Endothelium-Dependent Responses in Hypertension

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The endothelium controls the tone of the underlying vascular smooth muscle by releasing relaxing factors. These include prostacyclin, nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF). In certain types of hypertension, endothelium-dependent relaxations are curtailed, because of a reduced production and/or action of endothelium-derived NO and EDHF. In the spontaneously hypertensive rat, endothelium-dependent relaxations are reduced, because of the endothelium-dependent production of vasoconstrictor prostanoids (endoperoxides and, in some cases, thromboxane A₂). These prostanoids may be produced in the vascular smooth muscle cells rather than in the endothelium. The endothelial dysfunction observed in hypertensive blood vessels is likely to be a consequence rather than a cause of the disease process. (*Hypertens Res* 1995; 18: 87-98)

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In 1980, Furchgott and Zawadzki reported the obligatory role of the endothelial cells in relaxations of isolated rabbit aortas in response to acetylcholine (1). Their initial observation has been rapidly expanded to a wide variety of blood vessels, including human arteries (2) (Fig. 1), and it is now obvious that the endothelial cells can respond to numerous neurohumoral mediators (Fig. 2) by releasing relaxing factors. The endothelium contributes to the local control of vasomotor tone also in the intact organism (3-13). The endothelium-derived relaxing factors include, besides prostacyclin (14), two cyclooxygenase-independent diffusible substances (Fig. 3): (a) nitric oxide (NO), or a closely related compound, derived from the metabolism of L-arginine through the action of the constitutive NO-synthase (15-19); and (b) endothelium-derived hyperpolarizing factor (EDHF), which presumably is a labile metabolite of arachidonic acid possibly produced through the P450-pathway (20-24). The release of EDRF/NO by the endothelial cells can be triggered by activation of cell membrane receptors, linked to NO-synthase by G-proteins that differ depending on the agonist used (Fig. 4) (8, 25-28). Endothelium-derived factors, in particular NO, not only help to control locally the tone of vascular smooth muscle cells, but also modulate their growth, and brake the tendency of platelets to adhere and aggregate, as well as the adherence of other blood components (Fig. 4) (8, 13, 19, 29-31).

Shortly after the discovery of the role of endothelium-derived relaxing factor (1), De Mey and Vanhoutte demonstrated that endothelial cells can also direct the underlying vascular smooth muscle to contract (32, 33). Endothelium-dependent contractions can be explained either by the withdrawal of the release of EDRFs, or by the produc-

tion of diffusible vasoconstrictor substances (EDCF) (5, 6, 11, 12, 34-40). The EDCFs identified to date include superoxide anions which presumably act by scavenging NO (41), endoperoxides, thromboxane A₂ and the peptide endothelin-1 (Fig. 5). The latter is more likely to play a role, if any, in long-term modulation of vascular tone and structure than in moment-to-moment changes in the degree of contraction of vascular smooth muscle (42-49).

Under several pathological conditions, the endothelial cells are dysfunctional (3, 4, 8, 26, 27, 31, 50-53). Usually this dysfunction expresses itself as an impairment in endothelium-dependent relaxations, due either to a reduced release (or action) of EDRFs, and/or a greater propensity to evoke endothelium-dependent contractions. This is particularly the case in hypertension. This brief review updates earlier discussions of the endothelial dysfunction, in terms of vasomotor control, of the hypertensive blood vessel wall, with special focus on work in the area of endothelium-dependent contractions from the authors' laboratory. The putative role of endothelin in hypertension has been discussed elsewhere (48, 49).

Endothelium-Dependent Relaxations

Since the original report by Konishi and Su (54) that endothelium-dependent relaxations are reduced in arteries from spontaneously hypertensive rats, the phenomenon has been documented repeatedly in blood vessels from different experimental models of the disease (7, 8, 52, 53, 55-58). Similarly, in the forearm circulation of hypertensive patients, the vasodilator response to the intra-arterial injection of acetylcholine is blunted (*e.g.* 59-61) albeit not observed in all studies (62). These findings concur

to suggest that the release of EDRF is abnormal in hypertensive blood vessels.

Inhibitors of NO synthases cause a sustained increase in arterial pressure in the intact animal (*e.g.* 63-65) and peripheral vasoconstriction in normotensive humans (66, 67). Thus endothelium-derived NO may contribute to the overall regulation of arterial blood pressure by virtue of its ability to relax vascular smooth muscle. This interpretation rests

heavily on the assumption that the effect of NO-synthase inhibitors is due solely to the suppression of the direct vasodilator effect of NO. Such an assumption may be hazardous in view of the demonstration that NO can modulate other factors which contribute to cardiovascular homeostasis. Examples are the inhibitory effect of EDRF/NO on the release of renin (Fig. 6) (68-70) and that of endothelin (71), or the modulatory effect of EDRF/NO on the excretory function of the kidney (72-74). However, since inhibitors of NO-synthases cause less vasoconstriction in the forearm of hypertensive than normotensive subjects (75-77), a reduced efficiency of the L-arginine NO pathway may contribute to the increased peripheral resistance characteristic of chronic hypertension. A similar conclusion may explain the blunted response to endothelium-dependent vasodilators in isolated blood vessels from hypertensive animals. Thus, for example, in the aorta of the hypertensive salt-sensitive Dahl rat, the endothelium-dependent relaxation to acetylcholine is diminished (Fig. 7), suggesting a reduced release of EDRF (78). However, the response of the vascular smooth muscle to exogenous donors of NO is also reduced (Fig. 8) (78), which implies that the lower efficiency of the L-arginine NO pathway is due in part to an impaired responsiveness of the hypertensive vascular smooth muscle to EDRF.

