Endothelial Dysfunction in Hypertension

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The endothelium modulates the tone of the underlying vascular smooth muscle by releasing relaxing factors, including prostacyclin, nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF). In most types of hypertension, endothelium-dependent relaxations are impaired because of a reduced production and/or action of endothelium-derived NO and EDHF. In essential hypertension, endothelium-dependent relaxations are reduced because of a concomitant release of vasoconstrictor prostanoids (endoperoxides and thromboxane A2). These prostanoids may be produced in the vascular smooth muscle rather than in the endothelium. The endothelial dysfunction observed in hypertension is likely to be a consequence rather than a cause of the disease, representing premature aging of the blood vessels due to the chronic exposure to the high blood pressure. The endothelial dysfunction can be improved by antihypertensive therapy, favoring the prevention of the occurrence of vascular complications in hypertension. J Atheroscler Thromb, 1998; 4: 118-127.

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The endothelium is a monolayer of squamous cells in direct contact with the blood. Due to this anatomical position, it is a primary target for injuries such as hypertension, hyperlipidemia, diabetes mellitus and ischemia (1, 2). Since the demonstration by Furchgott and Zawadzki of the obligatory role of the endothelium in the relaxation to acetylcholine in the isolated rabbit aorta (3), it has become obvious that the endothelium modulates the degree of constriction of the underlying vascular smooth muscle. It does so by liberating several relaxing and contracting substances (Fig. 1).

The endothelium-derived relaxing factors (EDRFs) include, beside prostacyclin (PGI2), (a) nitric oxide (NO), or closely related compound, derived from L-arginine through the action of endothelial NO synthase (eNOS) (4), and (b) endothelium-derived hyperpolarizing factor (EDHF) (5). The release of EDRF/NO by endothelial cells can be triggered by activation of cell membrane receptors, which are linked to eNOS by G proteins that differ depending on the agonists used (1, 6, 7) (Fig. 2). Endothelium-derived NO (EDNO) inhibits not only constriction of the underlying vascular smooth muscle cells but also their proliferation, platelet aggregation, and adherence of blood cells (platelets and white blood cells) to the endothelium (1, 2) (Figs. 1 and 2). The nature of EDHF still remains uncertain, however, epoxyeicosatetraenoic acids, metabolites of P450 monoxygenase pathway of arachidonic acid, have recently been proposed as a possible candidate for EDHF (8). EDHF appears to substantially contribute to endothelium-dependent relaxations, especially in microvessels, both in animals (9) and humans (10). The endothelium-derived contracting factors (EDCFs) include superoxide anions which may act by scavenging NO (11), endoperoxides (PGH2), thromboxane A2, and endothelin-1 (12) (Fig. 1). Indeed, endothelium-dependent contractions can be explained either by the production of those EDCF, or the withdrawal of the release of EDRFs (1, 2).

Under several pathological conditions, endothelial cells become dysfunctional, primarily due either to a reduced...
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Shear stress

Ach

ADP

PGI2

EDRF

EDHF

K+ channel

1cGMP

1cAMP

Contraction

Proliferation

Smooth muscle cells

Endothelial cells

Cytokines

Thrombin

Hypoxia

SHT

TxA2

EDCF

EDCFs

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Fig. 1. Current concept of endothelial modulation of the contraction and proliferation of the underlying vascular smooth muscle. Endothelial cell synthesize and release several vasodilators and vasoconstrictors, which inhibit or stimulate the contraction and proliferation of the underlying vascular smooth muscle. Vasodilators include endothelium-derived relaxing factor (EDRF; most likely nitric oxide (NO)), prostacyclin (PGI2), and endothelium-derived hyperpolarizing factor (EDHF). Vasoconstrictors include endothelin, superoxide anion (O2-), and prostaglandins (thromboxane A2 (TxA2) and prostaglandin H2 (PGH2)). AA = arachidonic acid; Ach = acetylcholine; ADP = adenosine diphosphate; A23187 = the calcium ionophore; SHT = serotonin; PDGF = platelet-derived growth factor; M = muscarinic receptor; P = P2y-purinergic receptor; S = serotonergic receptor; TK = receptor for PDGF that is coupled to tyrosine kinase. (from Ref. (1) with permission)

production (or action) of EDRFs and/or a greater propensity to evoke endothelium-dependent contractions. This is especially the case in hypertension. This brief review summarizes the current knowledge on the endothelial dysfunction in hypertension (1, 2, 13, 14).

