AJH 1990; 3:317-330

Imbalance of Endothelium-derived Relaxing and Contracting Factors

A New Concept in Hypertension?

T. F. Lüscher

The endothelium has a strategical anatomical position between the circulating blood and vascular smooth muscle cells. It has recently been recognized that endothelial cells play an important regulatory role in the circulation. The cells metabolize or activate vasoactive hormones (ie, norepinephrine, serotonin, bradykinin, angiotensin II), produce substances involved in coagulation and can release endothelium-derived relaxing factors and contracting factors. Nitric oxide and prostacyclin are vasodilators and inhibitors of platelet function. Endothelin is the most potent vasoconstrictor substance known. Thus, the endothelium can profoundly affect platelet adhesion and aggregation, vascular smooth muscle tone and possibly also vascular smooth muscle growth. Under physiological conditions, endothelium-derived relaxing factors appear to dominate. In contrast, in hypertensive and atherosclerotic arteries the release of endothelium-derived relaxing factors and/or the responsiveness of vascular smooth muscle cells to the relaxing factors is reduced, while that of endothelium-derived contracting factors is augmented. This imbalance of endothelium-derived relaxing and contracting factors may be important in the pathogenesis of hypertension and its cardiovascular complications. Am J Hypertens 1990; 3:317-330

KEY WORDS: **Acetylcholine, angiotensin, atherosclerosis, endothelin, nitric oxide platelets, prostaglandins, vascular occlusion.**

The functional importance of endothelial cells as regulators of cardiovascular function has been increasingly recognized in the last decade.[1-4] It has been known for years that endothelial cells play an important role in the regulation of movement of fluids from the blood to the tissue.[5] The endothelium also plays a metabolic role by clearing the blood of hormones such as norepinephrine and serotonin. The converting enzyme of the endothelial cell membrane transforms angiotensin I into angiotensin II and inactivates the vasodilator bradykinin.[2-7] Further, endothelial cells can profoundly affect coagulation, platelet function and fibrinolysis.[1,8-11] More recently, it has been recognized that endothelial cells produce vasoactive factors which can inhibit or activate vascular smooth muscle cells (Figure 1)[1,2,12-15] This article updates previous reviews[4,16-18] and focuses on the possibility that endothelial cells act both as mediators and targets of hypertension and its complications.

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This research was supported by grants from the Swiss National Research Foundation (No. 3. 8891-0.86 and 32-25468.88), the Swiss Cardiology Foundation and Schweizerische Rentenanstalt. Dr. Lüscher is a recipient of a career development award of the Swiss National Research Foundation (SCORE-grant No. 3231-025150).

This article is based on data first presented at the American Society of Hypertension annual meeting, May 10 to 12, 1989.

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STRUCTURAL CHANGES OF THE ENDOTHELIUM IN HYPERTENSION

In rat models of hypertension endothelial cells exhibit morphological changes.[4,19-23] Indeed, although the integrity of the endothelium is preserved, an increased

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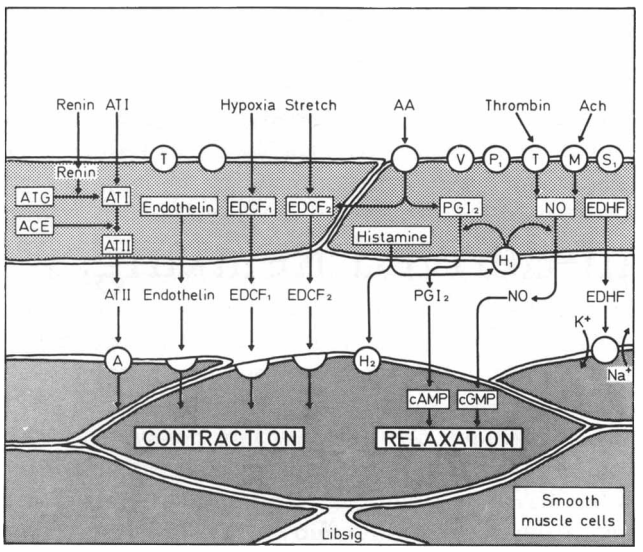

FIGURE 1. *Endothelium-derived vasoactive substances. AA = Arachidonic acid; ACE = Angiotensin converting enzyme; ATG = Angiotensinogen; ACh = Acetylcholine; ATI/II = Angiotensin I/II; cAMP/cGMP = cyclic adenosine/guanosine monophosphate; EDCF = Endothelium-derived constricting factor(s); EDHF = Endothelium-derived hyperpolarizing factor; NO = Nitric oxide; PGI_2 = Prostacyclin. Modified from Ref. 18.*

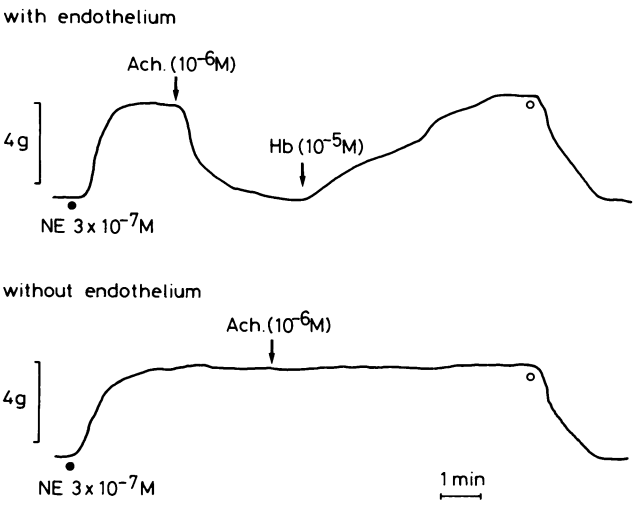

FIGURE 2. *Endothelium-dependent relaxation to acetylcholine in a human internal mammary artery (NE, norepinephrine; original recording). The relaxation to acetylcholine (Ach) can be reversed by hemoglobin (Hb; 10^{-5} mol/L; upper panel). From Ref. 50, by permission.*

permeability, number, volume and replication rate of endothelial cells has been noted.[4,19–23] Transmission electronmicroscopy studies have shown that the number of cytoplasmatic organelles, such as the rough endoplasmatic reticulum, polyribosomes, microtubules, mitochondria and Golgi complexes, as well as the number of actin microfilament bundles and the length and complexity of tight junctions, is augmented in experimental hypertension.[20–22] Focal expansion of the subendothelial space due to accumulation of granular matrix occurs early during the hypertensive process and becomes more pronounced as the disease progresses.[21,23]

ENDOTHELIUM-DERIVED VASOACTIVE SUBSTANCES

Endothelium-derived Relaxing Factors Furchgott and Zawadzki[2] first demonstrated that the relaxation of isolated arteries induced by acetylcholine is endothelium-dependent and mediated by the labile substance endothelium-derived relaxing factor (EDRF) (Figure 2). Hemoglobin, oxygen-derived free radicals and antioxidants are effective inhibitors of EDRF.[24–28] The factor is a potent vasodilator and inhibitor of platelet adhesion and aggregation (Figure 3).[2,8,9,29] In vascular smooth muscle cells and platelets EDRF stimulates soluble guanylate cyclase and in turn increases the intracellular levels of cyclic guanosine 3′,5′-monophosphate (cyclic GMP) (Figure 1).[8,9,29,30] Substances interfering with the production of cyclic GMP (methylene blue, LY 83583) inhibit endothelium-dependent relaxations.[3,4,24] The similarity of the actions of EDRF and nitrovasodilators indicated that endothelial cells might produce an endogenous nitrate. Indeed, after independent proposals by Furchgott[31] and Ignarro,[32] Palmer, Ferrige and Moncada demonstrated that endothelial cells in culture release nitric oxide in response to bradykinin.[33] Nitric oxide has a similar half-life and mode of action as EDRF and the amounts released from endothelial cells in culture after stimulation with bradykinin appear sufficient to explain the biological activity of the factor in vascular tissues and in platelets. L-Arginine is the precursor substance from which nitric oxide is cleaved by specific enzymes.[34,35] EDRF is released in response to flow (ie, shear stress),[36,37] platelet-derived products (ie, adenosine diphosphate, thrombin, serotonin)[38–41] and certain hormones and autacoids (ie, bradykinin, histamine, noradrenalin, substance P and vasopressin).[42–46] Aggregating platelets release large amounts of adenine nucleotides and serotonin which can evoke endothelium-dependent relaxations in isolated blood vessels.[39–41] Adenosine diphosphate is the main mediator of the response to aggregating platelets in human and canine coronary arteries,[41,47] while in the pig serotonin is mediating the effect (Figure 3).[48] Thrombin which is formed after activation of the coagulation cascade evokes potent endothelium-dependent relaxations also in human blood vessels.[49,50] In addition to EDRF, platelet-derived products and thrombin can also stimulate the endothelial production of prostacyclin (PGI_2) and tissue plasminogen activator.[10] Indeed, PGI_2—a vasodilator and inhibitor of platelet function which stimulates cyclic adenosine 3′,5′-monophosphate (cyclic AMP)[1,4,11]—and EDRF potentiate each other's vascular and antiaggregatory effects even at subthreshold con-