Not all reduced endothelium-dependent relaxations to acetylcholine (and other agonists) observed in arteries from hypertensive animals and humans can be attributed to abnormalities in the L-arginine NO pathway. Indeed, in arteries of the SHR, the blunted responses are observed despite a normal release of EDRF (79-81). A normal responsiveness to endothelium-dependent vasodilators can be restored by inhibitors of cyclooxygenase (Fig. 9) (79). Thus,

Fig. 1. Endothelium-dependent relaxations induced by acetylcholine in isolated human renal arteries (left panel) and peripheral arteries (right panel), under control conditions or after incubation with indomethacin (10^{-5} M), during contraction with norepinephrine (renal arteries) or prostaglandin $F_{2\alpha}$ (peripheral arteries). Asterisks denote a statistically significant effect of indomethacin (From Ref. Lüscher et al: Endothelium-dependent relaxations in human arteries. Mayo Clinic Proceedings 1987; 62: 601-606, by permission).

Fig. 2. Neurohumoral mediators which cause the release of endothelium-derived relaxing factor (EDRF) through activation of specific endothelial receptors (circles). In addition, EDRF can be released independently of receptor-operated mechanisms by the calcium ionophore A23187 and by increases in shear stress (not shown). A=adrenaline (epinephrine); AA=arachidonic acid; ACh=acetylcholine; ADP=adenosine diphosphate; α =alpha adrenergic receptor; AVP=arginine vasopressin; B=kinin receptor; ET=endothelin receptor; H=histaminergic receptor; 5-HT=serotonin (5-hydroxytryptamine), serotonergic receptor; M=muscarinic receptor; NA=noradrenaline (norepinephrine); P=purinergic receptor; T=thrombin receptor; VP=vasopressinergic receptor. (Adapted from Lüscher and Vanhoutte, 1990).

Fig. 3. Role of the increase in cytosolic calcium concentration in the release of EDRF(s). Endothelial receptor (R) activation and the Ca^{2+} -ionophore A23187 increase the cytosolic concentration of calcium in the endothelial cell; following interaction with calmodulin, this activates NO-synthase and the (unidentified) metabolic pathway leading to the release of endothelium-derived hyperpolarizing factor (EDHF). NO causes relaxation by activating the formation of cyclic GMP (cGMP) from GTP. EDHF causes hyperpolarization and relaxation by opening K^+ channels. Any increase in cytosolic calcium (including that induced by the calcium ionophore A23187) causes the release of relaxing factors when agonists activate the endothelial cells. An increase in inositol phosphate (IP_3) may contribute to the increase in cytoplasmic Ca^{2+} by releasing it from the sarcoplasmic reticulum (SR). (Adapted from Vanhoutte et al, 1993).

in this model of hypertension, the reduced endothelium-dependent relaxations are explained best by the concomitant release of NO and EDCF, with the latter "scavenging" the former (see below). Likewise, a reduced release of EDHF may contribute to abnormal endothelium-dependent responses, since in the aortas of both renal and spontaneously hypertensive rats, endothelium-dependent hyperpolarizations are reduced (82, 83).

Fig. 5. A number of physicochemical stimuli, neurohumoral mediators, and the calcium ionophore A23187 can evoke endothelium-dependent contractions in certain blood vessels, presumably because they evoke the release of endothelium-derived contracting factor(s) (EDCF). AA = arachidonic acid; ACh = acetylcholine; ADP = adenosine diphosphate; 5-HT = serotonin (5-hydroxytryptamine), serotonergic receptor; M = muscarinic receptor; P = purinergic receptor. (Adapted from Lüscher and Vanhoutte, 1990).

Fig. 4. Postulated signal transduction processes in a control porcine endothelial cell. Activation of the cell causes the release of EDRF-NO, which has important protective effects in the vascular wall. α = alpha-adrenergic; 5-HT = serotonin-receptor; ET = endothelin receptors; B = bradykinin receptor; P = purinoceptor; G = coupling proteins; cAMP = cyclic adenosine monophosphate; NO = nitric oxide; LDL = low density lipoproteins. (Adapted from Flavahan and Vanhoutte, 1995).

Fig. 6. The endothelial cells of the juxtaglomerular apparatus may exert an inhibitory effect on renin release through the release of endothelium-derived relaxing factor(s) (EDRF). If this was activated by increases in shear forces, the endothelium may constitute the "intrarenal baroreceptor", β = beta-adrenoceptor, and NE = norepinephrine. (From Lüscher and Vanhoutte, by permission).

Fig. 7. Endothelium-dependent relaxations in response to acetylcholine in thoracic aortas of Dahl rats. The endothelial responses in salt-sensitive rats maintained on a diet containing 0.1% NaCl do not differ from those in salt-resistant rats. However, when the salt content is increased to 8% NaCl (open squares), the endothelium-dependent relaxation induced by acetylcholine in the now hypertensive Dahl salt-sensitive rat is decreased. (From Lüscher, Raj and Vanhoutte, 1987, by permission of the American Heart Association).

Fig. 8. Relaxations in response to sodium nitroprusside in thoracic aortic rings without endothelium taken from Dahl-salt-sensitive rats fed a high sodium diet (8% NaCl) or a low sodium diet (0.1% NaCl). The IC_{50} values of sodium nitroprusside were significantly higher in rats fed 8% NaCl as compared with animals fed 0.1% NaCl. (From Lüscher, Raj and Vanhoutte, 1987, by permission of the American Heart Association).

Causes or Consequences?