Morphological Changes of the Endothelium

Morphological changes of the endothelium and the subendothelial layer occurs in hypertension despite of the well-preserved integrity of the cells (15, 16). Endothelial cells of hypertensive blood vessels are more voluminous, bulge into the lumen and exhibit a greater variation in size, shape and orientation as compared to those of normal blood vessels (15, 16). The cell replication rate and number of endothelial cells is increased (17). In addition, the adherence of circulating blood cells to the endothelial cell layer is augmented (18, 19). The adhering cells are mainly granulocytes, monocytes and lymphocytes, and in the cerebral circulation of the rat also platelets (18, 19).

Endothelium-Dependent Relaxations

Endothelium-dependent relaxations to acetylcholine are reduced in the aorta of rats with spontaneous hypertension (20, 21), renal hypertension, salt-induced hypertension, coarctation of the aorta and DOCA salt-induced hypertension (22-25). In the spontaneously hypertensive rat (SHR), the reduced response to acetylcholine is related to the production of a cyclooxygenase-dependent EDCF (most likely an endoperoxide) (26-28), while in most of other forms of experimental hypertension the reduced production of EDNO predominates (1, 2, 13, 14).

In mesenteric resistance arteries of the rat, EDNO accounts only in part for the endothelium-dependent relaxations to acetylcholine (9), and only part of the response is reduced in adult SHR (29). In the mesenteric artery of the SHR, endothelium-dependent relaxations to acetylcholine are impaired due to a reduced EDHF-mediated response, while the EDNO-mediated component of the relaxation seems to be preserved (30).

In patients with essential hypertension, the vasodilator response to acetylcholine in the forearm is blunted (31-33), although not observed in all studies (34). These results suggest that the production of EDRF/NO is impaired in hypertensive patients. The reduced dilatation to acetylcholine was not improved by the treatment with L-arginine (precursor of EDNO), in one (35) but augmented in
Chronic administration of L-arginine analogues to normotensive rats results in a progressive increase in blood pressure (e.g. 37, 38), and the analogues also cause peripheral vasoconstriction in normotensive humans (e.g. 39, 40). Thus EDNO may contribute to the overall regulation of arterial blood pressure by virtue of its ability to relax vascular smooth muscle. This interpretation is mainly based on the assumption that the effect of the NOS inhibitors is due solely mediated by the inhibition of the direct vasodilator effect of EDNO. However, this may not be the case because NO can modulate in an inhibitory fashion other factors which contribute to cardiovascular homeostasis, including the release of renin and of endothelin, the excretory function of the kidney, and the sympathetic nervous system (1, 2, 13, 14). L-arginine analogues are also known to exert non-specific cardiovascualr effects which are not related to the synthesis of NO (e.g. 41-45). Thus it is conceivable that multiple effects other than simple inhibition of EDNO synthesis are involved when the analogues are used chronically in vivo. Mice lacking the gene for eNOS exhibits mild hypertension (46), however, genetic studies in humans do not support a linkage between eNOS and the occurrence of essential hypertension (47).

Several mechanisms may be involved in the impaired endothelium-dependent relaxations in hypertension (Fig. 3) (1, 2, 13, 14). It is likely that they all can contribute to some extent, depending on the type and stage of hypertension, the agonist tested, and the blood vessel and species of animals studied (Fig. 3) (1, 2, 13, 14). Among them, the effect of endothelial regeneration is of importance since endothelial replication rate is markedly increased in hypertension (17). Regenerating endothelial cells have an altered morphological appearance and an impaired capacity to release EDRF/NO in response to serotonin, aggregating platelets, and $\alpha_2$-agonist, while endothelium-dependent relaxations to bradykinin, ADP, and the calcium ionophore A23187 are well preserved (48-50). The endothelium-dependent relaxations to the former agonists are pertussis toxin-sensitive, while those to the latter agonists are not. Indeed, ADP-ribosylation by pertussis toxin is reduced in regenerated endothelial cells, demonstrating the dysfunction of Gi-protein in regenerated endothelial cells (51). The expression of endothelial Gi-protein in human coronary arteries is reduced with aging, hypertension, and hyperlipidemia (52). Another important mechanism is a concomitant release of EDCF (26-28). Indeed, the reduced endothelium-dependent relaxations are explained best by the normal release of EDNO (26, 53) and the concomitant release of a cyclooxygenase_dependent EDCF (most likely an endoper-
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**Figure 3.** Possible mechanisms underlying the reduced endothelium-dependent relaxations in hypertension. These include abnormalities in (1) endothelial signal transduction system, (2) endothelium-derived contracting factors (EDCFs), (3) intimal thickening, and (4) smooth muscle cell response. Depending on the type and stage of hypertension, vessels and species tested, and agonists used, these mechanisms may contribute to the impairments to a variable extent. Particularly, the former two mechanisms are important in the reduced endothelium-dependent relaxations in hypertension.

