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Endothelium-Derived Relaxing and Contracting Factors

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Abstract Key discoveries in the past decade revealed that the endothelium can modulate the tone of underlying vascular smooth muscle by the synthesis/release of potent vasorelaxant (endothelium-derived relaxing factors; EDRF) and vasoconstrictor substances (endothelium-derived contracting factors; EDCF). It has become evident that the synthesis and release of these substances contribute to the multitude of physiological functions the vascular endothelium performs. Accumulating evidence suggests that at least one of the EDRFs is identical with nitric oxide (NO) or a labile nitroso compound, which is produced from L-arginine by an NADPH- and Ca^{2+}-dependent enzyme, arginine oxidase. The existence of more than one chemically distinct EDRF has been proposed, including an endothelium-derived hyperpolarizing factor (EDHF). The target of EDRF (NO) is soluble guanylate cyclase (increase in cyclic GMP) while EDHF appears to activate a K^+-channel in vascular smooth muscle. Recent data suggest that muscarinic receptor subtypes selectively mediate the release of EDRF(NO) (M_2) and EDHF (M_1). EDRF(NO) affects not only the underlying vascular smooth muscle, but also platelets, inhibiting their aggregation and adhesion to the endothelium. The antiaggregatory effect of EDRF is synergistic with prostacyclin, so their combined release may represent a physiological mechanism aimed at preventing thrombus formation. An additional proposed biological function of EDRF(NO) is cytoprotection by virtue of scavenging superoxide radicals. The endothelium can also mediate vasoconstriction by the release of a variety of endothelium-derived contracting factors (EDCF). Other than the unique peptide endothelin, the nature of EDCFs has not yet been firmly established. Autoregulation of cerebral and renal blood flow and hypoxic pulmonary vasoconstriction may represent the physiological role of endothelium-dependent vasoconstriction. Growing evidence indicates that the endothelium can serve as a unique mechanoreceptor, sensing and transducing physical stimuli (e.g., shear forces, pressure) into changes in vascular tone by the release of EDRFs or EDCFs. In physiological states, a delicate balance exists between endothelium-derived vasodilators and vasoconstrictors. Alterations in this balance can result in local (vasospasm) and generalized (hypertension) increase in vascular tone and also in facilitated thrombus formation. Endothelial dysfunction may also contribute to the pathophysiology of angiopathies associated with hypercholesterolemia and atherosclerosis.

Key words: atherosclerosis, EDHF, endothelial dysfunction, endothelin, flow, hypoxia, leukocytes, mechanoreception, muscarinic receptor subtypes, nitric oxide, platelets, pressure, S-nitroso-L-cysteine, thrombosis, vasospasm

It has been known for a long time that the endothelium has unique properties which prevent the formation of thrombi. The traditional view, that this important task is achieved because of the physical characteristics (smooth surface, physical barrier, etc.) of the endothelium has been changed dramatically in the past two decades. It is now well established that the endothelial cell plays an active role in a variety of physiological functions including maintenance of the fluidity of the blood, modulation of the tone of underlying vascular smooth muscle, inflammatory and immunological processes, etc.

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Studies in the past 10 to 15 years revealed that the endothelial cell is a storehouse of biologically active substances, which are involved in the multitude of functions the endothelium performs. The discovery that the endothelium synthesizes and releases potent vasoactive factors opened a new era in cardiovascular sciences. Although much remains to be done before the true importance of all the newly characterized factors will be understood, the present knowledge already allows speculation on some of the important physiological and pathological processes where endothelium-derived vasoactive factors may play a role. In addition to describing these factors this review will summarize some of their potential functions, including modulation of vascular tone, prevention of platelet aggregation and thrombus formation, and cytopro-

tection. The mechanosensor-function of the endothelium and its potential physiological importance as well as the pathological consequences of endothelial dysfunction will also be reviewed.

ENDOTHELIUM-DERIVED VASOACTIVE FACTORS: MODULATION OF VASCULAR TONE BY THE ENDOTHELIUM

Endothelium-Derived Relaxing Factors

The first endothelial vasoactive substance was discovered in the late '70s, when it was demonstrated human vascular endothelial cells synthesize prostacyclin, a potent vasorelaxant and platelet inhibitory metabolite of arachidonic acid [1] (Fig. 1).

After the cornerstone observation of Furchgott and Zawadzki in 1980 [2] that the presence of endothelium is obligatory for acetylcholine-induced vasorelaxation, bioassay studies demonstrated that a very labile, diffusible, non-prostanoid substance(s) (called endothelium-derived relaxing factor, or EDRF) mediates endothelium-dependent vasorelaxation [3] (Fig. 1).

EDRF is released under basal conditions and upon stimulation [4]. Among the many physiologic stimuli that can elicit the release of EDRF are platelet products, thrombin, hormones, neurotransmitters, local autacoids, changes in oxygen tension, and increase in flow (shear stress) [4].