Since the endothelial abnormality observed in the aorta of the hypertensive salt-sensitive Dahl-rat is reversed by appropriate antihypertensive therapy (84), the reduced endothelium-dependent relaxation must be viewed as a consequence, rather than a cause, of the high blood pressure. Likewise, in hypertensive patients, the peripheral vasoconstrictor response to NO-inhibitors of NO-synthases is normalized by anti-hypertensive agents (77). In the arteries of the young SHR, the relaxation to acetylcholine is comparable to that observed in blood vessels from young normotensive controls, but they

become progressively impaired as the arterial blood pressure increases (85-87). This suggests that the partial loss of endothelium-dependent relaxation may result from premature aging of the blood vessel wall exposed chronically to the higher distending pressure. If this were so, one again would conclude that the curtailment of endothelium-dependent relaxations is not likely to play a primary role in the initiation of hypertension. However, at the later stages of the disease, it could contribute to the maintenance of an elevated peripheral vascular resistance, or favor the occurrence of complications such as atherosclerosis (8, 52). Premature aging also

Fig. 9. In the aorta with endothelium (contracted with norepinephrine) of the spontaneously hypertensive rat (SHR), indomethacin prevents the secondary rise in tension caused by higher concentrations of acetylcholine. In the presence of an inhibitor of cyclooxygenase, the effect of acetylcholine is no longer different (*) in preparations from spontaneously hypertensive and normotensive Wistar-Kyoto (WKY) rats. These experiments demonstrate that the blunted relaxation to higher concentrations of acetylcholine in the hypertensive aorta is due to the release of cyclooxygenase-dependent contracting factor. (From Lüscher and Vanhoutte 1986, by permission of the American Heart Association).

would help to explain the reduction in endothelium-dependent hyperpolarisation observed in hypertensive arteries (82, 88), as this type of response decreases markedly with age, even in normotensive animals (88, 89).

Therapeutic Implications

If a reduced release of EDRF/NO, and/or EDHF, contributes to the maintenance of hypertension, or accelerates the occurrence of vascular complications, therapeutic agents reinforcing the ability of the endothelial cells to produce relaxing factors may be beneficial. Of the available antihypertensive agents, this seems to be particularly the case for converting enzyme inhibitors, which augment the release of both NO and EDHF triggered by endogenously formed or exogenously added bradykinin (Fig. 10) (90-92).

Endothelium-Dependent Contractions

In 1986, Lüscher and Vanhoutte (79) reported that in quiescent rings of SHR aortas, acetylcholine causes endothelium-dependent contractions (Fig. 11). Later work demonstrated the occurrence of similar endothelium-dependent contractions in response to arachidonic acid (93), serotonin (94, 95), endothelin (96-98), and adenine nucleotides (99). The release of EDCF caused by serotonin and ADP presumably helps to explain why the presence of the endothelium does not reduce the contractions to aggregating platelets in the aorta of the SHR, as it does in blood vessels from normotensive controls (94). The release of EDCF presumably also ex-

Fig. 10. ACE inhibitors and endothelium-derived vasodilator mediators. By inhibiting ACE (converting enzyme) which is abundantly expressed at the surface of endothelial cells, ACE inhibitors prevent both the generation of the potent vasoconstrictor angiotensin II, and the degradation of the powerful vasodilator bradykinin. Bradykinin, through activation of endothelial B₂-kinin receptors, induces the release of endothelium-derived hyperpolarizing factor (EDHF) and/or NO. (From Vanhoutte et al, 1993, by permission).

plains why serotonin causes cyclooxygenase-dependent vasoconstrictions in the heart and kidneys of SHR, but not of normotensive rats (100, 101).

The endothelial muscarinic receptors mediating the endothelium-dependent contractions to acetylcholine belong to the same subtype as those initiating the release of EDRF in the same preparation (Fig. 12) (102). A cyclooxygenase-dependent EDCF is involved, since the response can be prevented by indomethacin. As mentioned already (Fig. 9), the release of this EDCF explains the abnormal relaxations to acetylcholine observed in the aorta (79) and small resistance arteries (103, 104) of the SHR. The same may hold for certain types of human hypertension, because in essential hypertensive patients the local administration of indomethacin normalizes the vasodilator response to intra-arterial acetylcho-

line (61).

The endothelium-dependent contractions of the SHR aorta are augmented by inhibitors of nitric oxide synthase and by oxyhemoglobin (which scavenges NO), but not by methylene blue (which inhibits soluble guanylate cyclase and the relaxations caused by NO) (Fig. 14). The logical interpretation from those findings is that the EDCF released from the hypertensive endothelium interacts chemically with NO, and that both mediators inactivate each other (105, 106). Such a chemical interaction certainly would explain the blunting of endothelium-dependent relaxations (Fig. 9), despite a normal release of EDRF (79) and a normal responsiveness of the smooth muscle of the SHR aorta to exogenous NO-donors in the absence of endothelium (79).

Fig. 11. Isometric tension recordings in quiescent rings with (upper) and without (lower) endothelium of spontaneously hypertensive (left) and normotensive (right) rats. Acetylcholine causes endothelium-dependent contractions (note the phasic activity during the contraction) only in arteries from the hypertensive strain. (From Lüscher and Vanhoutte, 1990, by permission).

Fig. 12. Inhibition by the preferential M_3 muscarinic antagonist 4-DAMP of the contractions (left, in quiescent rings in the presence of NG -nitro-L-arginine) and relaxations (right, in rings contracted with norepinephrine in the presence of indomethacin) to acetylcholine in the SHR-aorta. The experiments were performed on parallel rings with endothelium, under control conditions (●) and in the presence of 4-DAMP ($10^{-9}M$ (△), $10^{-8}M$ (□), $10^{-7}M$ (○)). (Data from Boulanger et al, 1994).

Fig. 13. Effect of a thromboxane synthase inhibitor (dazoxiben; 10^{-4} M; ○) and thromboxane-endoperoxide receptor antagonists (AH23, 848, 10^{-6} M, ◇; SQ 29, 548, 10^{-6} M, ■ and R 68,070, 5×10^{-5} M, □) on endothelium-dependent contractions to acetylcholine in quiescent aortic rings of spontaneously hypertensive rats. The response to acetylcholine, represented in control conditions by full circles (●), is prevented by antagonists of the thromboxane-endoperoxide receptors but not affected by the inhibitor of thromboxane synthase. (From Auch-Schwelk et al, 1989, by permission of the American Heart Association).

