Editorial Comment

The Endothelium

Target and Promoter of Hypertension?

Thomas F. Lüscher

In spite of its strategic anatomic position between the circulating blood and vascular smooth muscle cells, the endothelium until recently has not attracted much attention. Moncada et al[1] were the first to demonstrate that endothelial cells are a rich source of prostacyclin. Furchgott and Zawadzki[2] later showed that endothelial cells release a potent relaxing factor when stimulated with acetylcholine. The control of vascular function by the endothelium, however, proved to be much more complex than anticipated as the cells not only release different vasodilator substances but also mediate contraction of the underlying vascular smooth muscle. Indeed, shortly after the discovery of endothelium-derived relaxing factor, it became evident that the endothelium facilitates contractions to hypoxia in canine blood vessels.[3] Sandwich experiments convincingly demonstrated this response to be mediated by a diffusible endothelium-derived substance ($EDCF_1$) (Figure 1).[4] Other physical stimuli such as quick stretch and pressure also evoke endothelium-dependent contractions, at least in certain blood vessels.[5,6] In addition, endothelium-dependent contractions are elicited by arachidonic acid and thrombin in canine veins[3] and by arachidonic acid, acetylcholine, nicotine, and norepinephrine in cerebral arteries of the dog.[7,8]

Subsequent to the description of endothelium-dependent contractions, research interest focused on the nature of the endothelium-derived contracting factors. It soon became clear that different mediators must be involved. Indeed, in the canine coronary artery endothelium-dependent contractions to hypoxia proved to be resistant to all pharmacological interventions except calcium antagonists.[4] In contrast, endothelium-dependent contractions to arachidonic acid in canine veins could be prevented by inhibitors of cyclooxygenase.[9] This latter observation suggested that one of the endothelium-derived contracting factors ($EDCF_2$) represents a cyclooxygenase product. Thromboxane A_2 was the most obvious candidate as it possesses vasoconstrictor properties.[10] Indeed, in cerebral blood vessels of the dog, the endothelium-dependent contractions evoked by arachidonic acid are associated with an increased formation of thromboxane A_2. Both events can be reduced or prevented by endothelium removal and by inhibitors of cyclooxygenase or thromboxane synthetase.[11] However, in canine veins endothelium-dependent contractions to arachidonic acid are resistant to inhibitors or thromboxane synthetase suggesting that an arachidonic acid metabolite other than thromboxane A_2 is involved. Thus, at least two contracting substances must be formed during activation of the cyclooxygenase pathway in endothelial cells.

Activation of the cyclooxygenase pathway facilitates not only the formation of prostaglandins but also that of oxygen-derived free radicals. Endothelial cells in culture produce superoxide anions under certain conditions,[12] and the radicals evoke contraction of isolated blood vessels such as the canine basilar artery and the rat aorta.[13,14] In the canine basilar artery, endothelium-dependent contractions to the calcium ionophore A23187 can be prevented by both indomethacin and superoxide dismutase, a scavenger of oxygen-derived free radicals.[13] Prostaglandin H_2 synthetase possesses hydroperoxidase activity, which results in the production of superoxide anions.[15] Hence, superoxide is likely to be the endothelium-derived contracting factor formed after activation of the cyclooxygenase pathway in the canine cerebral circulation.

To complicate things further, Hickey et al[16] reported that media of endothelial cells in culture contain a potent vasoconstrictor peptide. Yanagisawa et al[17] subsequently identified this substance as the 21–amino acid peptide endothelin. In cultured endothelial cells, thrombin, adrenaline, transforming growth factor β, arginine vasopressin, interleukin 1, and the calcium ionophore induce the expression of preproendothelin messenger RNA.[18] Thrombin has been shown to elicit the release of endothelin from endothelial cells in culture and from the intact porcine aorta.[19,20] Endothelin can be excluded because the mediator of the endothelium-dependent contractions requiring the activity of cyclooxygenase as indomethacin does not reduce its effects.[18] Although ischemia causes externalization of endothelin-1 binding sites in rat cardiac membranes,[21] it is unlikely that the peptide is responsible for the endothelium-

From the Department of Medicine, Division of Cardiology and Department of Research, Laboratory of Vascular Physiology, University Hospital, Basel, Switzerland.

Address for correspondence: Thomas F. Lüscher, MD, Department of Medicine, Division of Cardiology, University Hospital, CH-4031 Basel, Switzerland.