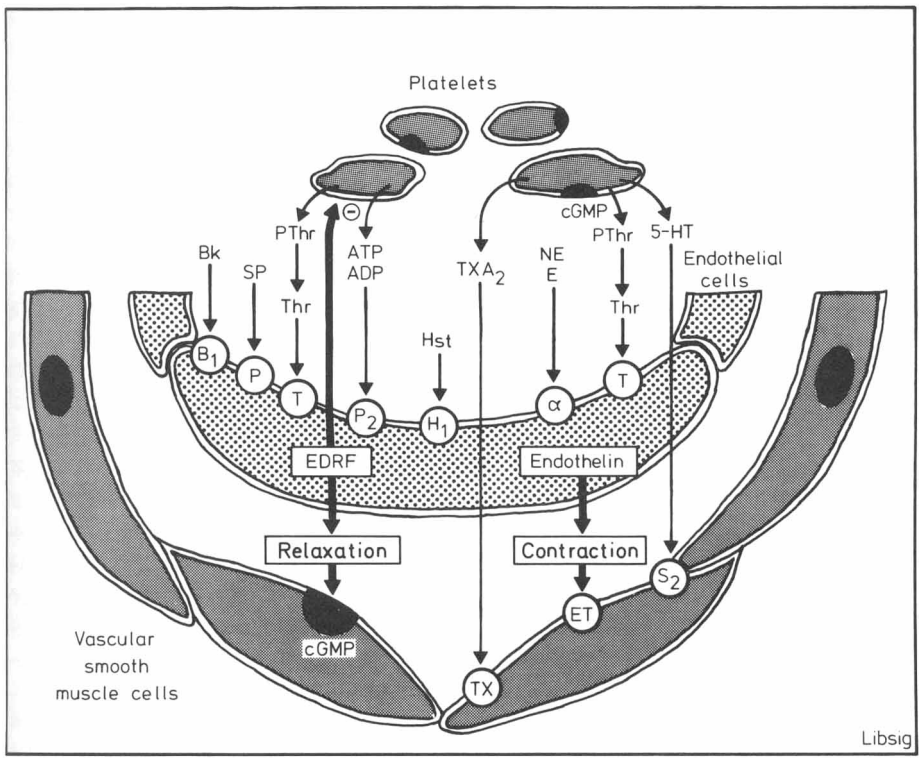

FIGURE 3. *Endothelium and platelet-vessel wall interaction. The production and release of endothelium-derived relaxing factor (EDRF) and endothelin-1 can be stimulated by platelet-derived substances such as thrombin, and in the case of the former (in human arteries) also by adenosine tri- and diphosphate (ATP/ADP), histamine (HST), bradykinin (Bk) and substance P (SP). EDRF causes vascular relaxation and inhibition of platelet function (—), while endothelin-1 mainly causes profound contraction of blood vessels. Modified from Ref. 18.*

centrations.[8,51] Thus, at sites where platelets and/or the coagulation cascade are activated, potent vasodilators, inhibitors of platelet function and stimulators of fibrinolysis are released which may provide a local protective mechanism against vasospasm, ischemia and thrombus formation.

It is likely that besides EDRF and PGI_2 the endothelium releases an endothelium-derived hyperpolarizing factor (EDHF) (Figure 1).[52] In the canine femoral artery, endothelium-dependent relaxations to acetylcholine are reduced after inhibition of sodium–potassium ATPase by ouabain.[53] Acetylcholine causes endothelium-dependent increases in membrane potential of vascular smooth muscle cells which can be blocked by ouabain.[54] Exogenous nitric oxide, however, does not hyperpolarize vascular smooth muscle cells nor does hemoglobin prevent the increase in membrane potential evoked by acetylcholine.[55] Thus, another substance must be involved. Endothelium-dependent hyperpolarization of vascular smooth muscle cells may facilitate relaxation and attenuate the responsiveness of the vascular wall to certain vasoconstrictor hormones.

Endothelium-derived Contracting Factors Endothelial cells are also a source of contracting factors (EDCF) (Figure 1).[3,4,7,12,15] Endothelial cells in culture produce a vasoconstrictor which has recently been characterized as the 21 amino acid peptide endothelin by Yanagisawa et al (Figure 3).[15,56,57] Three forms of the peptide exist, endothelin-1 (formerly human or porcine), endothelin-2 and endothelin-3 (formerly rat endothelin).[58–60] Thrombin, epinephrine, transforming growth factor β, the calcium ionophore A23187 and phorbol ester express preproendothelin messenger RNA in cultured endothelial cells (Figure 3).[15,58–60] Thrombin releases endothelin from porcine endothelial cells in culture.[61] A putative converting enzyme cleaves the final peptide from its precursor (proendothelin). Endothelin binds to specific membrane receptors on vascular smooth muscle cells where it activates phospholipase C (and in turn stimulates the formation of inositol trisphosphate and diacylglycerol),[62,63] phospholipase A_2,[64] increases intracellular calcium and evokes long-lasting contractions.[15,65] In isolated arteries, endothelin is a very potent vasoconstrictor with a half-maximal effective concentration which is one to two orders of magnitude lower than that of angiotensin II and norepinephrine.[15,66–68] When infused in vivo, endothelin causes a rapid and transient decrease in blood pressure followed by prolonged hypertension.[15,69,70] The vasodilator action of endothelin has been attributed to the release of PGI_2 and/or EDRF.[71] While EDRF, nitrovasodilators and isoproterenol can reverse endothelin-induced contractions, calcium antagonists are less effective in most vascular preparations.[15,65,68,72,73]

In the canine coronary artery, hypoxia causes endothelium-dependent contractions.[13] It is unlikely that the endothelium-derived contracting factor released under these conditions ($EDCF_1$) is endothelin, since the re-

sponse to hypoxia is faster and can be prevented by calcium antagonists.[73] Indeed, endothelin is not stored in endothelial cells.[15]

In addition, the endothelium and vascular wall possess an independent renin-angiotensin system which may provide high local concentrations of the potent vasoconstrictor angiotensin II (Figure 1).[7] Thromboxane A_2 and another cyclooxygenase-dependent endothelium-derived contracting factor ($EDCF_2$) can also be produced in endothelial cells of certain vascular beds (Figure 1).[3,4,14,74]

ENDOTHELIUM AND BLOOD PRESSURE CONTROL

It is likely that the endothelium contributes to the regulation of peripheral vascular resistance. Indeed, infusion of hemoglobin—an inhibitor of EDRF[24]—in healthy human subjects increases arterial blood pressure.[75] Similarly, infusion of L-*N*-monomethylarginine, a false precursor substance of EDRF,[34,35] increases blood pressure in experimental animals (Figure 4).[76] Endothelium-dependent contractions to stretch[74,77] may contribute to autoregulation. Intravenous infusion of endothelin causes profound and longlasting increases in peripheral vascular resistance and blood pressure.[15,69-71]

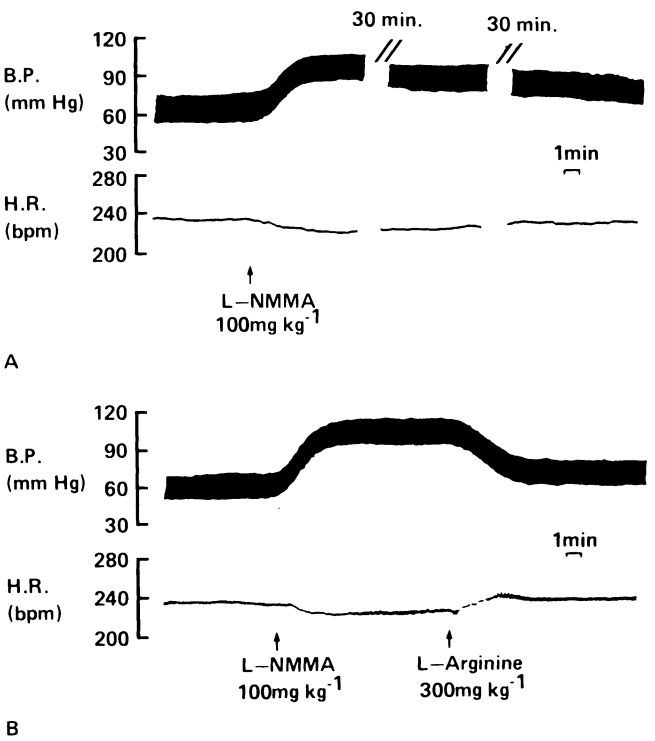

FIGURE 4. *Effect of* L-N^G-*monomethyl arginine (L-NMMA; 100 mg/kg, intravenously) on blood pressure (B.P.) and heart rate (H.R.) in the anesthetized rabbit. L-NMMA evokes a marked and sustained rise in blood pressure and a slight decrease in heart rate (A). Infusion of* L-*arginine reverses the effects of L-NMMA (B). From Ref. 76, by permission.*

The circulating levels of endothelin-1 in healthy normal subjects, however, are very low (ie, 1.5 pg/mL).[78,79] Hence it is likely that the peptide exerts its action locally and/or that it is secreted primarily towards the abluminal side of the blood vessel wall. Even subthreshold concentrations of the peptide may, by potentiating the effects of norepinephrine, contribute to the regulation of blood pressure.[80-82] Thus, alterations in the release of endothelium-derived relaxing and contracting factors in hypertension may contribute to the increased peripheral vascular resistance.