The responses to acetylcholine, arachidonic acid, and adenine nucleotides are not prevented by inhibitors of thromboxane synthase, but are abolished by antagonists of endoperoxide/thromboxane receptors (Fig. 13) (79, 93, 99, 107-109). Similar conclusions have been reached concerning the contraction to arachidonic acid observed in aortas with the endothelium of rats with aortic coarctation (110). This prompted the suggestion that the EDCF involved is an endoperoxide (108, 109). Estimations of the release of prostaglandin H_2 have confirmed this interpretation (111, 112). The larger release of prostaglandin H_2 in the SHR may result from a greater production as suggested by cyclooxygenase expression (111) and/or an altered metabolism of the endoperoxide into prostanoids (110). In addition to a larger production of endoperoxides, an altered responsiveness of the hypertensive vascular smooth muscle contributes to endothelium-dependent contractions. Indeed, SHR aortas without endothelium contract more vigorously to endoperoxides than those of normotensive controls (111). In the case of endothelium-dependent contractions to endothelin, the endothelial mediator involved appears to be thromboxane A_2 (97, 98).

The endothelium-dependent contractions of the SHR aorta to acetylcholine are inhibited by indomethacin and meclofenamate (non-selective inhibitors of cyclooxygenase) (79) and tenidap, a preferential inhibitor of cyclooxygenase-1, (COX-1), but not by NS398, a preferential inhibitor of cyclooxygenase-2 (COX-2) (111, 112). These findings suggest that activation of COX-1 is involved in the response. This interpretation is strengthened by data demonstrating in the SHR aorta, compared with that of normotensive controls, a greater expression of COX-1, both at the messenger RNA and protein levels, and a larger production of endoperoxide(s) upon stimulation with acetylcholine (111, 112). However, the COX-1 expression in the

Fig. 14. Effect of inhibitors of endothelium-dependent relaxations on endothelium-dependent contractions to acetylcholine in quiescent aorta from spontaneously hypertensive rats. L-NMMA, N^G -monomethyl-L-arginine (inhibitor of NO production). (From Auch-Schwelk et al, 1990, by permission of the American Heart Association).

SHR aorta was comparable before and after removal of the endothelium (111, 112). This implies that the exaggerated production of endoperoxides in the hypertensive arteries may originate in vascular smooth muscle rather than the endothelium. This interpretation is in line with earlier work demonstrating that oxygen-derived free radicals cause contractions, which have been attributed to the production of endoperoxides and are larger in aortic rings without endothelium of the SHR than of normotensive controls (113). If the endoperoxides mediating the contractions to acetylcholine are formed by vascular smooth muscle cells, the endothelium-dependency of the response would have to be explained by the release of an endothelial mediator, inactivated by NO, that diffuses to the underlying smooth muscle to stimulate COX-1. Oxygen derived free radicals would be likely candidates (114), since they scavenge EDRF/NO (41) and cause cyclooxygenase-dependent contractions which are augmented in the SHR aorta without endothelium (107, 113). The role of free radicals acting as an endothelial signal would be compatible, in view of their extremely short half-life, with the repeated failure to bioassay the EDCF produced by the SHR aorta, under conditions where NO can be easily demonstrated (79). However, superoxide dismutase plus catalase, or deferoxamine do not inhibit endothelium-dependent contractions to acetylcholine in the SHR aorta (113).

Causes or Consequences?

In the spontaneously hypertensive rat, the occurrence of endothelium-dependent contractions is age-dependent, as are the cyclooxygenase-dependent vasoconstrictions evoked by serotonin in the kidney (101, 115). The phenomenon is observed also in older normotensive animals (99). In human arteries obtained from older patients, there is an indomethacin-sensitive blunting of the relaxation to

NORMOTENSIVE (WKY) **HYPERTENSIVE (SHR)**

Fig. 15. Stimulation of the thromboxane/endoperoxide receptor (TBA_2-R) is involved in endothelium-dependent contractions to ACh in the aorta of the SHR. However, the nature of the substance released from the endothelium in response to ACh is unknown. Two possibilities are suggested: (1) endoperoxides (PGH_2) are generated in the endothelium in response to acetylcholine or (2) the endothelium releases an EDCF (possibly oxygen-derived free radicals) which stimulate the production of endoperoxides in the vascular smooth muscle. NO=nitric oxide; COX-1=cyclooxygenase 1.

acetylcholine (Fig. 1, left), which is not seen in blood vessels from younger individuals (Fig. 1, right). In the aorta of younger normotensive rats, which unlike the SHR do not exhibit endothelium-dependent contractions to arachidonic acid, such response can be induced by incubation with interleukin 2; the cytokine does not affect the endothelium-dependent contractions of aortas of SHR of a comparable age (93). Taken in conjunction, these different observations suggest that the occurrence of the cyclooxygenase-dependent EDCF response may, again, reflect the premature aging of the blood vessel wall, accelerated by the hypertensive process. The experiments with interleukin 2 (93) imply further that in the aorta of the adult SHR, the EDCF-response is fully expressed, as it apparently cannot be upregulated by the cytokine. This interpretation is supported by molecular biology studies that demonstrate, in conjunction with a greater activity of cyclooxygenase, a larger expression of the enzyme mRNA in the SHR aorta compared to that of normotensive rats of the same adult age (111, 112).

Therapeutic Implications

Endothelium-dependent contractions are inhibited by Ca^{2+} channel inhibitors (116, 117), and by the diuretic vasodilator indapamide (118); it is tempting to assume that this action may contribute to the antihypertensive properties of these compounds. The improvement in arterial compliance that they cause in the SHR has been related to the antagonism of endothelium-dependent contractions (119). Likewise, in several models of hypertension, inhibitors of cyclooxygenase or antagonists of thromboxane/endoperoxide receptors lower arterial blood pressure (119, 120). Inhibitors of cyclooxygenase (but not of thromboxane synthase) and antagonists of endoperoxide/thromboxane receptors normalize arteriolar myogenic responses in the SHR

(121). Taken in conjunction with the normalisation of the forearm response to intra-arterial acetylcholine seen with indomethacin in essential hypertensives (see above) (61), these observations may prompt a closer, prospective look at the effects of cyclooxygenase inhibitors and antagonists of endoperoxide/thromboxane receptors on the course or the vascular complications of hypertension.