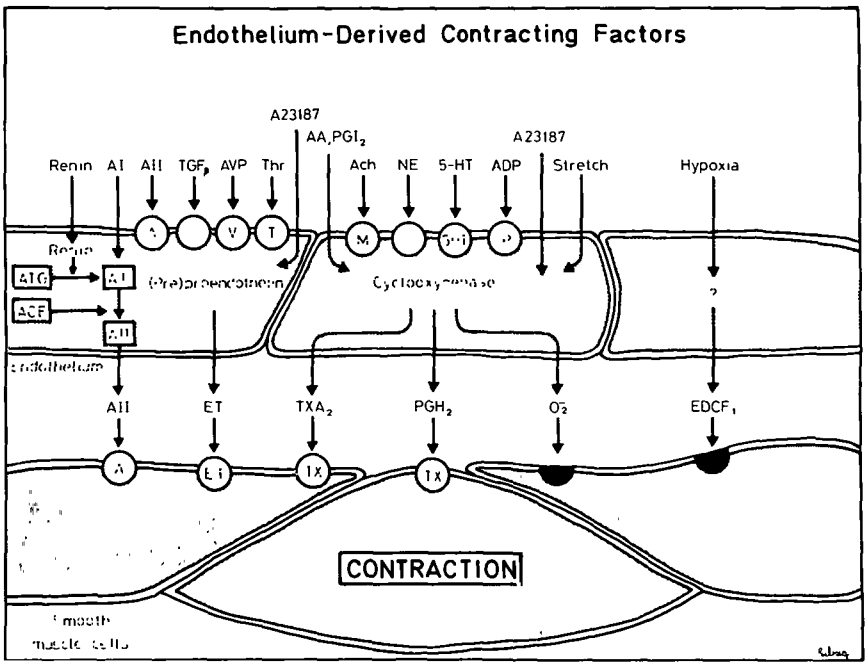

FIGURE 1. *Schematic diagram showing endothelium-derived contracting factors produced in the blood vessel wall. Canine coronary arteries release an endothelium-derived contracting factor ($EDCF_1$) during hypoxia. The cyclooxygenase pathway, after stimulation of various receptors (open circles) and physical forces can form thromboxane A_2 (TXA_2), prostaglandin H_2 (PGH_2), and superoxide radical (O_2^-). Circulating hormones such as angiotensin II (AII), epinephrine (E), and arginine vasopressin (AVP) and coagulation factors such as thrombin (Thr) and transforming growth factor β (TGFβ) can stimulate the production of endothelin (ET). In addition, an endothelial renin angiotensin system may exist in the endothelial cells that provide locally formed AII. AI, angiotensin I; AA, arachidonic acid; A23187, Calcium ionophore; ACE, angiotensin converting enzyme; Ach, Acetylcholine; ADP, Adenosine diphosphate; ATG, angiotensin; NE, norepinephrine; 5-HT, serotonin (5-hydroxytryptamine).*

dependent contractions occurring during hypoxia as the response is faster in its time course and resistant to the inhibitor of protein synthesis cycloheximide.[22] Thus, it is apparent that the endothelium can release at least five different endothelium-derived contracting factors and possibly also angiotensin II (Figure 1).[23,24]

The endothelium is the most obvious target of high blood pressure. Endothelial cells sense physical forces such as blood flow and align the long axis of their cell body and nuclei along its direction. In hypertension, striking morphological changes of the intimal layer occur.[23] Physical forces such as shear stress, stretch, and pressure elicit functional changes of endothelial cells. Thus, it is not surprising that endothelium-dependent relaxations to acetylcholine are reduced in hypertensive arteries.[25] More surprising was the fact that indomethacin normalized the reduced endothelium-dependent relaxations in the aorta of the spontaneously hypertensive rat.[26] These results suggested that the primary defect in this form of hypertension does not involve a decreased release of endothelium-derived relaxing factor but rather the concomitant release of cyclooxygenase-dependent contracting factors. As in canine veins, the endothelium-dependent contractions can be prevented by inhibitors of cyclooxygenase but not those of thromboxane or prostacyclin synthetase, which suggests that a prostanoid other than thromboxane A_2 or prostacyclin mediates the response.[25] This conclusion was further strengthened by the observation that, in the perfused aorta of the spontaneously hypertensive rat, very little thromboxane A_2 is released and its production remains unaffected by acetylcholine.[26] More recently, superoxide anions have been excluded as superoxide dismutase did not prevent the response.[14] In this issue of *Hypertension*, Kato et al[27] provide evidence that prostaglandin H_2 may be the endothelium-derived contracting factor released by acetylcholine in the aorta of the spontaneously hypertensive rat (Figure 1). Indeed, exogenous prostaglandin H_2 evokes contractions of the rat aorta at much lower concentrations than do prostaglandin E_2 and D_2. Thus, the amounts of the latter prostanoids released appear insufficient to explain the response. The fact that the contractions to exogenous prostaglandin H_2 can be prevented by thromboxane receptor antagonists, but not by inhibitors of the synthesis of the prostanoid is in accord with similar effects of the drugs on endothelium-dependent contractions to acetylcholine.[27,28] Unresolved questions are whether the hypertensive endothelium releases more prostaglandin H_2 or whether the smooth muscle cells are more sensitive to the vasoconstrictor effects of this prostanoid.[25]