Nature of EDRF. The discovery that similar to nitric oxide (NO) the effect of EDRF is mediated by an increase in smooth muscle cyclic GMP content [5], and hemoglobin [6] and superoxide anions [7] inactivate both NO and EDRF, it was proposed that EDRF and NO are the same [8,9]. The demonstration of NO production by endothelial cells provided more direct evidence for this hypothesis [10].

However, several findings argue against the concept that EDRF is identical with NO. Nitric oxide and EDRF are differentially retained by

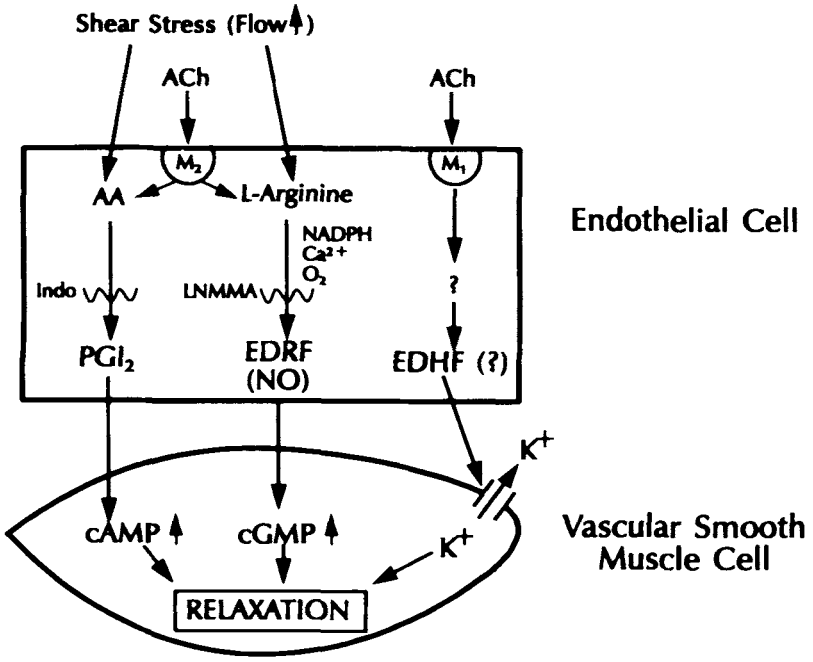

Fig. 1. Endothelium-derived relaxing factors and their effect on vascular smooth muscle. Prostacyclin (PGI_2) is a vasodilator metabolite of arachidonic acid (AA) which stimulates smooth muscle adenylate cyclase and elevates cyclic AMP (cAMP). Inhibitors of cyclooxygenase (e.g., indomethacin; Indo) prevent the synthesis/release of PGI_2. Endothelium-derived relaxing factor (EDRF) is synthesized from L-arginine in endothelial cells via an enzyme (arginine-oxidase) requiring NADPH, oxygen (O_2), and calcium ions (Ca^{2+}). Arginine analogues (e.g., LNMMA) inhibit the synthesis of EDRF. EDRF may be identical with nitric oxide (NO) or with a labile nitroso compound (R-NO). EDRF(NO) stimulates soluble guanylate cyclase and elevates cyclic GMP (cGMP) level in vascular smooth muscle. Endothelial cells also release a putative hyperpolarizing factor (EDHF), which probably acts via activation of a potassium (K^+) channel. The nature of EDHF is not known yet. Acetylcholine (ACh) (by acting on M_2-muscarinic receptor) and shear forces stimulate the synthesis/release of PGI_2 and EDRF(NO). Activation of M_1-muscarinic receptors stimulates synthesis/release of EDHF.

anionic exchange columns [11] and EDRF (but not NO) shows striking stability during chromatography and lyophilization [12]. Neither the spectrophotometric method nor the chemiluminescence method (using an acidic reducing reflux chamber) used for the detection of NO can distinguish it from nitrite or labile nitroso species; thus neither method can determine whether EDRF was a labile precursor of NO or free NO itself. Using a modified chemiluminescence technique (without reflux chamber) in which NO is directly measured in the effluent, it was found that the concentration of NO generated by endothelial cells could not account for the relaxation observed in bioassay preparations [13].

Analyzing whether EDRF and NO are the same, we found that when solutions of EDRF and NO with similar relaxing activity were passed through a hemoglobin-agarose column, only the authentic NO solution gives an NO-Hb electron paramagnetic resonance (EPR) signal [14]. This important difference between free NO and EDRF was substantiated by recent observations using the EPR technique. No NO-Hb formation was found in the medium of cultured endothelial cells stimulated by various agonists (Dr. M. Peach, personal communication). EDRF released from perfused canine carotid arteries by acetylcholine could not be detected as a paramagnetic dinitrosyl ion complex by EPR [15]. These findings could be explained if EDRF released from endothelial cells is a nitric oxide containing substance (and not free NO) which does not produce detectable (by EPR) NO-Hb at a concentration which has the same bioactivity (i.e., vasorelaxation) as free NO.