Conclusion

The hypertensive blood vessel wall exhibits reduced endothelium-dependent relaxations. Depending on the cause of the hypertension, this can be due to a reduced release or effect of EDRF/EDHF. In essential hypertension, the endothelial dysfunction resides in a greater propensity to release EDCF. The latter may be a short-lived radical that diffuses to the underlying vascular smooth muscle to activate their over-expressed cyclooxygenase (COX-1) to yield endoperoxides which are responsible for the endothelium-dependent contractions. These endothelial abnormalities probably are the consequence of the chronic exposure of the blood vessel wall to high blood pressure, and may reflect its premature aging.

References

1. Furchgott RF, Zawadzki JV: The obligatory role of the endothelial cells in relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; **288**: 373-376.
2. Lüscher TF, Cooke JP, Houston DS, Neves RJ, Vanhoutte PM: Endothelium-dependent relaxations in human arteries. *Mayo Clin Proc* 1987; **62**: 601-606.
3. Vanhoutte PM: State of the Art Lecture: endothelium and responsiveness of vascular smooth muscle. *J Hypertens* (suppl 5) 1987; **5**: S115-S120.
4. Vanhoutte PM: The endothelium-modulator of vascular smooth-muscle tone. *N Engl J Med* 1988;

- 319 (August 25): 512-513.
5. Vanhoutte PM: State of the Art Lecture: endothelium and control of vascular function. *Hypertension* 1989; **13** (No.6, Part 2): 658-667.
6. Furchgott RF, Vanhoutte PM: Endothelium-derived relaxing and contracting factors. *FASEB J* 1989; **3**: 2007-2018.
7. Vanhoutte PM: Endothelium-derived relaxing and contracting factors. *Adv Nephrol* 1990; **19**: 3-16.
8. Lüscher TF, Vanhoutte PM: The Endothelium: modulator of cardiovascular function. Boca Raton, CRC Press, Inc., 1990, pp 1-228.
9. Feletou M, Vanhoutte PM: Endothelium, endothelin and local hemodynamic control. *J Nephrol* 1990; **1**: 17-27.
10. Vanhoutte PM: Hypercholesterolaemia, atherosclerosis and release of endothelium-derived relaxing factor by aggregating platelets. *Eur Heart J* 1991; **12** (suppl E): 25-32.
11. Shepherd JT, Vanhoutte PM: Endothelium-derived relaxing (EDRF) and contracting factors (EDCF) in the control of cardiovascular homeostasis: the pioneering observations, in Rubanyi, GM (ed): Cardiovascular Significance of Endothelium-Derived Vasoactive Factors. Mount Kisco, NY, Futura Publishing Co., 1991, pp 39-64.
12. Pearson PJ, Vanhoutte PM: Vasodilator and vasoconstrictor substances produced by endothelium. *Rev Physiol Biochem Pharmacol* 1993; **122**: 1-67.
13. Vane JR: The Croonian Lecture, 1993: the endothelium: maestro of the blood circulation. *Philos Trans R Soc Lond B* 1994; **343**: 225-246.
14. Moncada S, Vane JR: Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A₂ and prostacyclin. *Pharmacol Rev* 1979; **30**: 293-331.
15. Furchgott RF, Cherry PD, Zawadzki JV: Endothelium-dependent relaxation of arteries by acetylcholine, bradykinin and other agents, in Vascular Neuroeffector Mechanisms: 4th Int Symp, in Bevan JA, Fujiwara M, Maxwell RA, Mohri K, Shibata S, Toda N (eds): New York, Raven Press, 1983; 37-43.
16. Ignarro LJ, Byrns RE, Wood KS: Biochemical and pharmacological properties of endothelium-derived relaxing factor and its similarity to nitric oxide radical, in Vanhoutte PM (ed): Vasodilatation: Vascular Smooth Muscle, Peptides, Autonomic Nerves and Endothelium. New York, Raven Press, 1988; pp 427-436.
17. Palmer RMJ, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; **327**: 524-526.
18. Palmer RMJ, Moncada S: A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. *Biochem Biophys Res Commun* 1989; **158**: 348-352.
19. Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; **43**: 109-142.
20. Feletou M, Vanhoutte PM: Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br J Pharmacol* 1988; **93**(3): 515-524.
21. Vanhoutte PM: The end of a quest? *Nature* 1987; **327**: 459-460.
22. Komori K, Vanhoutte PM: Endothelium-Derived Hyperpolarizing Factor. *Blood Vessels* 1990; **27**: 238-245.
23. Nagao T, Vanhoutte PM: Endothelium-derived hyperpolarizing factor and endothelium-dependent relaxations. *Am J Respir Cell Mol Biol* 1993; **8**: 1-6.
24. Vanhoutte PM: The other endothelium-derived vasoactive factors. *Circulation* 1993; **87**(suppl V): V-9-V-17.
25. Flavahan NA, Shimokawa H, Vanhoutte PM: Pertussis toxin inhibits endothelium-dependent relaxations to certain agonists in porcine coronary arteries. *J Physiol* 1989; **408**: 549-560.
26. Flavahan NA, Vanhoutte PM: G-Protein and Endothelial Responses. *Blood Vessels* 1990; **27**: 218-229.
27. Vanhoutte PM, Shimokawa H: Endothelium-derived relaxing factor(s) and coronary vasospasm. *Circulation* 1989; **80** (No. 1): 1-9.
28. Flavahan NA, Vanhoutte PM: Endothelial cell signaling and Endothelial Dysfunction. *Am J Hypertens* 1995 (in press).
29. Scott-Burden T, Vanhoutte PM: The endothelium as a regulator of vascular smooth muscle proliferation. *Circulation* 1993; **87** (suppl V): V-51-V-55.
30. Scott-Burden T, Vanhoutte PM: Regulation of smooth muscle cell growth by endothelium-derived factors. *Tex Heart Institute J* 1994; **21**(1): 91-97.
31. Vanhoutte PM, Scott-Burden T: The endothelium in health and disease. *Tex Heart Institute J* 1994; **21**(1): 62-67.
32. De Mey JG, Vanhoutte PM: Heterogeneous behavior of the canine arterial and venous wall: importance of the endothelium. *Circ Res* 1982; **51**: 439-447.
33. De Mey JG, Vanhoutte PM: Anoxia and endothelium dependent reactivity of the canine femoral artery. *J Physiol* 1983; **335**: 65-74.
34. Rubanyi GM, Vanhoutte PM: Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. *J Physiol* 1985; **364**: 45-56.
35. Vanhoutte PM: Endothelium and the control of vascular tissue. *News Physiol Sci* 1987; **2**: 18-22.
36. Vanhoutte PM: Endothelium-dependent contractions in arteries and veins. *Blood Vessels* 1987; **24**: 141-144.
37. Vanhoutte PM: Endothelium-derived relaxing and contracting factors. *Adv Nephrol* 1990; **19**: 3-16.
38. Vanhoutte PM, Lüscher TF: Endothelium-dependent vasoconstriction, in Rubanyi GM, Vanhoutte PM (eds): Endothelium-derived relaxing Factors. Karger Basel, 1990; pp 1-7.
39. Vanhoutte PM, Lüscher TF, Thomas F: Graser endothelium-dependent contractions. *Blood Vessels* 1991; **28** (No. 1-3): 74-83.
40. Vanhoutte PM, Gräser T, Lüscher TF: Endothelium-derived contracting factors, in GM Rubanyi (ed): Endothelin. Oxford, Oxford University Press, 1992, pp 3-16.
41. Rubanyi GM, Vanhoutte PM: Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor(s). *Am J Physiol* 1986; **250**: H822-H827.
42. Yanagisawa M, Kurihara H, Kimura S, et al: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; **332**: 411-415.
43. Masaki T, Kimura S, Yanagisawa M, Goto K: Molecular and cellular mechanisms of endothelin regulation. *Circulation* 1991; **84**: 1457-1468.
44. Masaki T, Yanagisawa M, Goto K: Physiology and pharmacology of endothelins. *Med Res Rev* 1992; **12**(4): 391-421.
45. Sakurai T, Yanagisawa M, Masaki T: Molecular characterization of endothelin receptors. *TIPS* 1992; **13**(3): 103-108.
46. Schini VB, Vanhoutte PM: Endothelin-1: a potent vasoactive peptide. *Pharmacol Toxicol* 1991; **69**: 1-7.
47. Sakurai S, Goto K: Endothelins: vascular actions and