ENDOTHELIUM-DEPENDENT RESPONSES IN HYPERTENSION

Endothelium-dependent Relaxations Acute and severe increases in blood pressure can cause endothelial damage.[83] In the canine coronary artery, this is associated with an endothelium-dependent enhancement of the vasoconstrictor effects to serotonin, while the relaxations to acetylcholine are unaffected.[83] In the cat cerebral circulation, acute hypertension abolishes endothelium-dependent relaxations to acetylcholine.[84]

In the aorta of rats with chronic spontaneous hypertension, renal hypertension, salt-induced hypertension, coarctation and DOCA-salt-induced hypertension, endothelium-dependent relaxations to acetylcholine and the calcium ionophore A23187 are attenuated (Figure 5).[4,85-91] Similar defects occur in the carotid artery, in mesenteric resistance arteries, but not the renal artery of the spontaneously hypertensive rat.[92-97] Endothelium-

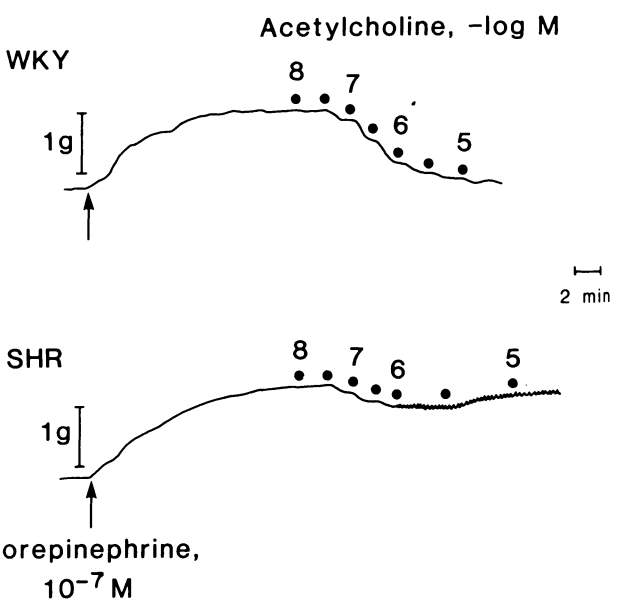

FIGURE 5. *Endothelium-dependent relaxations to acetylcholine in the aorta of normotensive Wistar-Kyoto rats (WKY; top panel) and spontaneously hypertensive rats (SHR; lower panel). Note the reduced relaxation in the SHR. Original recording, from Lüscher and Vanhoutte 1988, by permission.*

dependent relaxations are impaired regardless of the agonist used to contract the resistance arteries (norepinephrine, serotonin or endothelin).[95-98] In the forearm circulation of hypertensive patients, the vasodilator response to acetylcholine, but not that to sodium nitroprusside, is reduced.[99,100]

The reduction of endothelium-dependent relaxations to acetylcholine is directly related to the level of systolic blood pressure (Figure 6).[4,90,101] In the Dahl rat, antihypertensive therapy normalizes blood pressure and the attenuated endothelium-dependent relaxations.[102] In rats with aortic coarctation endothelium-dependent relaxations are normal in the aortic segment distal to the stenosis suggesting that the defect is a consequence rather than a cause of the high blood pressure.[91] In the spontaneously hypertensive rat, endothelium-dependent responses become progressively impaired as blood pressure increases.[94,103] Whether or not this defect is secondary or primary in nature remains to be determined.

In contrast to the rat, endothelium-dependent relaxations to acetylcholine are augmented in the aorta and in the perfused hindlimb of mice with psychosocial hypertension.[104] Similar results have been reported in the femoral artery of the spontaneously hypertensive rat.[85] In the hindlimb circulation of the rabbit, hypertension does not induce marked changes in vascular reactivity; the maximal relaxation evoked by acetylcholine, however, is reduced.[105] Whether or not these differences between rats, mice and rabbits are related to differences in the severity, duration or form of hypertension, or due to species differences, remains to be clarified.

As regards to the sequellae of hypertension, an increased interaction between circulating blood cells and the blood vessel wall are of particular interest (Figure 3).[3,4] Indeed, hypertension is associated with an increased adherence of granulocytes, monocytes and lymphocytes to the endothelium.[18,106] In cerebral arteries of the spontaneously hypertensive rat, the adhesion of platelets to the endothelium is augmented.[107] Endothelium-dependent relaxations to adenosine diphosphate —which is released from aggregating platelets[3,4,41]— are impaired in the aorta[108] and carotid artery[90,92] of hypertensive rats. Endothelium-dependent relaxations to thrombin are markedly reduced in Dahl hypertensive, but not in spontaneously hypertensive rats.[90,108] In the aorta of normotensive rats, the contractions evoked by aggregating platelets are inhibited by the endothelium, while in spontaneously hypertensive rats the inhibitory capacity of the endothelium against platelet-induced contractions is attenuated indicating an impaired protective function of the endothelium.[108] This is particularly unfavorable, since hypertensive platelets are preactivated and aggregate more readily to various stimuli than those of normotensive controls.[109]

Endothelium-dependent Contractions Aortic rings from normotensive rats contract in response to stretch, a response which is augmented in DOCA-hypertensive animals (Figure 7).[77] The stretch-induced contraction can be inhibited by calcium antagonists and by removal of the endothelium suggesting that hypertension promotes stretch-induced endothelium-dependent contractions.

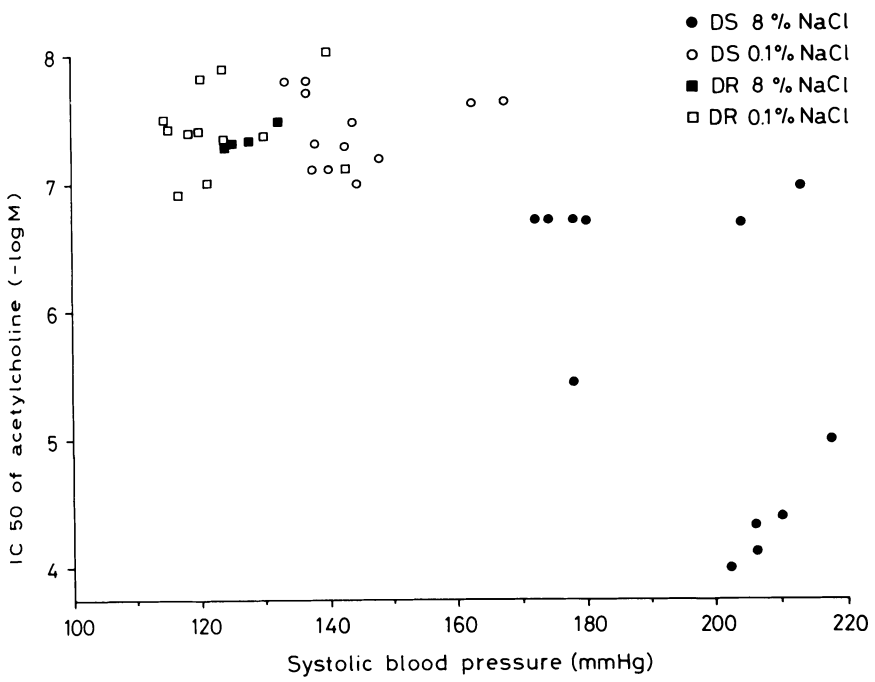

FIGURE 6. *Correlation between systolic blood pressure and the concentration of acetylcholine needed to induce a half maximal relaxation in the aorta of Dahl salt-sensitive (circles) and salt-resistant rats (squares) on either a high (8% NaCl; closed symbols) or low salt diet (0.1% NaCl; open symbols). These experiments demonstrate that endothelium-dependent relaxations become progressively impaired as blood pressure rises. Data from Ref. 102, by permission.*

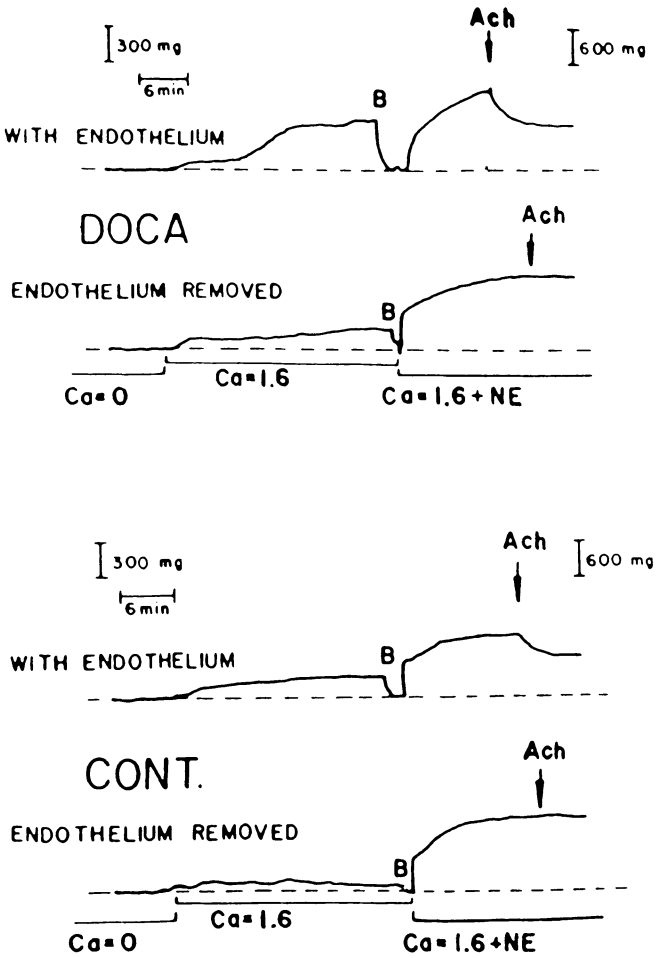

FIGURE 7. *Endothelium-dependent contractions to stretch in the aorta of normotensive (CONT.) and hypertensive rats (DOCA). The response is absent in calcium free solution (Ca = 0) and is augmented in the hypertensive animals. From Ref. 77, by permission of the American Heart Association.*