A possible candidate for such substance is a nitrosothiol. The most commonly available thiol compounds in mammalian tissues are cysteine and cystine and the nitrosothiol formed by the reaction of cysteine and NO is S-nitroso-L-cysteine (cysNO). This hypothesis has been supported by recent findings showing that cysNO but not NO shows similarities to EDRF [16].

We also tested whether, in contrast to NO, cysNO behaves similar to EDRF in the EPR test (i.e., no detectable NO-Hb at concentrations of cysNO which evoke vasorelaxation) [17]. Although authentic NO and cysNO relaxed canine coronary artery rings with similar potency, the lower detection limit by EPR of cysNO was more than two orders of magnitude higher than that of NO. At concentrations which cause vasorelaxation similar to EDRF, only NO but not cysNO could be detected as NO-Hb by EPR. These findings suggest that cysNO (or other S-nitrosothiols with similar characteristics), but not free NO, is likely to be the EDRF released by ACh from the endothelium of canine femoral arteries. This possibility is further supported by the demonstration that bradykinin increased the efflux of ^{35}S from cultured bovine pulmonary endothelial cells preincubated with ^{35}S-cysteine [17].

L-arginine is the precursor of EDRF. Although it is still uncertain whether EDRF is free NO or a labile nitroso compound from which NO is liberated (or both), it is widely accepted that L-arginine is the substrate required for formation of NO and EDRF (Fig. 1).

Vascular endothelial cells in culture synthesize NO from the terminal guanido nitrogen atom(s) of L-arginine [18]. This reaction is specific, since other analogs of L-arginine, including its D-enantiomer, are not substrates. Furthermore, N^G-monomethyl-L-arginine (L-NMMA) inhibits the reaction in a dose-dependent and enantiomerically specific manner. This inhibition can be reversed by L-arginine in a way which suggests a competition between the inhibitor and the substrate. Using homogenates of porcine vascular endothelial cells, the enzyme responsible for this reaction was partially characterized: it is NADPH-dependent, requires a divalent cation, and forms L-citrulline as a coproduct [19].

Endothelium-derived hyperpolarizing factor. In addition to the "classical" EDRF (NO), the existence of other, chemically different, nonprostanoid relaxing mediators [20], including an endothelium-derived hyperpolarizing factor (EDHF) [21] was proposed (Fig. 1).

In a bioassay system using perfused segments of canine femoral arteries (donor) and superfused canine coronary artery rings (acceptor) acetylcholine caused a biphasic release of EDRF [3]. Catecholamines affected the two phases differently: they prevented the secondary sustained response but only moderately depressed the initial transient phase. In contrast, treatment of the endothelium in the perfused segment with quinacrine (a phospholipase A_2 inhibitor) prevented the first transient phase but had no effect on the later sustained relaxation. Subsequent bioassay experiments demonstrated that three different inhibitors of the metabolism of arachidonic acid (quinacrine, NDGA, *metyrapone*) selectively prevented the first but not the second phase of the biphasic concentration-

relaxation curve to acetylcholine [20]. These findings suggested the existence of two chemically different endothelium-derived relaxing factors.

In similar bioassay experiments low concentrations of acetylcholine caused only transient relaxations while higher concentrations of acetylcholine caused sustained decreases in tension [22]. Atropine (non-selective muscarinic receptor antagonist) inhibited the two phases of the biphasic concentration-relaxation curve with similar potencies. Pirenzepine (selective antagonist of M_1-muscarinic receptors) inhibited both phases in a competitive manner but exhibited significantly higher potency against the first phase. Compound McN-A-343 (selective M_1-muscarinic receptor agonist) induced only transient relaxations, whereas carbachol (selective M_2-muscarinic receptor agonist) caused sustained relaxations. These findings suggested the existence of two muscarinic receptor subtypes (M_1 and M_2) on vascular endothelium, which may mediate the release of different EDRFs.

This suggestion was confirmed by electrophysiological experiments, showing that parallel with sustained vasorelaxation, acetylcholine evokes transient hyperpolarization of rabbit saphenous artery smooth muscle cells [23]. Oxotremorine (M_2-agonist) caused vasorelaxation, but did not alter the membrane potential. Atropine prevented both responses with similar potency, but pirenzipine inhibited the transient hyperpolarization with higher potency than the relaxations. It was concluded, that similar to canine femoral arteries [22], two types of muscarinic receptors are located on endothelial cells of the rabbit saphenous artery, and stimulation of either receptor would release a different endothelium-derived relaxing substance: stimulation of the M_1-receptor subtype triggers the release of EDHF (causing relaxation by activating a K^+-channel), and that of the M_2-receptor subtype would release EDRF(NO) (causing relaxation via cyclic GMP) [24].