Hypertension is not the only condition that is associated with an increased occurrence of endothelium-dependent contractions. Aging also promotes endothelium-dependent contractions. In old Wistar-Kyoto rats, the response becomes as prominent as that in adult spontaneously hypertensive rats.[29] Similarly, the circulating levels of endothelin increase

with age.[30] Thus, the occurrence of pronounced endothelium-dependent contractions may reflect the premature aging of the hypertensive blood vessel wall.

What is the importance of endothelium-dependent contractions in hypertension? An imbalance of endothelium-dependent relaxations and contractions in hypertensive blood vessels may contribute to the increased peripheral vascular resistance or to cardiovascular complications. With respect to the latter, it is of interest that the endothelium-derived contracting factor also appears to be released by aggregating platelets in the aorta[31] and in response to serotonin in the aorta and in isolated hearts[31,32] as well as in response to both serotonin and adenosine diphosphate (both platelet-derived products) in the cerebral circulation of the spontaneously hypertensive rat.[33] Indomethacin normalizes endothelium-dependent relaxations to acetylcholine in mesenteric resistance arteries of the rat,[34,35] which suggests that these functional changes of the endothelium also occur in blood vessels where peripheral vascular resistance is regulated. Unfortunately, in contrast to platelet-derived mediators, the role of acetylcholine as an agonist for the release of endothelium-derived contracting factor remains elusive. Indeed, acetylcholine is not a circulating hormone capable of activating the endothelium from the intraluminal side. Acetylcholine released from cholinergic nerves, however, may reach the endothelium in resistance vessels[36] but not in larger arteries. Hence, it will be of crucial importance to further delineate the release mechanisms for endothelium-derived contracting factors in normotensive and hypertensive arteries in vitro and in vivo.

Thus, we are faced with the fact that the endothelium, even with its structural inconspicuity within the blood vessel wall, can profoundly affect cardiovascular function. Under physiological conditions it appears to play a protective role in the circulation by the release of substances inhibiting contraction and platelet activation. As we grow older, become hypertensive, hyperlipidemic,[37] and eventually develop atherosclerosis,[38] the role of these protective mechanisms appears to diminish, and contracting factors come more into play, almost as if the endothelium serves as the unifying target and common denominator of cardiovascular disease.