Acetylcholine causes endothelium-dependent contractions in the aorta of the adult spontaneously hypertensive rat, but not in normotensive Wistar-Kyoto rats of the same age (Figure 8).[87,110,111] In old rats (12 months of age), when the contractions become most pronounced in the hypertensive animals, acetylcholine does evoke endothelium-dependent contractions in normotensive rats as well suggesting that endothelium-dependent contractions reflect the premature aging of the hypertensive arterial wall.[112]

The endothelium-dependent contractions to acetylcholine occur with higher concentrations of acetylcholine than those needed to release EDRF.[87] Endothelium-dependent contractions to acetylcholine can be prevented by inhibitors of phospholipase A_2 and cyclooxygenase suggesting that the metabolism of arachidonic acid is involved (Figures 8 and 9).[87] Similarly, in mesenteric resistance arteries of spontaneously hypertensive rats, endothelium-dependent relaxations to acetylcholine are reversed at higher concentrations and this can be prevented by inhibitors of cyclooxygenase.[96,97] The metabolite of arachidonic acid is not likely to be prostacyclin, since inhibitors of prostacyclin synthetase are ineffective.[87] In the perfused aorta, small amounts of prostaglandin $F_{2\alpha}$, E_2 and—in the spontaneously hypertensive rat—of thromboxane B_2 are released.[114] Thromboxane receptor antagonists, but not thromboxane synthetase inhibitors, however, inhibit the contractions suggesting that a cyclooxygenase product other than thromboxane activates the receptor.[111] The fact that attempts to bioassay the endothelium-derived contracting factor ($EDCF_2$) released by acetylcholine have failed, indicates that a very labile substance such as endoperoxide intermediates or oxygen-derived free radicals must be involved or that the substance is only released towards the abluminal side of the blood vessel wall. Superoxide radicals contract the rat aorta and the response is augmented in hypertension.[110] Scavengers of oxygen-derived free radicals such as superoxide dismutase do not prevent endothelium-dependent contractions to acetylcholine as they do in brain blood vessels.[110] Thus, the nature of this endothelium-derived relaxing factor ($EDCF_2$) remains elusive.

In the aorta of the spontaneously hypertensive rat, the contractions evoked by aggregating platelets are enhanced in rings with, but not in those without, endothelium.[108] Serotonin exerts similar effects, particularly if $5HT_2$-serotonergic receptors of the vascular smooth muscle are blocked by ketanserin to unmask the endothelial effects of the monoamine.[108] Serotonin increases coronary flow and dilates cerebral microvessels of normotensive rats, but decreases coronary flow and constricts cerebral microvessels in hypertensive rats.[114,115] This effect of serotonin in the hypertensive coronary and cerebral circulation can be inhibited by indomethacin. Inhibition of cyclooxygenase also normalizes the attenuated vasodilator effects of adenosine diphosphate in hypertensive cerebral microvessels. These results are consistent with the concept that in hypertension an endothelium-derived contracting factor dependent on cyclooxygenase ($EDCF_2$) is released in response to platelet-derived products which offsets the effects of EDRF.

Whether or not these altered responses to platelet-derived products contribute to the increased peripheral vascular resistance in hypertension is uncertain. However, it may relate to the vascular complications such as ischemic stroke, myocardial infarction and peripheral vascular disease.[4]

Causes of Endothelial Dysfunction The alterations of endothelium function differ in different vascular beds and different models of hypertension.[4] Hypertension can interfere with the release and/or action of endothelial substances. As judged from bioassay experiments in the rat aorta, the luminal release of EDRF and PGI_2

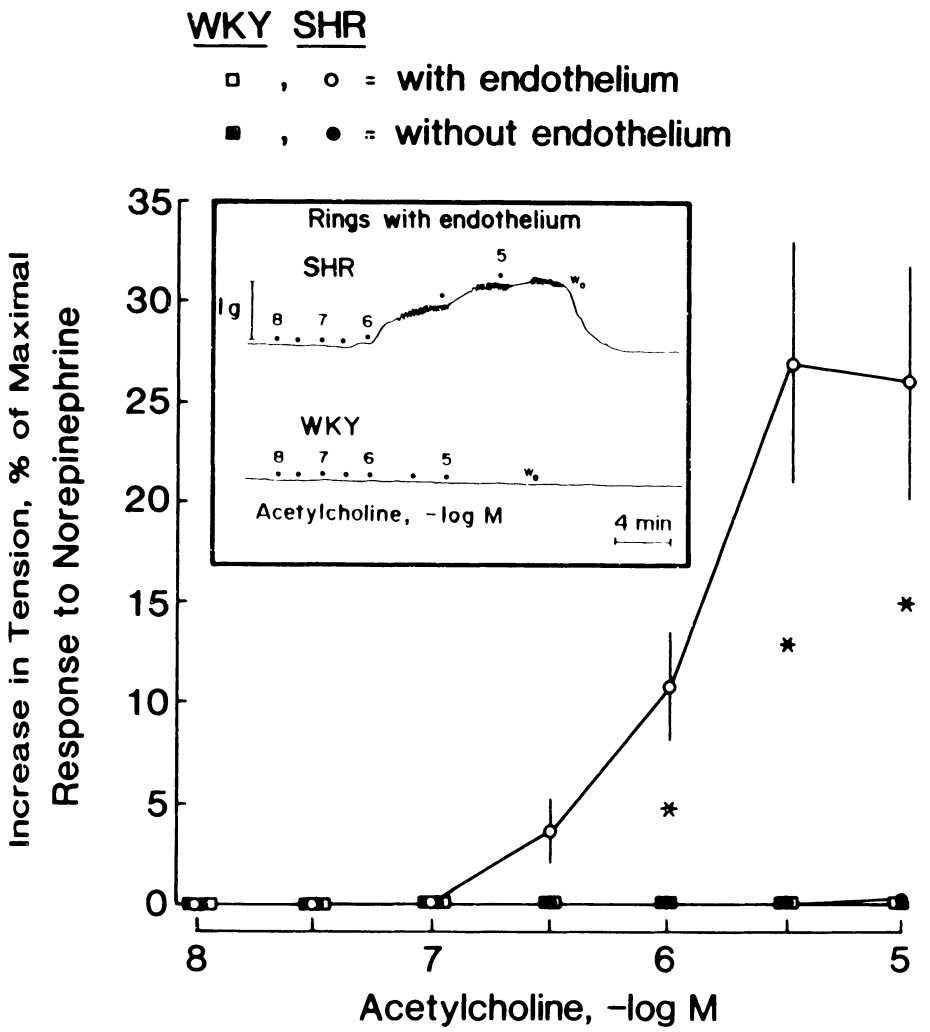

FIGURE 8. *Endothelium-dependent contractions to acetylcholine in the aorta of adult spontaneously hypertensive rats (SHR; open symbols and top inset). In normotensive Wistar-Kyoto rats (WKY) of that age, the response does not occur. From Ref. 87, by permission of the American Heart Association.*

induced by acetylcholine or histamine is normal in spontaneous, renal and DOCA-salt hypertension.[113,116,117] A reduced abluminal release of the factor, however, remains a possibility. Indirect evidence suggests that in the human forearm and in salt-induced hypertension of the rat the release of EDRF might be reduced.[90,99,100] Whether or not the basal release of the factor is decreased in the spontaneously hypertensive rat is uncertain.[113,117]

Subendothelial thickening of the hypertensive blood vessel wall[19-23] is unlikely to interfere with the diffusion of EDRF towards vascular smooth muscle. Indeed, if a diffusion barrier were important, a parallel right-ward shift of the concentration response curve to acetylcholine should be expected. However, endothelium-dependent relaxations are most prominently impaired at higher concentrations of the agonists (Figure 5).[85-91] Further, extensive intimal thickening does not interfere with endothelium-dependent relaxations in the carotid artery of normotensive rabbits.[118]

Sodium nitroprusside—which, like EDRF, activates guanylate cyclase[30]—has been used to assess the responsiveness of hypertensive blood vessels to EDRF.[4] Normal, reduced and enhanced relaxations to sodium nitroprusside have been reported.[85-97,119,120] Differences in the anatomical location of the blood vessel, the age of the animal and of the duration, severity and form of hypertension may be important. Indeed, in the aorta of the rat, the relaxations to sodium nitroprusside and sodium nitrate decrease with age.[120] The presence of endothelial cells inhibits the effects of nitrovasodilators and in the aorta of the spontaneously hypertensive rat and this accounts at least in part for the reduced response.[120] In the carotid artery and in the aorta of these animals the relaxations to sodium nitroprusside are impaired, while in the renal and in hypertensive mesenteric resistance arteries, the response is not attenuated.[94,95,97] Thus, depressed endothelium-dependent relaxations do occur in hypertensive arteries even in the presence of a normal response to nitrovasodilators indicating a predominant endothelial defect.