Chen et al. [21] postulated the existence of an EDHF by demonstrating that inhibitors of EDRF, such as oxyhemoglobin and methylene blue inhibited vasorelaxation by ACh, but did not alter the endothelium-dependent smooth muscle hyperpolarization.

The different sensitivity of the mechanical and electrophysiological responses to oxyhemoglobin and methylene blue indicates that the hyperpolarizing factor must be chemically different from EDRF. However, the nature of this putative substance(s), EDHF, is still unknown.

Endothelium-Derived Contracting Factors

The observation that removal of the endothelium from canine femoral arteries significantly reduced the contractions evoked by several agonists and hypoxia was the first indication that the endothelium not only mediates relaxation of the underlying vascular smooth muscle but can also facilitate smooth muscle contraction [25]. A subsequent bioassay study provided the first evidence for the existence of a diffusible endothelium-derived contracting factor released from canine coronary and femoral arteries during hypoxia [26]. Ample experimental evidence, accumulated over the past years, has clearly established the existence of endothelium-dependent vasoconstriction mediated by a variety of endothelium-derived contracting factor(s) (EDCF) [27] (Fig. 2).

Endothelium-dependent vasoconstriction can be stimulated by naturally occurring substances (e.g., acetylcholine, arachidonic acid, norepinephrine, prostaglandin H_2, thrombin), pharmacological agents (e.g., calcium ionophores, nicotine, high K^+), physical forces (stretch, pressure), and hypoxia [27].

The endothelium-derived contracting factor(s) mediating endothelium-dependent vasoconstriction belong to three major categories: vasoconstrictor metabolites of arachidonic acid or free radicals (superoxide anion was postulated to be one of the EDCFs) ($EDCF_1$); the EDCF released by severe hypoxia, the nature of which has not been identified yet ($EDCF_2$); and a potent peptidergic vasoconstrictor substance produced by endothelial cells in culture ($EDCF_3$) which in contrast to the prompt and reversible contraction triggered by the other EDCFs, causes slowly developing, and long-lasting vasoconstriction [28].

The mechanism of vasoconstriction by EDCFs involves facilization of Ca^{2+}-influx and stimulation of phosphoinositol turnover ($EDCF_2$ and $EDCF_3$) in vascular smooth muscle [27] (Figure 2). The exact mechanism of $EDCF_1$-induced vascular contraction remains to be determined.

Endothelin. The potent peptidergic vasoconstrictor ($EDCF_3$) has been recently isolated and identified as endothelin, a unique polypeptide containing 21 amino acids [29].

Endothelin is produced by cultured endothelial cells under basal conditions and in response

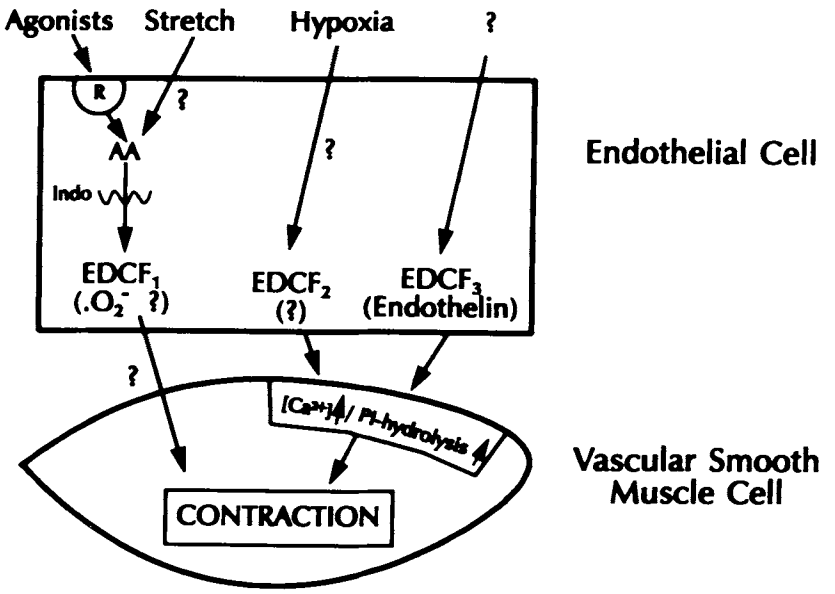

Fig. 2. Endothelium-derived contracting factors (EDCFs) that mediate endothelium-dependent vasoconstriction. There are at least three different EDCFs. The release of one can be inhibited by blockers of cyclooxygenase (e.g., indomethacin; Indo), which is presumably a metabolite of arachidonic acid (AA) or a free radical, like superoxide anion ($EDCF_1$). The second EDCF is released by severe hypoxia ($EDCF_2$), which is indomethacin insensitive. The nature of this factor is not known yet. Endothelial cells also synthesize and release a very potent peptidergic vasoconstrictor ($EDCF_3$), which was recently identified as the 21 amino acid peptide, endothelin. It is still uncertain which stimuli activate its release in vivo. $EDCF_2$ and endothelin facilitate Ca^{2+}-influx and stimulate phosphatydilinositol (PI) hydrolysis in vascular smooth muscle. The mechanism of action of $EDCF_1$ is unknown (?).

to a variety of stimuli (e.g., thrombin) in a constitutive manner; it can be detected in the culture medium only several hours after stimulation. Because of its high vasoconstrictor potency and long-lasting action, endothelin is a potential candidate to trigger and maintain vasospastic episodes. The interested reader is referred to a recent review [30] which summarizes the progress made in the past two years with this new peptide family.