AA = arachidonic acid; Ach = acetylcholine; ADP = adenosine diphosphate; cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate; EDHF = endothelium-derived hyperpolarizing factor; EDRF/NO = endothelium-derived relaxing factor/nitric oxide; eNOS = endothelial nitric oxide synthase; SHT = serotonin; PGH₂ = prostaglandin H₂; S = serotonergic receptor; T = thrombin receptor; TK = receptor for PDGF that is coupled to tyrosine kinase; TXA₂ = thromboxane A₂.

oxide), with the latter scavenging the former (26-28). A reduced release of EDHF may also contribute to the impaired endothelium-dependent relaxations in hypertension, particularly in resistance vessels, where EDHF plays a more important role than in large conduit arteries under normal conditions (9, 30, 54).

### Endothelium-Dependent Contractions

In the aorta of the adult SHR, acetylcholine causes indomethacin-sensitive, endothelium-dependent contractions, which are weak or absent in normotensive Wistar-Kyoto rats of the same age (26). Similar endothelium-dependent contractions are obtained with arachidonic acid (55), serotonin (56, 57), endothelin (58, 59), and adenine nucleotides (56, 60). In the isolated perfused heart from normotensive rats, serotonin moderately increases coronary blood flow, while the monoamine markedly decreases flow in hearts from SHR, which can be inhibited by indomethacin (61). These results suggest that a cyclooxygenase-dependent EDCF is liberated in response to serotonin. In patients with hypertension, a cyclooxygenase-dependent vasoconstrictor mechanism participates in the blunting of endothelium-dependent vasodilation in essential hypertension but not in secondary forms of hypertension (62). The endothelial muscarinic receptors mediating the endothelium-dependent contractions to acetylcholine belong to the M₃ subtype as those initiating the release of EDRF/NO in the same preparation (63).

The endothelium-dependent contractions of the SHR aorta are augmented by inhibitors of L-arginine analogues and by oxyhemoglobin (which scavenges NO), but not by methylene blue (which inhibits soluble guanylate cyclase) and the relaxations caused by NO (64). This implies that EDCF released from the hypertensive endothelium interacts chemically with NO, and both factors inactivate each other (64, 65). Such a chemical interaction between EDCF and NO explains the blunting of endothelium-dependent relaxations despite a normal ability of the
endothelial cells to release EDRF/NO in SHR (26-28, 53) (Fig. 4).

The endothelium-dependent contractions to several agonists in the SHR aorta are not prevented by inhibitors of thromboxane synthase, but are abolished by antagonists of endoperoxide/TxA$_2$ receptors (26-28, 56-60). This suggests that the EDCF involved is an endoperoxide (27, 28). Measurement of the release of PGH$_2$ confirmed this interpretation (66). The larger release of PGH$_2$ in the SHR aorta may result from a greater production, as suggested by the augmented expression of cyclooxygenase (66) and/or an altered metabolism of the endoperoxide into prostanoids (67). In addition to the larger production of endoperoxides, an increased responsiveness of the hypertensive vascular smooth muscle in the SHR aorta may also contribute to the endothelium-dependent contractions (66). In the case of endothelium-dependent contractions to endothelin, the EDCF involved appears to be TxA$_2$ (59).

The indomethacin-sensitive, endothelium-dependent contractions of the SHR aorta to acetylcholine are inhibited by a preferential inhibitor of cyclooxygenase-1 (COX-1), but not by a preferential inhibitor of cyclooxygenase-2 (COX-2) (66). These results suggest that activation of COX-1 is involved in the responses. This interpretation is further supported by the demonstration of a greater expression of COX-1, at the levels of both mRNA and protein, in the SHR aorta compared to that of normotensive rats (66). Interestingly, however, the increased expression of COX-1 in the SHR aorta is unaltered before and after endothelium removal, indicating that the increased production of endoperoxides in the hypertensive arteries may originate in the vascular smooth muscle rather than the endothelium (66). Thus, the endothelium-dependency of the response would be explained by the release of an endothelial factor, inactivated by NO, that diffuses to the underlying smooth muscle to stimulate COX-1 (14) (Fig. 4). Oxygen-derived free radicals would be likely candidates (68), since they scavenge EDRF/NO (69) and cause cyclooxygenase-independent contractions which are augmented in the SHR aorta (70). Indeed, free radicals have extreme short half-life, and no experiments have yet succeeded to bioassay the EDCF produced by the SHR aorta, where NO can be easily demonstrated. However, superoxide dismutase plus catalase, or deferoxamine, do not inhibit the endothelium-dependent contractions to acetylcholine in the SHR aorta (70), and superoxide dismutase does not restore endothelium-dependent vasodilatation in patients with essential hypertension (71). This suggests that free radicals other than superoxide anions or hydroxyl radicals may be involved in the responses.