- clinical implications. *Drugs* 1993; **46**(5): 795-804.
48. Vanhoutte PM: Is endothelin involved in the pathogenesis of hypertension? *Hypertension* 1993; **21**: 747-751.
 49. Vanhoutte PM: A matter of life and breath, *Nature* 1994; **368**: 693-694.
 50. Vanhoutte PM, Lüscher TF: Vascular endothelium and hypertension. *J Cardiovasc Pharmacol* 1987; **10** (suppl 4): S19-S24.
 51. Vanhoutte PM, Eber B: Endothelium-derived relaxing and contracting factors (Relaxierende und kontrahierende endothelfaktoren). *Wien Klin Wochenschr* 1991; **14**: 405-411.
 52. Lüscher TF, Vanhoutte PM: Dysfunction of the release of endothelium-derived relaxing factor, in Simionescu N, Simionescu M (eds): *Endothelial Cell Dysfunctions*. New York, Plenum Publishing Corporation, 1992, pp 65-102.
 53. Lüscher TF, Vanhoutte PM, Boulanger C, Dohi, Y and Bühler FR: Endothelial dysfunction in hypertension, in Rubanyi GM (ed): *Cardiovascular Significance of Endothelium-Derived Vasoactive Factors*, Mount Kisco, NY, Futura Publishing Co., 1991; pp 199-221.
 54. Konishi M, Su C: Role of endothelium in dilator responses of spontaneously hypertensive rat arteries. *Hypertension* 1983; **5**: 881-886.
 55. Bell DR: Vascular smooth muscle responses to endothelial autacoids in rats with chronic coarctation hypertension. *J Hypertens* 1993; **11**: 65-74.
 56. Boegehold MA: Enhanced arteriolar vasomotion in rats with chronic salt-induced hypertension. *Microvasc Res* 1993; **45**: 83-94.
 57. Mourlon Legrand MC, Benessiano J, Levy BI: Endothelium, mechanical compliance, and cGMP content in the carotid artery from spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 1993; **21**(suppl 1): S26-S30.
 58. Tuncer M, Vanhoutte PM: Response to the endothelium-dependent vasodilator acetylcholine in perfused kidneys of normotensive and spontaneously hypertensive rats. *Blood Pressure* 1993; **2**: 217-220.
 59. Linder L, Kiowski W, Bühler FR, Lüscher TF: Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation *in vivo*. *Circulation* 1990; **81**: 1762-1767.
 60. Panza JA, Quyyumi AA, Brush JE, Epstein SE: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990; **323**: 22.
 61. Taddei S, Virdis A, Mattei P, Salvetti A: Vasodilatation to acetylcholine in primary and secondary forms of human hypertension. *Hypertension* 1993; **21**: 929-933.
 62. Cockcroft JR, Chowiecnyk PJ, Benjamin N, Ritter JM: Preserved endothelium-dependent vasodilatation in patients with essential hypertension. *N Engl J Med* 1994; **330**: 1036-1040.
 63. Rees DD, Palmer RM, Moncada S: The role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* 1989; **86**: 3375-3378.
 64. Sigmon DH, Beierwaltes WH: Nitric oxide influences blood flow distribution in renovascular hypertension. *Hypertension* 1994; **23**(suppl I): I-34-I-39.
 65. Arnal JF, Warin L, Michel JB: Determinants of aortic cyclic guanosine monophosphate in hypertension induced by chronic inhibition of nitric oxide synthase. *J Clin Invest* 1992; **90**: 647-652.
 66. Vallance P, Collier J, Moncada S: Effects of endothelium derived nitric oxide on peripheral arterial tone in man. *Lancet* 1989; **ii**: 997-1000.
 67. Haynes WG, Noon JP, Walker BR, Webb DJ: Inhibition of nitric oxide synthesis increases blood pressure in healthy humans. *J Hypertens* 1993; **11**: 1375-1380.
 68. Vidal MJ, Romero JC, Vanhoutte PM: Endothelium-derived relaxing factor inhibits renin release. *Eur J Pharmacol* 1988; **149**: 401.
 69. Beirwaltes WH: Nitric oxide participates in calcium-mediated regulation of renin release. *Hypertension* 1994; **23**(suppl I): I-40-I-44.
 70. Vanhoutte PM, Boulanger CM, Vidal MJ, Mombouli JV: Endothelium-derived mediators and the renin-angiotensin system. in: *Renin-Angiotensin System Textbook*. London, Gower Medical Publishing, 1993, pp 29.1-29.15.
 71. Boulanger CM, Lüscher TF: Release of endothelin from the porcine aorta; inhibition by endothelium-derived nitric oxide. *J Clin Invest* 1990; **85**: 587-590.
 72. Biondi ML, Dousa T, Vanhoutte PM, Romero JC: Evidences for the existence of endothelium-derived relaxing factor in the renal medulla. *Am J Hypertens* 1990; **3**: 876-879.
 73. Lahera V, Salazar J, Salom MG, Romero JC: Deficient production of nitric oxide induces volume-dependent hypertension. *J Hypertens* 1992; **10**(suppl 7): S173-S177.
 74. Ruilope LM, Lahera V, Rodicio JL, JC Romero: Participation of nitric oxide in the regulation of renal function: possible role in the genesis of arterial hypertension. *J Hypertens* 1994; **12**: 625-631.
 75. Calver A, Collier J, Moncada S, Vallance P: Effect of local intraarterial N^G -monomethyl-L-arginine in patients with hypertension: the nitric oxide dilator mechanism appears abnormal. *J Hypertens* 1992; **10**: 1025-1031.
 76. Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA: Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation* 1993; **87**: 1468-1474.
 77. Lyons D, Webster J, Benjamin N: The effect of anti-hypertensive therapy on responsiveness to local intra-arterial N^G -monomethyl-L-arginine in patients with essential hypertension. *J Hypertens* 1994; **12**: 1047-1052.
 78. Lüscher TF, Raji L, Vanhoutte PM: Endothelium-dependent vascular responses in normotensive and hypertensive Dahl-rats. *Hypertension* 1987; **9**: 157-163.
 79. Lüscher TF, Vanhoutte PM: Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension* 1986; **8**: 344-348.
 80. Lüscher TF, Romero JC, Vanhoutte PM: Bioassay of endothelium-derived vasoactive substances in the aorta of normotensive and spontaneously hypertensive rats. *J Hypertens* 1987; **4**(suppl 6): S81-S83.
 81. Sawada Y, Sakamaki T, Nakamura T, Sato K, Ono Z, Murata K: Release of nitric oxide in response to acetylcholine is unaltered in spontaneously hypertensive rats. *J Hypertens* 1994; **12**: 745-750.
 82. Van de Voorde J, Vanheel B, Leusen I: Endothelium-dependent relaxation and hyperpolarization in aorta from control and renal hypertensive rats. *Circ Res* 1992; **70**(1): 1-8.
 83. Fujii K, Tominaga M, Ohmori S, et al: Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Circ Res* 1992; **70**(4): 660-669.