In the aorta of the spontaneously hypertensive rat, endothelium-dependent relaxations are decreased due to the simultaneous occurrence of endothelium-depen-

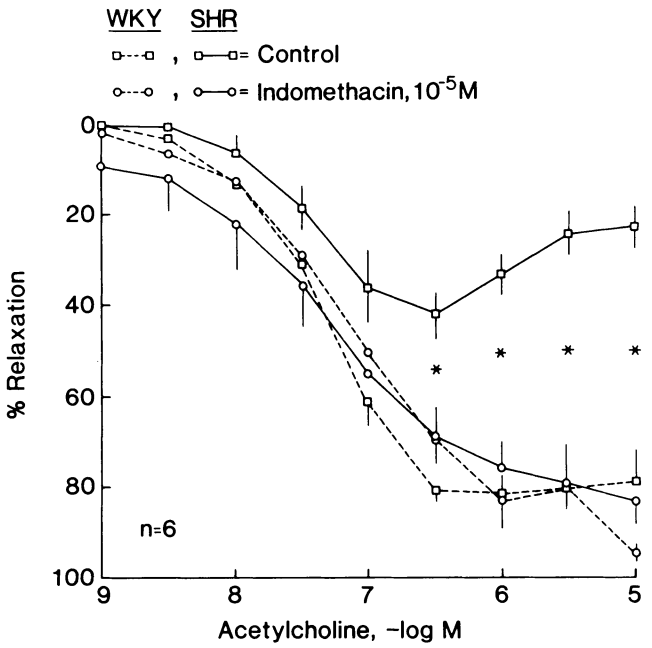

FIGURE 9. *Effect of indomethacin (to inhibit the formation of vascular prostaglandins) on endothelium-dependent relaxations to acetylcholine in the aorta of normotensive (WKY; dashed lines) and spontaneously hypertensive (SHR; solid lines) rats. These experiments demonstrate that indomethacin can normalize the reduced endothelium-dependent relaxations in the aorta of the SHR. From Ref. 87, by permission of the American Heart Association.*

dent contractions which can be blocked by indomethacin (Figure 9).[87] In the aorta of the Dahl rat,[90] in the carotid artery of the spontaneously hypertensive rat[92] and most likely also in the forearm circulation of hypertensive patients,[100] a cyclooxygenase-dependent endothelium-derived relaxing factor ($EDCF_2$) is not released by acetylcholine, since the response to the muscarinic agonist is unaffected by indomethacin. In these blood vessels, the predominant lesion appears to be a reduced production and/or attenuated responsiveness to EDRF. Locally generated endothelin would—if produced in sufficient amounts in vivo[78,79]—attenuate endothelium-dependent relaxations in hypertensive arteries, while in normotensive rats EDRF inhibits the contractile effects of the peptide.[98] This imbalance between endothelium-derived relaxing and contracting factors would be most pronounced in arteries with an augmented sensitivity to the peptide, such as the renal artery of the spontaneously hypertensive rat.[121]

ATHEROSCLEROSIS

Although in the rat hypertension is associated with morphological changes of the endothelium—in contrast to atherosclerosis—intimal lipid accumulation does not occur in the absence of hyperlipoproteinemia.[106,122] In men, however, hypertension and hyperlipidemia are major risk factors for the development of atherosclerotic vascular disease and they are often associated in the same patient. In the rabbit aorta low-density lipoproteins (LDL), but not high-density lipoproteins (HDL), inhibit endothelium-dependent relaxations to acetylcholine.[123] In cultured endothelial cells LDL interferes with the basal and receptor-operated release of EDRF.[124,125] A reduced production of EDRF would be associated with an increased adherence of platelets to the vessel wall[9] and in turn locally increased levels of platelet-derived growth factor (PDGF).[122] This would promote vascular growths particularly since EDRF may act as a antiproliferative factor.[126] Thus, a reduced release of EDRF[127] may also contribute to the formation of the atherosclerotic plaque. In atherosclerotic arteries, including the human coronary artery, endothelium-dependent relaxations to acetylcholine, adenosine diphosphate, bradykinin, substance P and aggregating platelets are reduced (Figure 10).[47,128-131] Selective infusion of acetylcholine into the left anterior descending coronary artery causes paradoxical contractions in patients with coronary artery disease.[132] A reduced release of EDRF, structural changes of the intima and media, and—at least in the porcine coronary artery—the production of an endothelium-derived contracting factor ($EDCF_2$) contribute to these changes in atherosclerotic arteries.[4,47,127-132] Thus, hypertension and hyperlipidemia are associated with an imbalance of endothelium-derived relaxing and contracting factors and this may reduce the antispastic, antithrombotic and antiproliferative properties of the endothelial layer. Thus, endothelium dysfunction may represent a common denominator of vascular injury induced by cardiovascular risk factors.

ANTIHYPERTENSIVE THERAPY AND VASCULAR PROTECTION

A normalization of endothelium-dependent relaxations with antihypertensive therapy[101,102] might contribute to the ability of the drugs to prevent or reduce cardiovascular complications of hypertension. In Dahl rats with salt-induced hypertension, reserpine, hydrochlorothiazide and hydralazine normalize blood pressure and the attenuated endothelium-dependent relaxations to acetylcholine, adenosine diphosphate and thrombin.[102] Changes in transmural pressure during antihypertensive therapy must play an important role, since the effect is related to the degree of blood pressure reduction.[4,101] The inhibitor of converting enzyme enalapril, which does not lower blood pressure in Dahl rats, does not affect endothelium-dependent relaxations.[4] On the other hand, in the aorta of the rabbit, but not in that of the rat, the relaxations induced by hydralazine are in part endothelium-dependent.[133] Chronic therapy with captopril or hydralazine, but not with enalapril, augments endothelium-dependent relaxations to acetylcholine even in normotensive rats.[134] Thus, changes in transmural pressure and—at least in part—direct pro-

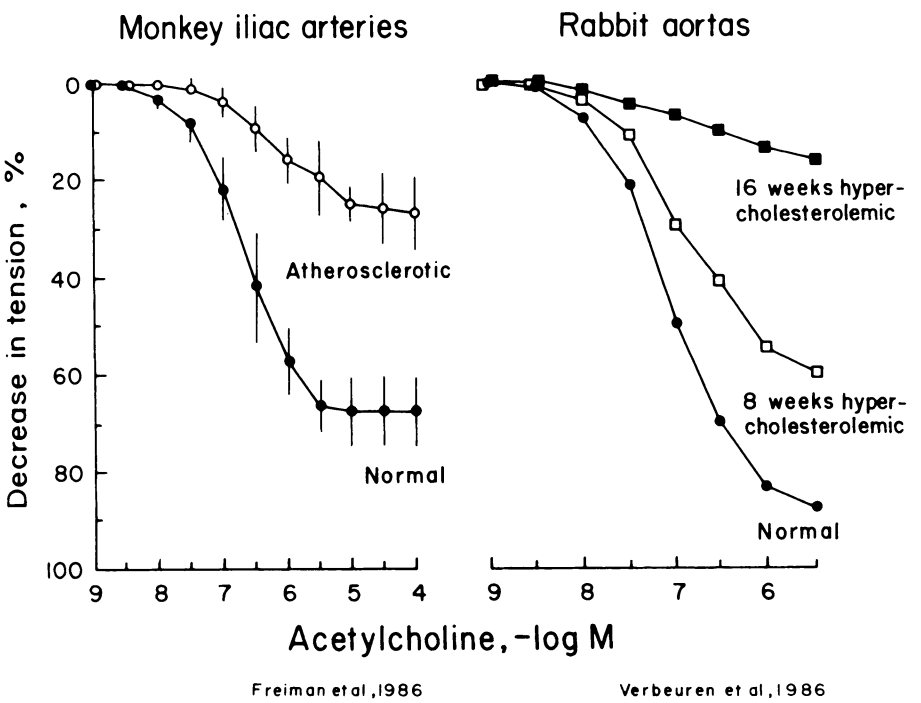

FIGURE 10. *Endothelium-dependent relaxations to acetylcholine in atherosclerotic arteries. Atherosclerosis was induced by a high cholesterol diet. In the iliac artery of atherosclerotic monkeys (left; open circles) and in the aorta of rabbits with hypercholesteremia (right; squares), the response to the muscarinic agonist is reduced in preparations with endothelium. From Ref. 4, by permission.*

tective vascular effects of some drugs restore endothelium-dependent relaxations in hypertension.

Other factors than high blood pressure can cause morphological changes of the intima. Indeed, in the rat, the changes are more pronounced with high than with low levels of mineralcorticoids.[19,20] In man, high renin hypertension is associated with a higher incidence of stroke and myocardial infarction than low renin hypertension.[135] In hypertensive rats, high dietary sodium is an important determinant of intimal injury.[136] In man, a high dietary potassium intake protects against stroke-associated deaths.[137] This effect of high dietary potassium is independent of other dietary variables or cardiovascular risk factors. Indeed in hypertensive Dahl rats high dietary potassium supplementation reduces the incidence of strokes and renal vascular injury independent of its effects on blood pressure.[138,139] High dietary potassium supplementation enhances endothelium-dependent relaxations to acetylcholine in hypertensive Dahl rats at least in part independently of blood pressure, suggesting a vascular protective effect of the diet (Figure 11).[141]

In populations with a high dietary fish consumption the death rate from coronary artery disease is low.[141] In the pig, cod-liver oil delays the development of coronary atherosclerosis.[142] This vascular protective effect of cod-liver oil is independent of its effects on plasma lipid levels and probably mediated primarily by eicosapentanoic acid.[141,142] In the coronary artery and in coronary microvessels of the pig, dietary supplementation with fish oil or eicosapentanoic acid enhances endothelium-dependent relaxation to platelet-derived products and other autocoids.[143,144] In cultured porcine endothelial cells eicosapentanoic acid augments the release of EDRF evoked by bradykinin and adenosine diphosphate, but not that induced by the calcium ionophore A23187.[145] This may be important both in the prevention of athero-