Of the three members of the endothelin family, endothelin-1 (ET-1) and endothelin-3 (ET-3) circulate in the blood.
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and thus are potentially important as homeostatic hormones (72). If endothelin were involved in the pathogenesis of hypertension, one would expect that the levels of the peptide are increased or that the vasoconstrictor responses to it are potentiated (1). However, the circulating levels of the peptides are not increased in rats with various types of hypertension except malignant cases of hypertension (73, 74). In patients with essential hypertension, the circulating levels of endothelin are modestly increased in some (75, 76) but not in other (77, 78) studies. Similarly, the changes in pressor responsiveness to endothelin with hypertension are controversial (1, 72). In SHR, the increase in blood pressure and the peripheral vasoconstrictor responses evoked by endothelin are reported to be normal (79), reduced (80) or marginally augmented (81). In isolated aorta of the SHR, the sensitivity to endothelin is enhanced (74), normal (82), or reduced (83). In addition, in heterozygous mice with deletion of the endothelin gene, the arterial blood pressure is higher, rather than lower, than in normal animals (84). Thus, there are few convincing evidence that an augmented production of, or an increased sensitivity to, endothelin contributes to the pathogenesis of most forms of hypertension (1, 72).

Causes or Consequences?

Several lines of evidence indicate that the reduced endothelium-dependent relaxations in hypertension are a consequence, rather than a cause, of the disease, representing premature aging of the blood vessels due to the chronic exposure to the high blood pressure. First, the reduced responses can be reversed by appropriate antihypertensive therapy (85, 86). Second, in hypertensive patients, the peripheral vasoconstrictor responses to L-arginine analogues are normalized by antihypertensive therapy (87). Third, in the arteries of SHR, the endothelium-dependent relaxations to acetylcholine are normal when they are young, and thereafter progressively decreased as the arterial pressure increases with aging (88, 89). Fourth, endothelium-dependent relaxations, including those mediated by EDHF, can be reduced with aging (1, 10, 90-92). The effect of endothelial regeneration may play an important role in this phenomenon (1, 48-50). Similarly, endothelium-dependent contractions in hypertension may reflect premature aging of blood vessels due to the chronic exposure to the high blood pressure. First, in the SHR, the occurrence of endothelium-dependent contractions is age-dependent (93, 94). Although the responses also occur in normotensive older rats, they are more pronounced in SHR of the same age (26). Second, an indomethacin-sensitive blunting of the relaxation to acetylcholine is noted in human arteries obtained from older patients, but not in those from younger patients (95).

Thus, the altered endothelium-dependent responsiveness may not play a primary role in the initiation of hypertension. However, it could contribute to the maintenance of high blood pressure and facilitate the occurrence of vascular complications such as atherosclerosis (1, 2, 13, 14).

Therapeutic Implications

As mentioned above, antihypertensive therapy should be regarded as the most important strategy to treat the altered endothelium-dependent responsiveness. Besides salt restriction, body weight control, and physical therapy including exercise, the use of antihypertensive agents should be considered when the combination of these non-pharmacological therapies is not sufficient to lower blood pressure. Among the antihypertensive agents, angiotensin-converting enzyme inhibitors (ACE-I) appear to be most beneficial, since they potentiate the release of both EDNO and EDHF triggered by endogenously formed bradykinin (96, 97). This mechanism of action of ACE-I has an advantage in vivo since shear stress activates the local kallikrein-bradykinin system in the arterial wall, which can yield enough kinins to activate the endothelial cells to release relaxing factors, particularly when ACE is inhibited (98). Other agents that also potentiate endothelium-dependent relaxations include fish oil (eicosapentaenoic acid), antioxidants, estrogens, and L-arginine (1, 99-103). In addition to those agents, calcium-channel inhibitors can inhibit endothelium-dependent contractions (104). Likewise, inhibitors of cyclooxygenase (e.g. aspirin) and antagonists of thromboxane/endoperoxide receptors (but not of thromboxane synthase) may be beneficial to prevent the occurrence of vascular complications in hypertension.

Conclusion

Endothelium-dependent relaxations are reduced in hypertension, due either to either a reduced production (or action) of EDNO/EDHF and/or a greater propensity to evoke endothelium-dependent contractions. Multiple mechanisms may be involved in the reduced responses, depending on the type of hypertension. In essential hypertension, the endothelial dysfunction is largely caused by a concomitant release of EDCF, which may be a short-lived free radical. The endothelial dysfunction in hypertension may be a consequence of the chronic exposure to high blood pressure, exhibiting premature aging of blood vessels. Antihypertensive therapy appears to improve the endothelial function and to help to prevent the occurrence of vascular complications.

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