact endothelium can protect the vascular bed from catastrophic occlusion. If, however, platelets aggregate near dysfunctional endothelial cells, or areas where endothelial cells have been denuded, the products of platelet aggregation lead to unopposed smooth muscle contraction by direct action on smooth muscle cells. Thus, this elimination of the protective role of the endothelium may lead to exaggerated vasoconstriction and could potentiate thrombus formation. It is also well-known that reperfusion after ischemia produces oxygen radicals that may contribute to the tissue damage. In addition to direct tissue injury, these radicals may potentiate vasoconstriction, spasm, and thrombus formation by destroying EDRF. In the cerebral circulation of the cat, oxygen radicals have been shown to destroy EDRF. Furthermore, testing the hypothesis directly with a global ischemia reperfusion model, Wei has demonstrated that the dilation to acetylcholine was abolished after ischemia and reperfusion injury in vivo. Endothelium-independent dilation was unchanged before and after the ischemia reperfusion injury. Topical SOD plus catalase or deferoxamine preserved the endothelium-dependent dilation after the ischemia reperfusion injury. Mayhan et al have also shown that reperfusion after ischemia results in loss of acetylcholine-mediated dilation in the cerebral microcirculation of the cat. In the coronary circulation of the dog, Van-Benthuyzen et al showed in an ex vivo study that 60 minutes of ischemia followed by 60 minutes of reperfusion abolished acetylcholine-mediated dilation, whereas endothelium-independent dilation was unaltered by the injury. Thus, in these models in two different vascular beds, ischemia/reperfusion injury inhibits EDRF function. The exact consequences of the loss of EDRF in ischemia/reperfusion injury has not yet been separated from the many other pathological events occurring simultaneously. However, it certainly suggests that radical scavenging strategies...
may help preserve the protective role of the endothelium in this type of injury.

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