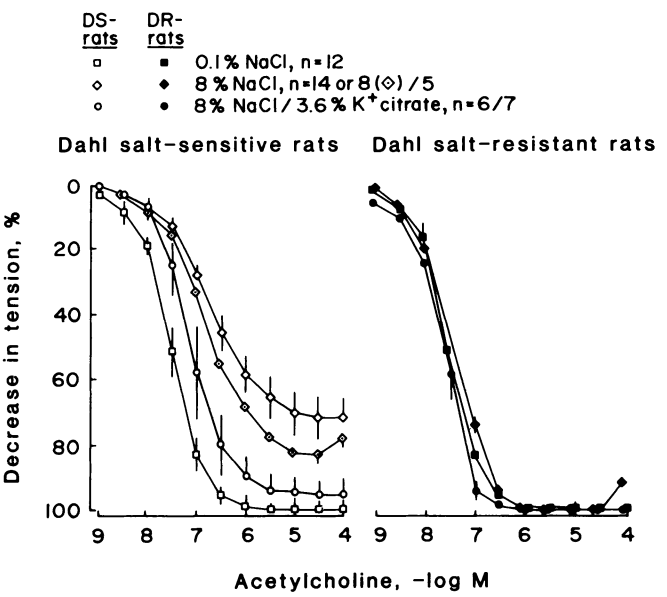

FIGURE 11. *Effects of high dietary potassium supplementation on endothelium-dependent relaxations to acetylcholine in the aorta of hypertensive Dahl rats. The response is attenuated in rats fed high dietary sodium (open diamonds; left). In hypertensive rats supplemented with high dietary potassium (8% NaCl plus 3.6% K^+ citrate; open circles), endothelium-dependent relaxations are augmented both as compared to all hypertensive rats (open diamonds) and to a subgroup of rats fed 8% NaCl matched for blood pressure (dotted diamonds). DS = Dahl salt-sensitive; DR = Dahl salt-resistant. From Ref. 140, by permission of the American Heart Association.*

sclerosis and for the regulation of local blood flow. Indeed, dietary eicosapentanoic acid lowers blood pressure in people.[146]

ACKNOWLEDGMENT

The author wishes to thank Sabine Bohnert and Bernadette Libsig, Department of Research, University Hospital, Basel, for their assistance.

REFERENCES

1. Moncada S, Vane JR: Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. Pharmacol Rev 1979;30:293-331.
2. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;299:373-376.
3. Vanhoutte PM, Rubanyi GM, Miller VM, et al: Modulation of vascular smooth muscle contraction by the endothelium. Ann Rev Physiol 1986;48:307-320.
4. Lüscher TF: Endothelial Vasoactive Substances and Cardiovascular Disease. Basel, S. Karger Publisher AG, 1988, pp 1-133.
5. Shepherd JT, Vanhoutte PM: The Human Cardiovascular System. New York, Raven Press, 1979.
6. Ng KKF, Vane JR: Conversion of angiotensin I to angiotensin II. Nature 1967;216:762-766.
7. Dzau VJ: Significance of the vascular renin-angiotensin pathway. Hypertension 1986;8:553-559.
8. Radomski MW, Palmer RMJ, Moncada S: The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. Br J Pharmacol 1987;92:639-646.
9. Radomski MW, Palmer RMJ, Moncada S: Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. Lancet 1987;ii:1057-1068.
10. Chesterman CN: Vascular endothelium, haemostasis and thrombosis. Blood Reviews 1988;2:88-94.
11. Moncada S, Herman AG, Higgs EA, et al: Differential formation of prostacyclin (PGX or PGI_2) by layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. Thrombosis Res 1977;11:323-344.
12. De Mey JG, Vanhoutte PM: Anoxia and endothelium-dependent reactivity in canine femoral artery. J Physiol (London) 1983;335;65-74.
13. Rubanyi GM, Vanhoutte PM: Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. J Physiol (London) 1985;364:45-56.
14. Miller VM, Vanhoutte PM: Endothelium-dependent contractions to arachidonic acid are mediated by products of cyclooxygenase in canine veins. Am J Physiol 1985;248:H432-H437.
15. Yanagisawa M, Hurihara H, Kimura S, et al: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411-415.
16. Lüscher TF, Diederich D, Bühler FR, et al: Interactions between platelets and the vessel wall: Role of endothelium-derived vasoactive substances, *in* Laragh JH, Brenner B (eds): Hypertension: Pathophysiology, Diagnosis and Management, New York, Raven Press, 1989, pp 637-648.
17. Lüscher TF, Yang Z, Diederich D, et al: Endothelium-derived vasoactive substances: potential role in hypertension and atherosclerosis and vascular occlusion. J Cardiovasc Pharmacol 1989;14(suppl 6):63-69.
18. Lüscher TF: Endothelium-derived relaxing and contracting factors: potential role in coronary artery disease. Eur Heart J 1989;10:847-857.
19. Gabbiani G, Glemes G, Guelpa C, et al: Morphologic and functional changes of the aortic intima during experimental hypertension. Am J Pathol 1979;96:399-422.
20. Huttner I, Gabbiani G: Vascular endothelium in hypertension, *in* Genest J, Kuchel O, Hamet P, Cantin M (eds): Hypertension. New York, McGraw-Hill, 1983, pp 473-488.
21. Limas C, Westrum B, Iwai J, et al: Aortic morphology in salt-dependent genetic hypertension. Am J Pathol 1982;107:378-394.
22. McGuire PG, Twietmeyer TA: Aortic endothelial junctions in developing hypertension. Hypertension 1985;7:483-490.
23. Haudenschild CC, Prescott MF, Chobanian AV: Effects of hypertension and its reversal on aortic intima lesions of the rat. Hypertension 1979;2:33-44.
24. Martin W, Villani GM, Jothianandan D, et al: Blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation of rabbit aorta by certain ferrous hemoproteins. J Pharmacol Exp Ther 1985;233:679-685.
25. Rubanyi GM, Lorenz RR, Vanhoutte PM: Bioassay of endothelium-derived relaxing factor(s): inactivation by catecholamines. Am J Physiol 1985;249:H95-H101.
26. Rubanyi GM, Vanhoutte PM: Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. Am J Physiol 1986;250:H822-H827.
27. Gryglewski RJ, Palmer RMJ, Moncada S: Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature 1986;320:454-456.
28. Moncada S, Palmer RMJ, Grygewski RJ: Mechanism of action of some inhibitors of endothelium-derived relaxing factor. Proc Natl Acad Sci 1986;83:9164-9168.
29. Busse R, Lückhoff A, Bassenge E: Endothelium-derived relaxant factor inhibits platelet activation. Naunyn-Schmiedeberg's Arch Pharmacol 1987;336:566-571.
30. Rapoport RM, Draznin MB, Murad F: Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. Nature 1983;306:174-176.
31. Furchgott RF: Studies on relaxation of rabbit aorta by sodium nitrite: the basis for the proposal that acid-activatable inhibitory factor from bovine retractor penis is inorganic nitrite and the endothelium-derived relaxing factor is nitric oxide, *in* Vanhoutte PM (ed): Vasodilatation. New York, Raven Press, 1988, pp 401-414.
32. Ignarro LJ, Byrns RE, Wood KS: Pharmacological and biochemical properties of endothelium-derived relaxing factor (EDRF): evidence that it is closely related to nitric oxide (NO) radical (abstr). Circulation 1986; 74(suppl II):287.