84. Lüscher TF, Vanhoutte PM, Raij L: Antihypertensive treatment normalizes decreased endothelium-dependent relaxations in salt-induced hypertension of the rat. *Hypertension* 1987; **9** [suppl III]: III-193-III-197.
85. Gray SD, De Mey JG: Vascular reactivity in neonatal spontaneously hypertensive rats. *Prog Appl Microcir* 1985; **8**: 173-180.
86. 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.
87. Dohi Y, Thiel M, Bühler FR, Lüscher TF: Activation of endothelial L-arginine pathway in resistance arteries: effect of age and hypertension. *Hypertension* 1990; **15**: 170-179.
88. Fujii K, Ohmori S, Tominaga M: Age-related changes in endothelium-dependent hyperpolarization in the rat mesenteric artery. *Am J Physiol* 1993; **265**(2 Pt2): h509-16.
89. Nakashima M, Vanhoutte PM: Age-dependent decrease in endothelium-dependent hyperpolarizations to endothelin-3 in the rat mesenteric artery. *J Cardiovasc Pharmacol* 1993; **22**(suppl 8): S352-S354.
90. Vanhoutte PM, Auch-Schwelk W, Biondi ML, Lorenz RR, Schini VB, Vidal MJ: Why are converting enzyme inhibitors vasodilators? *Br J Clin Pharmacol* 1989; **28**: 95S-104S.
91. Vanhoutte PM, Boulanger CM, Illiano C, Nagao T, Vidal M, Mombouli JV: Endothelium-dependent effects of converting-enzyme inhibitors. *J Cardiovasc Pharmacol* 1993; **22**(suppl 5): S10-S16.
92. Mombouli JV, Vanhoutte PM: Kinins and vascular endothelium. *Curr Sci Nephrol Hypertens* 1994; **3**: 481-484.
93. Boulanger CM, Vanhoutte PM: Interleukin-2 causes endothelium-dependent contractions to arachidonic acid. *Hypertension* 1993; **21**: 289-293.
94. Lüscher TF, Vanhoutte PM: Endothelium-dependent responses to platelets and serotonin in spontaneously hypertensive rats. *Hypertension* 1986; **8**(suppl II): II-55-II-60.
95. Auch-Schwelk W, Vanhoutte PM: Endothelium-derived contracting factor released by serotonin in the aorta of the SHR. *Am J Hypertens* 1991; **4**: 769-772.
96. Auch-Schwelk W, Vanhoutte PM: Contractions to endothelin in normotensive and spontaneously hypertensive rats: role of endothelium and prostaglandins. *Blood Pressure* 1992; **1**: 45-49.
97. Taddei S, Vanhoutte PM: Role of endothelium in endothelin-evoked contractions in the rat aorta. *Hypertension* 1993; **21**(1): 9-15.
98. Taddei S, Vanhoutte PM: Endothelium-dependent contractions to endothelin in the rat aorta are mediated by thromboxane A₂. *J Cardiovasc Pharmacol* 1993; **22**(suppl 8): S328-S331.
99. Mombouli JV, Vanhoutte PM: Purinergic endothelium-dependent and independent contractions in rat aorta. *Hypertension* 1993; **22**: 577-583.
100. Lüscher TF, Rubanyi GM, Aarhus LL, Edoute Y, Vanhoutte PM: Serotonin reduces coronary flow in the isolated heart of the spontaneously hypertensive rat. *J Hypertens* 1986; **4**(suppl 5): S148-S150.
101. Tuncer M, Vanhoutte PM: Serotonin releases a vasoconstrictor prostanoid in the kidney of the aging spontaneously hypertensive rat. *Blood Pressure* (in press).
102. Boulanger CM, Morrison KJ, Vanhoutte PM: M₃-Muscarinic receptors mediate both endothelium-dependent contraction and relaxation to acetylcholine in the aorta of the spontaneously hypertensive rat. *Br J Pharmacol* 1994; **112**: 519-524.
103. Luscher TF, Aarhus LL, Vanhoutte PM: Indomethacin improves the impaired endothelium-dependent relaxations in small mesenteric arteries of the spontaneously hypertensive rat. *Am J Hypertens* 1990; **3**: 55-58.
104. Takase H, Dohi Y, Kojima M, and Sato K: Changes in the endothelial cyclooxygenase pathway in Resistance arteries of spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 1994; **23**: 326-330.