ENDOTHELIUM AND PLATELETS: PROTECTION AGAINST PLATELET-INDUCED VASOSPASM AND PLATELET AGGREGATION

One of the primary functions of vascular endothelium is to maintain the normal fluidity of blood. Among other mechanisms, this is achieved by the synthesis and release of anticoagulant (e.g., thrombomodulin) fibrinolytic (e.g., t-PA), and platelet inhibitory (e.g., prostacyclin, EDRF) substances.

Prostacyclin is one of the most potent inhibitors of platelet aggregation [1]. In addition to vasorelaxation, EDRF(NO) inhibits platelet aggregation [31] and platelet adhesion [32] in vitro.

Although they have different mechanisms of action, EDRF and prostacyclin can interact synergistically to inhibit platelet aggregation. EDRF acts by elevation of cyclic GMP, whereas prostacyclin causes the elevation of cyclic AMP. The antiaggregating activity of both EDRF and authentic NO is potentiated by subthreshold concentrations of prostacyclin. Similarly, subthreshold concentrations of NO and EDRF potentiate the antiaggregating activity of prostacyclin [32].

Aggregating platelets cause contraction in rings of coronary artery isolated from dog, pig, and human hearts [33,34]. These contractions are enhanced if the endothelium had been removed. If the rings were first contracted, exposure to aggregating platelets caused relaxation in rings with endothelium, but further contraction was observed in rings without endothelium [33,34].

Similar to several vasoactive substances, platelet-induced endothelium-dependent relaxation is mediated by EDRF (i.e., methylene blue and hemoglobin prevent the relaxations) *and not by* prostacyclin (i.e., inhibition of cyclooxygenase had no effect on the response) [34]. Thus, aggregating platelets release substances that cause endothelium-independent vasoconstriction *and* in parallel trigger endothelium-dependent inhibition of smooth muscle contraction. It has been

proposed that adenosine diphosphate (ADP) and serotonin (5-HT) are the main platelet products which trigger the synthesis release of EDRF [32].

ENDOTHELIUM AND POLYMORPHONUCLEAR LEUKOCYTES (PMN): CYTOPROTECTIVE FUNCTION OF ENDOTHELIUM-DERIVED RELAXING FACTORS

Release of superoxide anion radical from activated PMNs contributes to the pathogenesis of various cardiovascular diseases [35]. It has been postulated that one of the biological actions of EDRF(NO) may be to provide a protective chemical barrier against cytotoxic free radicals. This hypothesis is based on the facts that superoxide anion interacts with EDRF and inactivates it [7] and that the oxidation potential of NO (believed to be identical with one of the EDRFs) makes it able to scavenge superoxide anion [36].

This hypothesis was tested experimentally using human PMNs activated by fMLP [37]. Authentic NO depressed the reduction of cytochrome *c* evoked by superoxide anion released from PMNs. The activity was concentration-dependent and occurred at dilutions of a saturated solution of NO that caused relaxation of isolated canine coronary arteries and inhibited aggregation of human platelets. The ability of NO to suppress reduction of cytochrome *c* by superoxide radicals generated by the xanthine oxidase + hypoxanthine reaction suggested that the most likely explanation for the observed phenomenon is scavenging (inactivating) of superoxide by anion NO [37]. In contrast, prostacyclin may act as inhibitor of adhesion of PMNs to the blood vessel wall (margination) [38]. These findings support the hypothesis that one important biological function of EDRF(NO) may be to provide a chemical barrier to cytotoxic free radicals (e.g., superoxide anion), and may act synergistically with prostacyclin to prevent tissue damage by activated PMNs.

MECHANORECEPTION BY THE ENDOTHELIUM

It has long been recognized that blood vessels have the ability to sense changes in mechanical forces: rapid elevation of intraluminal pressure triggers vasoconstriction, which contributes to the autoregulation of blood flow in certain organs. The site of mechanoreception in the vessel wall was thought to be the vascular smooth muscle. Recent studies have shown that the endothelium may also be the site of mechanoreception; its presence is essential to observe changes in vascular smooth muscle tone in several blood vessels evoked by increases in flow/shear stress [39,40] and stretch/pressure [41–43] (Figure 3).

Flow-Induced Endothelium-Dependent Vasodilatation

Increases in flow rate dilate large conduit arteries in vivo, which is dependent on functional endothelium [39]. Bioassay studies revealed that increases in mean flow rate or introduction of pulsatile flow stimulates the release of EDRF and prostacyclin from the endothelium of perfused canine arteries [40].