33. Palmer RMJ, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524–526.
34. Palmer RMJ, Ashton DS, Moncada S: Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988;333:664–666.
35. Palmer RMJ, Moncada S: A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. Biochem Biophys Res Comm 1989;158:348–352.
36. Rubanyi GM, Romero JC, Vanhoutte PM: Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986;250:H1145–H1149.
37. Pohl U, Holtz J, Busse R, et al: Crucial role of endothelium in the vasodilator response to increased flow in vivo. Hypertension 1986;8:37–44.
38. De Mey JG, Claeys M, Vanhoutte PM: Endothelium-dependent inhibitory effects of acetylcholine, adenosine diphosphate, thrombin and arachidonic acid in the canine femoral artery. J Pharm Exp Ther 1982; 222:166–173.
39. Cohen RA, Shepherd JT, Vanhoutte PM: Inhibitory role of the endothelium in the response of isolated coronary arteries to platelets. Science 1983;221:273–274.
40. Cohen RA, Shepherd JT, Vanhoutte PM: 5-Hydroxytryptamine can mediate endothelium-dependent relaxations of coronary arteries. Am J Physiol 1983; 245:H1077–H1080.
41. Houston DS, Shepherd JT, Vanhoutte PM: Adenine nucleotides, serotonin and endothelium-dependent relaxations to platelets. Am J Physiol 1985;248:H389–H395.
42. Cocks TM, Angus JA, Campbell JH, et al: Release and properties of endothelium-derived relaxing factor (EDRF) from endothelial cells in culture. J Cell Physiol 1985;123:310–320.
43. Van de Voorde J, Leusen I: Role of the endothelium in the vasodilator response of rat thoracic aorta to histamine. Eur J Pharmacol 1983;87:113–120.
44. Cocks TM, Angus JA: Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature 1983;305:627–630.
45. Zawadzki JV, Furchgott RF, Cherry P: The obligatory role of endothelial cells in the relaxations of arterial smooth muscle by substance P (abstr). Fed Proc 1981;40:689.
46. Katusic ZS, Shepherd JT, Vanhoutte PM: Vasopressin causes endothelium-dependent relaxations of the canine basilar artery. Circ Res 1984;55:575–579.
47. Förstermann U, Mügge A, Bode SM, et al: Response of human coronary arteries to aggregating platelets: importance of endothelium-derived relaxing factor and prostanoids. Circ Res 1988;63:306–312.
48. Shimokawa H, Aarhus LL, Vanhoutte PM: Porcine coronary arteries with regenerated endothelium have a reduced endothelium-dependent responsiveness to aggregating platelets and serotonin. Circ Res 1987; 61:256–270.
49. Lüscher TF, Cooke JP, Houston DS, et al: Endothelium-dependent relaxations in human peripheral and renal arteries. Mayo Clin Proc 1987;62:601–606.
50. Lüscher TF, Diederich D, Siebenmann R, et al: Difference between endothelium-dependent relaxations in arterial and in venous coronary bypass grafts. N Engl J Med 1988;319:462–467.
51. Shimokawa H, Flavahan NA, Lorenz RR, et al: Prostacyclin releases endothelium-derived relaxing factor and potentiates its action in coronary arteries of the pig. Br J Pharmacol 1988;95:1197–1203.
52. Vanhoutte PM: The end of the quest? Nature 1987;327:459–460.
53. De Mey J, Vanhoutte PM: Role of Na^+, K^+-ATPase in the vasodilator response to acetylcholine, *in:* Vanhoutte PM, Leusen I (eds): Vasodilatation. New York, Raven Press, 1981, pp 331–337.
54. Feletou M, Vanhoutte PM: Endothelium-dependent hyperpolarization of canine coronary smooth muscle. Br J Pharmacol 1988;93:515–524.
55. Komori K, Lorenz RR, Vanhoutte PM: Nitric oxide, acetylcholine, and electrical and mechanical properties of canine arterial smooth muscle. Am J Physiol 1988;255:H207–H212.
56. Hickey KA, Rubanyi GM, Paul RJ, et al: Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. Am J Physiol 1985;248:C550–C556.
57. Gillespie MN, Owasoyo JO, McMurtry IF, et al: Sustained coronary vasoconstriction provoked by a peptidergic substance released from endothelial cells in culture. J Pharm Exp Ther 1986;236:339–343.
58. Masaki T: The discovery, the present state, and the future prospects of endothelin. J Cardiovasc Pharmacol 1989;13(suppl 5):1–4.
59. Yanagisawa M, Inoue A, Takuwa Y, et al: The human preproendothelin gene: possible regulation by endothelial phosphoinositide turnover signaling. J Cardiovasc Pharmacol 1989;13(suppl 5):13–17.
60. Yanagisawa M, Inoue A, Ishikawa T, et al: Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. Proc Natl Acad Sci USA 1988;85:6964–6967.
61. Schini VB, Hendrickson H, Heublein DM, et al: Thrombin enhances the release of endothelin from cultured porcine aortic endothelial cells. Eur J Pharmacol 1989; (in press).
62. Hirata Y, Yoshimi H, Takata S, et al: Cellular mechanism of action by a novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. Biochem Biophys Res Comm 1988;154:868–875.
63. Resink TJ, Scott-Burden T, Bühler FR: Endothelin stimulates phospholipase C in cultured vascular smooth muscle cells. Biochem Biophys Res Comm 1989; 157:1360–1368.
64. Resink TJ, Scott-Burden T, Bühler FR: Activation of phospholipase A_2 by endothelin in cultured vascular smooth muscle cells. Biochem Biophys Res Comm 1989;158:279–286.
65. Wallnoefer A, Weir S, Ruegg U, et al: The mechanism of action of endothelin-1 as compared with other agonists

in vascular smooth muscle. J Cardiovasc Pharmacol 1989;13(suppl 5):23–31.

66. Brain SD, Crossman DC, Buckley TL, et al: Endothelin-1: demonstration of potent effects on the microcirculation of humans and other species. J Cardiovasc Pharmacol 1989;13(suppl 5):147–149.
67. Hiley CR, Douglas SA, Randall MD: Pressor effects of endothelin-1 and some analogs in the perfused superior mesenteric arterial bed of the rat. J Cardiovasc Pharmacol 1989;13(suppl 5):197–199.
68. Yang Z, Bühler DR, Diederich D, et al: Different effects of endothelin-1 on cAMP- and cGMP-mediated vascular relaxation in human arteries and veins: comparison with norepinephrine. J Cardiovasc Pharmacol 1989; 13(suppl 5):129–131.
69. Watanabe TX, Kumagaye S-I, Nishio H, et al: Effects of endothelin-1 and endothelin-3 on blood pressure in conscious hypertensive rats. J Cardiovasc Pharmacol 1989;13(suppl 5):207–208.
70. Clarke JG, Larkin SW, Benjamin N, et al: Endothelin-1 is a potent long-lasting vasoconstrictor in dog peripheral vasculature in vivo. J Cardiovasc Pharmacol 1989;13(suppl 5):211–212.
71. deNucci G, Thomas R, D'Orleans-Juste P, et al: Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. Proc Natl Acad Sci USA 1988;85:9797–9800.
72. Yang Z, Richard V, Lüscher TF: unpublished observation, 1989.
73. Vanhoutte PM, Auch-Schwelk W, Boulanger C, et al: Does endothelin-1 mediate endothelium-dependent contractions curing anoxia? J Cardiovasc Pharmacol 1989;13(suppl 5):124–128.
74. Katusic ZS, Shepherd JT, Vanhoutte PM: Endothelium-dependent contractions to stretch in canine basilar arteries. Am J Physiol 1987a;252:H671–H673.
75. Savitzky JP, Doczi J, Black J, Arnold JD: A clinical safety trial of stroma-free hemoglobin. Clin Pharmacol Ther 1978;23:73–80.
76. Rees DD, Palmer RMJ, Moncada S: Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc Natl Acad Sci USA 1989;86:3375–3378.
77. Rinaldi G, Bohr D. Endothelium-mediated spontaneous response in aortic rings of deoxycorticosterone acetate-hypertensive rats. Hypertension 1989;13:256–261.
78. Suzuki N, Matsumoto H, Kitada C, et al: Immunoreactive endothelin-1 in plasma detected by a sandwich-type enzyme immunoassay. J Cardiovasc Pharm 1989;13(suppl 5):151–153.
79. Ando K, Hirata Y, Schichiri M, et al: Presence of immunoreactive endothelin in human plasma. FEBS Lett 1989;245:164–166.
80. Tabuchi Y, Nakamaru M, Rakugi H, et al: Endothelin enhances adrenergic vasoconstriction in perfused rat mesenteric arteries. Biochem Biophys Res Comm 1989;159:1304–1308.
81. Godfraind T, Mennig D, Morel N, Wibo M: Effect of endothelin-1 on calcium channel gating by agonists in vascular smooth muscle. J Cardiovasc Pharmacol 1989;13(suppl 5):112–117.
82. Yang Z, Richard V, Von Segesses L, et al: Endothelin-1 potentiates contractions to norepinephrine and serotonin in human mammary and coronary arteries (abst). J Amer Coll Cardiol (in press).
83. Lamping KG, Dole WP: Acute hypertension selectively potentiates constrictor responses of large coronary arteries to serotonin by altering endothelial function in vivo. Circ Res 1987;61:904–913.
84. Kontos HA: Oxygen radicals in cerebral vascular injury. Circ Res 1985;57:508–516.
85. Konishi M, Su C: Role of endothelium in dilator responses of spontaneously hypertensive rat arteries. Hypertension 1983;5:881–886.
86. Winquist RJ, Bunting PB, Baskin EP, et al: Decreased endothelium-dependent relaxation in New Zealand genetic hypertensive rats. J Hypertens 1984;2:536–541.
87. Lüscher TF, Vanhoutte PM: Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. Hypertension 1986;8:344–348.
88. Van de Voorde J, Cuvelier C, Leusen I: Endothelium-dependent relaxation effects in aorta from hypertensive rats. Arch Int Physiol Biochem 1984;92:P10–P11.
89. Van de Voorde J, Leusen I: Endothelium-dependent and independent relaxation of aortic rings from hypertensive rats. Am J Physiol 1986;250:H711–H717.
90. Lüscher TF, Raij L, Vanhoutte PM: Endothelium-dependent responses in normotensive and hypertensive Dahl rats. Hypertension 1987;9:157–163.
91. Lockette WE, Otsuha Y, Carretero OA. Endothelium-dependent relaxation in hypertension. Hypertension 1986;8(suppl II):61–66.
92. Lüscher TF, Diederich D, Vanhoutte PM, et al: Endothelium-dependent responses in the common carotid and renal artery of normotensive and spontaneously hypertensive rats. Hypertension 1988;11:573–578.
93. Hongo K, Nakagomi T, Kassell NF, et al: Effects of aging and hypertension on endothelium-dependent vascular relaxation in rat carotid artery. Stroke 1988; 19:892–897.
94. De Mey JG, Gray SD. Endothelium-dependent reactivity in resistance vessels. Prog Appl Microcirc 1985;88:181–187.
95. Tesfamariam B, Halpern W: Endothelium-dependent and endothelium-independent vasodilation in resistance arteries from hypertensive rats. Hypertension 1988;11:440–444.
96. Lüscher TF, Aarhus LL, Vanhoutte PM: Indomethacin enhances the impaired endothelium-dependent relaxations in small mesenteric arteries of the spontaneously hypertensive rat. Am J Hypertens 1990;3:55–59.
97. Diederich D, Bühler FR, Lüscher TF: Endothelium-derived relaxing factor (EDRF) and nitric oxide (NO) in mesenteric resistance arteries of normotensive and hypertensive rats (abstr). Hypertension 1988;12:334.
98. Lüscher TF, Diederich D, Yang Z, Bühler FR: Endothelin overrides endothelium-derived relaxing factor in

hypertensive resistance arteries (abstr). Kidney Int 1989;35(1):331.