105. Ito T, Kato T, Iwama Y, et al: Prostaglandin H₂ as an endothelium-derived contracting factor and its interaction with endothelium-derived nitric oxide. *J Hypertens* 1991; **9**: 729-736.
106. Auch-Schwelk W, Katusic ZS, Vanhoutte PM: Nitric oxide inactivates endothelium-derived contracting factor in the rat aorta. *Hypertension* 1992; **19**: 442-445.
107. Auch-Schwelk W, Katusic Z, and Vanhoutte PM: Endothelium dependent contractions in the SHR aorta are inhibited by thromboxane A₂ receptor antagonists, in GM Rubanyi, PM Vanhoutte (eds): Endothelium-Derived Relaxing Factors. Karger, Basel, 1990, pp 39-43.
108. Auch-Schwelk W, Katusic ZS, Vanhoutte PM: Thromboxane A₂ receptor antagonists inhibit endothelium-dependent contraction. *Hypertension* 1990; **15**: 699-703.
109. Kato T, Iwama Y, Okamura K, Hashimoto H, Ito T: Satake T. Prostaglandin H₂ may be the endothelium-derived contracting factor released by acetylcholine in the aorta of the rat. *Hypertension* 1990; **15**: 475-481.
110. Lin L, Balazy M, Pagano PJ, Nasjletti A: Expression of prostaglandin H₂-mediated mechanism of vascular contraction in hypertensive rats: relation to lipoxigenase and prostacyclin synthase activities. *Circ Res* 1994; **74**: 197-205.
111. Boulanger CM, Ge T, Hughes H, Junquero D, Wu KK, Vanhoutte PM: Augmented expression of prostaglandin H synthase and contraction to prostaglandin H₂ in the aorta of spontaneously hypertensive rats. *Hypertension* 1994; **24**: 3, abstr. 56, 382.
112. Ge T, Hughes H, Junquero D, Wu KK, Vanhoutte PM, Boulanger CM: Augmented expression and activity of prostaglandin H synthase in SHR aorta. *FASEB J* 1994; **18**, 4, A39.
113. Auch-Schwelk W, Katusic ZS, Vanhoutte PM: Contractions to oxygen-derived free radicals are augmented in aorta of the spontaneously hypertensive rat. *Hypertension* 1989; **13**: 859-864.
114. Vanhoutte PM, Katusic ZS: Endothelium-derived contracting factor: endothelium and/or superoxide anion? *TiPS* 1988; **9**: 229-230.
115. Tuncer M, Vanhoutte PM: Role of prostanoids in the increased vascular responsiveness and delayed tachyphylaxis to serotonin in the kidney of the spontaneously hypertensive rats. *J Hypertens* 1991; **9**: 623-629.
116. Vanhoutte PM: Vascular endothelium and Ca²⁺-antagonists. *J Cardiovasc Pharmacol* 1988; **12**(suppl 6): S21-S28.
117. Vanhoutte PM: Role of calcium and endothelium in hypertension, cardiovascular disease and subsequent vascular events. *J Cardiovasc Pharmacol* (in press).
118. Boulanger CM, Mombouli JV, Vanhoutte PM: Indapamide inhibits endothelium-dependent contractions in the aorta of the spontaneously hypertensive rat. *Fundam Clin Pharmacol* 1994; **7**: 443-448.
119. Lévy BI, Lahcen EF, Pieddeloup C, Barouki F, Safar ME: Role of endothelium in the mechanical re-

- sponse of the carotid arterial wall to calcium blockade in spontaneously hypertensive and Wistar-Kyoto rats. *J Hypertens* 1993; **11**: 57-63.
120. Geoffroy J, Benzoni D, Vincent M, Sassard J: Thromboxane A₂ and development of genetic hypertension in the Lyon Rat strain. *Hypertension* 1990; **16**: 655-661.
 121. Mistry M, Nasjletti A: Contrasting effect of thromboxane synthase inhibitors and a thromboxane receptor antagonist on the development of angiotensin II-salt-induced hypertension in rats. *J Pharmacol Exp Ther* 1990; **253**: 90-94.
 122. Huang A, Sun D, Koller A: Endothelial dysfunction augments myogenic arteriolar constriction in hypertension. *Hypertension* 1993; **22**: 913-921.