It has been shown that the response of cultured endothelial cells to shear stress is opposite to that proposed for pressure, that is, membrane depolarization (see below), since shear stress causes membrane hyperpolarization and activation of the inward rectifying potassium channel of the endothelial cell membrane [44]. Opposing interactions between pressure and shear stress on the release of EDRF from canine carotid arteries was also reported [43] (Figure 3).

Stretch- and Pressure-Induced Endothelium-Dependent Vasoconstriction

Rapid stretch of isolated rings of canine basilar arteries evokes contraction which can be prevented by removal of the endothelium and significantly reduced by indomethacin [41]. Thus, the endothelium-dependent contractions to stretch in canine basilar arteries must be mediated, at least in part, by vasoconstrictor cyclooxygenase products released from endothelial cells (Figure 3).

Elevation of intraluminal pressure in a perfused segment of canine carotid artery evokes vasoconstriction which also can be prevented by removal of the endothelium [43]. In contrast to stretch-induced responses in rings of basilar artery, the endothelium-mediated contractions of perfused canine carotid artery segments in response to rapid increases in transmural pressure were not affected by indomethacin [43]. This then suggests that the contractile factor(s) produced in endothelial cells in response to stretch may be different in blood vessels from different anatomical origin. In bioassay studies, pressure-induced contraction of the donor carotid artery segment was accompanied by contraction of an artery ring, superfused by the effluent from the donor segment, indicating that

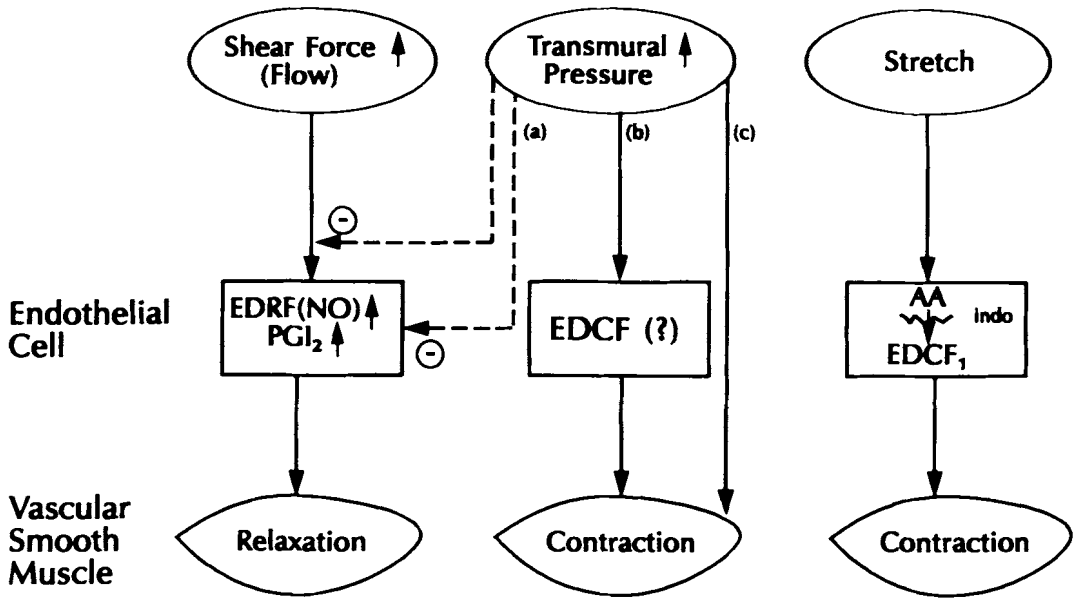

Fig. 3. Mechanoreception by the endothelium. The endothelial cell (EC) serves as a unique sensor of shear forces (left), transmural pressure (middle), and rapid stretch (right). By virtue of releasing vasoactive substances, EC can transduce changes in these physical forces into changes in vascular tone. Shear force (increases in blood flow) stimulates the release of PGI_2 and EDRF(NO) and causes vasodilation (see also Figure 1). Increase in transmural pressure evokes vasoconstriction via suppression of the release EDRF (e.g., canine carotid artery), facilitation of the release of a still unidentified EDCF (e.g., feline cerebral artery), and direct activation of smooth muscle. Rapid stretch of canine basilar arteries causes endothelium-dependent contraction by the release of a cyclooxygenase product of arachidonic acid (AA) ($EDCF_1$; see also Figure 2).

the phenomenon is mediated by a diffusible endothelium-derived vasoactive factor(s). Since methylene blue prevented the contractions, it was postulated that the cause of the phenomenon in this preparation is pressure-induced suppression of the release of EDRF [43] (Figure 3).