99. Panza JA, Quyyumi AA, Epstein SE: Impaired endothelium-dependent vascular relaxation in hypertensive patients (abstr). Circulation 1988;78(suppl II):473.
100. Linder L, Kiowski W, Bühler FR, Lüscher TF: unpublished observation, 1989.
101. Lüscher TF, Raij L, Vanhoutte PM: Effect of hypertension and its reversal on endothelium-dependent relaxations in the rat aorta. J Hypertens 1987;5(suppl 5):153-155.
102. Lüscher TF, Vanhoutte PM, Raij L: Antihypertensive therapy normalizes endothelium-dependent relaxations in salt-induced hypertension of the rat. Hypertension 1987;9(suppl III):193-197.
103. Criscione L, Bowell JR, Budet R, Engesser S: Reduced endothelium-dependent relaxations in spontaneously hypertensive rats is the result not the cause of hypertension (abst). Hochdruck 1988;8:38.
104. Webb RC, Vander AJ, Henry JP: Increased vasodilator responses to acetylcholine in psychosocial hypertensive mice. Hypertension 1987;9:268-276.
105. Wright CE, Angus JA: Effects of hypertension and hypercholesteremia on vasodilatation in the rabbit. Hypertension 1986;8:361-371.
106. Chobanian AV, Brecher PI, Haudenschild CC: Effects of hypertension and of antihypertensive therapy on atherosclerosis. Hypertension 1986;8(suppl I):15-21.
107. Hazama F, Ozaki T, Amano S: Scanning electron microscopic study of endothelial cells of cerebral arteries from spontaneously hypertensive rats. Stroke 1979; 10:245-252.
108. Lüscher TF, Vanhoutte PM: Endothelium-dependent responses to aggregating platelets and serotonin in spontaneously hypertensive rats. Hypertension 1986;8(suppl II):55-60.
109. De Clerck F: Blood platelets in human essential hypertension. Agents Actions 1986;18:563-580.
110. Auch-Schwelk W, Katusic Z, Vanhoutte PM: Contractions to oxygen derived free radicals are augmented in the aorta of the spontaneously hypertensive rat. Hypertension 1989;13:859-864.
111. Auch-Schwelk W, Katusic ZS, Vanhoutte PM: Endothelium-dependent contractions in the SHR aorta are inhibited by thromboxane A_2 receptor antagonists (abstr). J Vasc Med Biol 1989;1:76.
112. Koga T, Takata Y, Kobayashi K, et al: Age and hypertension promote endothelium-dependent contractions to acetylcholine in the aorta of the rat. Hypertension 1989; (in press).
113. Lüscher TF, Romero JC, Vanhoutte PM: Bioassay of endothelium-derived vasoactive substances in the aorta of normotensive and spontaneously hypertensive rats. J Hypertens 1986;4(suppl 6):81-83.
114. Lüscher TF, Rubanyi GM, Aarhus LL, et al: Serotonin reduces coronary flow in isolated hearts of the spontaneously hypertensive rat. J Hypertens 1986;4(suppl 5):148-150.
115. Mayhan WG, Faraci FM, Heistad DD: Impairment of endothelium-dependent responses of cerebral arterioles in chronic hypertension. Am J Physiol 1987; 253:H1435-H1440.
116. Van de Voorde J, Leusen I: Endothelium-dependent and independent relaxation effects on aorta preparations of renal hypertensive rats. Arch Int Physiol Biochem 1984;92:P35-P36.
117. Hoeffner U, Vanhoutte PM: Increases in flow reduce the release of endothelium-derived relaxing factor in the aorta of normotensive and spontaneously hypertensive rats. Am J Hypertens 1989;2:762-767.
118. Cocks TM, Manderson JA, Mosse PRL, et al: Development of a large fibromuscular intimal thickening does not impair endothelium-dependent relaxations in the rabbit carotid artery. Blood Vessels 1987;24:192-200.
119. Cohen ML, Berkowitz BA: Decreased vascular relaxation in hypertension. J Pharmacol Exp Ther 1976; 196:396-406.
120. Shirasaki Y, Su C, Lee TJ-F, et al: Endothelial modulation of vascular relaxation to nitrovasodilators in aging and hypertension. J Pharmacol Exp Ther 1986; 239:861-866.
121. Tomobe Y, Miyauchi T, Saito A, et al: Effects of endothelin on the renal artery from spontaneously hypertensive and Wistar Kyoto rats. Eur J Pharmacol 1988; 152:373-374.
122. Ross R: The pathogenesis of atherosclerosis—an update. N Engl J Med 1986;314:488-500.
123. Andrews HE, Bruckdorfer KR, Dunn RC, et al: Low-density lipoproteins inhibit endothelium-dependent relaxation in rabbit aorta. Nature 1987;327:237-239.
124. Bassenge E, Galle J: Suppression of EDRF-mediated vasodilatation by low density lipoproteins by two different mechanisms. J Vasc Biol Med 1989;1:77.
125. Boulanger C, Bühler FR, Lüscher TF: Low density lipoproteins impair the release of endothelium-derived relaxing factor from cultured porcine endothelial cells (abstr). Eur Heart J 1989;10:331.
126. Grag UC, Hassid A: Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured vascular smooth muscle cells. J Clin Invest 1989;83:1774-1777.
127. Shimokawa H, Vanhoutte PM: Impaired endothelium-dependent relaxation to aggregating platelets and related vasoactive substances in porcine coronary arteries in hypercholesterolemia and atherosclerosis. Circ Res 1989;64:900-914.
128. Bossaller C, Habib GB, Yamamoto H, et al: Impaired muscarinic endothelium-dependent relaxation and cyclic guanosine 5'-monophosphate formation in atherosclerotic human coronary artery and rabbit aorta. J Clin Invest 1987;79:170-174.
129. Förstermann U, Mügge A, Alheid U, et al: Selective attenuation of endothelium-mediated vasodilation in atherosclerotic human coronary arteries. Circ Res 1988;62:185-190.
130. Verbeuren TJ, Jordaens FH, Zonnekeyn LL, et al: Effect of hypercholesteremia in vascular reactivity in the rabbit. I. Endothelium-dependent relaxations in isolated arteries of control and hypercholesteremic rabbits. Circ Res 1986;58:552-564.

131. Freiman PC, Mitchell GG, Heistad DD, et al: Atherosclerosis impairs endothelium-dependent vascular relaxation to acetylcholine and thrombin in primates. Circ Res 1986;58:783–789.

132. Ludmer PL, Selwyn AP, Shook TL, et al: Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986; 315:1046–1051.

133. Spokas EG, Folco G, Quilley J, et al: Endothelial mechanism in the vascular action of hydralazine. Hypertension 1983;5(suppl I):107–111.

134. Schultz P, Tolins J, Raij L: Effect of oral administration of antihypertensive agents on endothelium dependent and endothelium independent vascular relaxation (abstr). J Amer Col Cardiol 1989;13:83A.

135. Laragh JH, Baer L, Brunner HR, et al: Renin, angiotensin and aldosterone system in pathogenesis and management of hypertensive vascular disease. Am J Med 1972;52:633–652.

136. Limas C, Westrum B, Limas CJ, et al: Effect of salt on the vascular lesions of spontaneously hypertensive rats. Hypertension 1980;2:477–489.

137. Khaw K-T, Barrett-Connor E: Dietary potassium and stroke-associated mortality. N Engl J Med 1987; 316:235–240.

138. Tobian L, Lange JM, Ulm KM, et al: Potassium prevents death from strokes in hypertensive rats without lowering blood pressure. J Hypertens 1984;2(suppl 3):363–366.

139. Tobian L, MacNeill D, Johnson MA, et al: Potassium protection against lesions of the renal tubules, arteries, and glomeruli and nephron loss in salt-loaded hypertensive rats. Hypertension 1984;6(suppl I):170–176.

140. Raij L, Lüscher TF, Vanhoutte PM: High dietary potassium augments endothelium-dependent relaxations in the Dahl rat. Hypertension 1988;12:562–567.

141. Ballard-Barbash R, Callaway CW: Marine fish oils: Role in prevention of coronary artery disease. Mayo Clin Proc 1987;62:113–118.

142. Weiner BH, Ockene IS, Levine PH, et al: Inhibition of atherosclerosis by cod-liver oil in a atherosclerotic swine model. N Engl J Med 1986;315:841–846.

143. Shimokawa H, Vanhoutte PM: Dietary cod-liver oil improves endothelium-dependent responses in hypercholesterolemic and atherosclerotic porcine coronary arteries. Circulation 1988;78:1421–1430.

144. Shimokawa I, Aarhus LL, Vanhoutte PM. Dietary $\omega 3$ polyunsaturated fatty acids augment endothelium-dependent relaxation to bradykinin in coronary microvessels of the pig. Br J Pharmacol 1988;95:1191–1196.

145. Boulanger C, Schini VB, Vanhoutte PM: Effect of chronic exposure to eicosapentaenoic acid on the release of relaxing factor and the production of cyclic GMP by cultured endothelial cells (abstr). J Vasc Med Biol 1989;1:79.

146. Knapp HR, FitzGerald GT: The antihypertensive effect of fish-oil. N Engl J Med 1989;320:1837–1843.