Elevation of intraluminal pressure in isolated segments of cat cerebral arteries depolarizes the vascular smooth muscle cells and the internal diameter of the vessel is either maintained or reduced [42]. When the endothelial lining of cerebral vessels is disrupted, the vessels dilate as transmural pressure is elevated and membrane potential does not change [42]. Bioassay studies demonstrated that pressure-induced endothelium-dependent contraction and membrane depolarization in feline cerebral arteries is mediated by diffusible factor(s) [45], the nature of which remains to be determined (Figure 3).

These observations suggest that the endothelium may serve as a unique mechanosensor of flow rate and pressure. Increases in flow rate (shear stress) stimulate the synthesis/release of EDRF in endothelial cells. Although the cellular mechanisms remain to be determined, this unique function of the endothelium may contribute to the local adjustment of vascular tone under various conditions, (i.e., negative feedback regulation of shear forces acting on endothelial cells). Several evidences suggest that active contraction of vascular smooth muscle in response to increases in pressure is mediated by the endothelium. Pressure-induced vasoconstriction may be mediated by reduced release of EDRF and/or EDHF, by facilitated release of endothelium-derived constrictor factors (EDCF), and by direct action on vascular smooth muscle cells (Figure 3). Thus the endothelium can serve as a pressure-transducer and may mediate or contribute to the "myogenic" response, originally thought to be of smooth muscle origin. This recently revealed regulatory mechanism may play a role in autoregulation of blood flow in the cerebral and renal vascular beds.

ENDOTHELIAL DYSFUNCTION

Endothelial dysfunction (characterized by an imbalance between the production of prostacyclin and thromboxane A_2, EDRFs and EDCFs, and pro- and anticoagulation factors) could lead to hyperreactivity of underlying vascular smooth

muscle and pathologically elevated vascular tone (vasospasm), and to activation, adhesion, and aggregation of platelets (thrombosis) (Figure 4). Some of these potential consequences have been demonstrated experimentally in a variety of animal models of endothelial injury and dysfunction and also in human coronary artery disease. A brief summary of findings in atherosclerosis will illustrate the existence and importance of this "syndrome."

Impairment of Endothelium-Dependent Vasodilation in Atherosclerosis and Hypercholesterolemia

Chronic feeding of cholesterol-enriched diet to certain animals leads to atherosclerotic lesions, similar to those observed in the human disease. Diet-induced experimental atherosclerosis impairs endothelium-dependent vasorelaxation [46], but generally leaves endothelium-independent relaxations intact. Similar to these animal experiments, atherosclerotic human coronary arteries (both in vitro and in vivo) show impaired endothelium-dependent dilation to intracoronary infusion of acetylcholine, but not to nitroglycerine [47]. Hypercholesterolemia was found to be one of the most important factors which predicts endothelial dysfunction in human coronary arteries [47].

Several possible mechanisms were proposed, including reduced synthesis of EDRF(s), altered membrane receptor coupling mechanisms af-

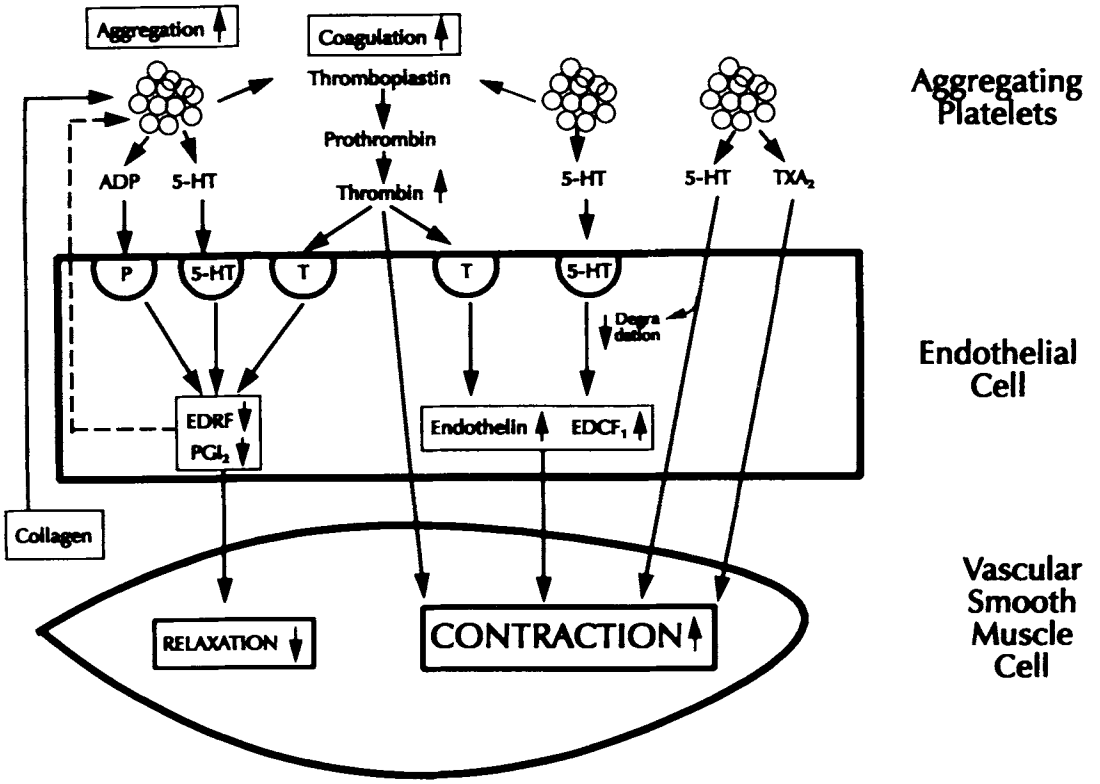

Fig. 4. Endothelial dysfunction and some of its pathological consequences. The protective functions of the endothelium (i.e., inhibition of platelet aggregation and platelet-induced vasoconstriction, suppression of vascular reactivity, etc.) are depressed or lost in pathological conditions (e.g., endothelial injury after angioplasty, hypercholesterolemia, and atherosclerosis). Dysfunctional endothelial cells loose their ability to synthesize/release prostacyclin (PGI_2) and EDRF (left), and to metabolically degrade vasoconstrictor substances (e.g., 5-HT). However, the synthesis/release of EDCFs is maintained or even enhanced in injured cells (middle). Platelet adhesion and aggregation is stimulated by contact with collagen in the damaged vessel wall, which is unopposed because of the lack of PGI_2 and EDRF (left). The predominance of proaggregant and procoagulant stimuli will lead to the formation of platelet thrombi, and the continuous formation by and release from platelets of thrombin, serotonin (5-HT), and thromboxane A_2 (TXA_2) can cause vasoconstriction by acting directly on vascular smooth muscle (right) and by facilitating the release of EDCFs (middle) from endothelial cells. Thus vasospasm and thrombosis may occur at sites of endothelial dysfunction where the protection by EDRF(s) is reduced or lost.

fecting synthesis of EDRF(s), and/or impaired diffusion or augmented destruction of EDRF(s) in the intima. Alternatively endothelium-dependent relaxation can be reduced by the concomitant augmented synthesis/release or action of endothelium-derived contracting factor(s). Thus there are a variety of mechanisms (probably acting in concert in a complex manner in the different animal models and in the human disease) which can contribute to impaired endothelium-dependent vasodilation.

Consequences of Endothelial Dysfunction in Coronary Artery Disease

Clinical and experimental observations suggest that atherosclerosis alters vascular reactivity. Coronary vessels with atherosclerosis are more susceptible to spasm induced by ergonovine and may be predisposed to spontaneous coronary vasospasm [47].

It seems likely that persistent coronary occlusion can result from and involve a number of critical vascular reactions. These include (1) local hyperreactivity of smooth muscle resulting from supersensitivity to constrictive stimuli or the loss of endothelium-dependent relaxation mechanisms, and (2) the presence of a thrombotic tendency due to hyperaggregable platelets and/or activated coagulation factors in a local environment where normal inhibitory mechanisms have been diminished or lost (Figure 4).

FUTURE PROSPECTS

The discovery of endothelium-derived vasoactive factors in the last decade and their potential contribution to many bodily functions in health and disease revolutionized cardiovascular sciences similar to the discovery of the control by autonomic nerves of the circulation almost a century ago. Although major progress has been made in the past years, several key questions need to be clarified in the future. For example, it remains to be determined whether the "classical" EDRF is identical with free NO or with a labile NO precursor, such as a nitrosothiol. Characterization of the endothelial metabolic pathway(s) leading to the synthesis of EDRF(NO) (e.g., arginine oxidase) and endothelin (e.g., endothelin converting enzyme) will be a key to designing substances that modify their function. The variety of functions described (vasorelaxation, antiplatelet activity, cytoprotection) and the fact that the L-arginine:NO pathway exists in a variety of cell types (e.g., PMNs, monocytes, brain cells, Kupfer cells, mesangial cells) suggest a much wider biological importance of this mediator than originally predicted. Experimental analysis of this possibility is an important task for future research. Identification of the chemical nature and mechanism of action of the EDRF(s), including EDHF, appears to be an exciting and important project. The newly discovered mechanosensor property of endothelial cells and the involvement of EDRFs and EDCFs in signal transduction opened new vistas for the understanding of "old" physiological phenomenon, such as the reactive (flow-induced) dilation of conduit arteries and the "myogenic" mechanism of autoregulation of blood flow. Finally, better characterization of the new syndrome of endothelial dysfunction will, without any doubt, contribute to unraveling some of the still remaining mysteries of many vascular disorders (e.g., atherosclerosis) and will enable the design of novel therapies for such diseases. Naturally, this represents only a partial list of topics for future research. It is the conviction of the author that scientists who will become or remain active in this exciting field will find satisfaction in their results, not only because of their theoretical interest, but more importantly because of their practical significance.

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