References

1. Moncada S, Herman AG, Higgs EA, Vale C: 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
2. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;299:3737–376
3. De Mey JG, Vanhoutte PM: Heterogenous behavior of the canine arterial and venous wall: Importance of the endothelium. *Circ Res* 1982;51:439–447
4. Rubanyi GM, Vanhoutte PM: Hypoxia released a vasoconstrictor substance from the canine vascular endothelium. *J Physiol* (Lond) 1985;364:45–56
5. Katusic ZS, Shepherd JT, Vanhoutte PM: Endothelium-dependent contractions to stretch in canine basilar arteries. *Am J Physiol* 1987;252:H671–H673
6. Harder DR: Pressure-induced myogenic activation of cat cerebral arteries is dependent on intact endothelium. *Circ Res* 1987;60:102–107
7. Katusic ZS, Shepherd JT, Vanhoutte PM: Endothelium-dependent contractions to calcium ionophore A23187, arachidonic acid and acetylcholine in canine basilar arteries. *Stroke* 1988;19:476–479
8. Shirahase H, Usui H, Kurahashi K, Fujiwara M, Fukui K: Endothelium-dependent contraction induced by nicotine in isolated canine basilar artery—possible involvement of a thromboxane A_2 (TXA_2) like substance. *Life Sci* 1988; 42:437–445
9. 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
10. Ellis EF, Oelz O, Roberts LJ II, Payne NA, Sweetman BJ, Nies AS, Oates JA: Coronary arterial smooth muscle contraction by a substance released from platelets: Evidence that it is thromboxane A_2. *Science* 1976;193:1135–1137
11. Shirahase H, Usui H, Kurahashi K, Fujiwara M, Fukui K: Possible role of endothelial thromboxane A_2 in the resting tone and contractile responses to acetylcholine and arachidonic acid in canine cerebral arteries. *J Cardiovasc Pharmacol* 1987;10:517–522
12. Rosen GM, Freeman SA: Detection of superoxide generated by endothelial cells. *Proc Natl Acad Sci USA* 1984;81: 7269–7273
13. Katusic ZS, Vanhoutte PM: Superoxide anion is an endothelium-derived contracting factor. *Am J Physiol* 1989; 257:H33–H37
14. 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
15. Kukreja RC, Kontos HA, Hess MZ, Ellis EF: PGH synthase and lipoxygenase generate superoxide in the presence of NADH and NADPH. *Circ Res* 1986;59:612–619
16. Hickey KA, Rubanyi GM, Paul RJ, Highsmith RF: Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. *Am J Physiol* 1985;248:C550–C556
17. Yanagisawa M, Kurihara H, Kimura S, Mitsui Y, Kobayashi M, Watanabe TX, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; 332:411–415
18. Yanagisawa M, Masaki T: Molecular biology and biochemistry of the endothelins. *Tr Pharmacol Sci* 1989;10:374–378
19. Schini V, Hendrickson H, Heublein D, Burnett J Jr, Vanhoutte PM: Thrombin enhances the release of endothelin from cultured porcine aortic endothelial cells. *Eur J Pharmacol* 1989;165:333–334
20. Boulanger C, Lüscher TF: Release of endothelin from the porcine aorta: Inhibition by endothelium-derived nitric oxide. *J Clin Invest* (in press)
21. Liu J, Casley DJ, Nayler WG: Ischaemia causes externalization of endothelin-1 binding sites in rat cardiac membranes. *Biochem Biophys Res Commun* 1989;164:1220–1225
22. Vanhoutte PM, Auch-Schwelk W, Boulanger C, Janssen PA, Katusic ZS, Komori K, Miller VM, Schini VB, Vidal M: Does endothelin-1 mediate endothelium-dependent contractions during anoxia? *J Cardiovasc Pharmacol* 1989;13(suppl 5):124–128
23. Lüscher TF: Endothelial vasoactive substances and cardiovascular disease. Basel, S. Karger Publisher AG, 1988, pp 1–133
24. Dzau VJ: Significance of the vascular renin-angiotensin pathway. *Hypertension* 1986;8:553–559
25. Lüscher TF, Vanhoutte PM: Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension* 1986;8;344–348
26. 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

27. Kato T, Iwama Z, Okumura K, Hashimoto H, Ito T, Satake T: Prostaglandin H_2 may be the endothelium-derived contracting factor released by acetylcholine in the aorta of the rat. *Hypertension* 1990;15:475–481
28. Auch-Schwelk W, Katusic ZS, Vanhoutte PM: Endothelium-dependent contractions in the SHR aorta are inhibited by thromboxane A_2 receptor antagonists (abstract). *J Vasc Med Biol* 1989;1:76
29. Koga T, Takata Y, Kobayashi K, Takishita S, Yamashita Y, Fujishima M: Age and hypertension promote endothelium-dependent contractions to acetylcholine in the aorta of the rat. *Hypertension* 1989;14:542–548
30. Miyauchi T, Yanagisawa M, Suzuki N, Iida K, Sugishita Y, Masahiko F, Saito T, Goto K, Masaki T: Venous plasma concentrations of endothelin in normal and hypertensive subjects (abstract). *Circulation* 1989;80:2280
31. Lüscher TF, Vanhoutte PM: Endothelium-dependent responses to aggregating platelets and serotonin in spontaneously hypertensive rats. *Hypertension* 1986;8(suppl II):II-55–II-60
32. Lüscher TF, Rubanyi GM, Aarhus LL, Vanhoutte PM: Serotonin reduces coronary flow in isolated hearts of the spontaneously hypertensive rat. *J Hypertens* 1986;4(suppl 5):148–150
33. Mayhan WG, Faraci FM, Heistad DD: Responses of cerebral arterioles to adenosine diphosphate, serotonin, and the thromboxane analogue U-46619 during chronic hypertension. *Hypertension* 1988;12:556–561
34. 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* (in press)
35. Watt AC, Thurston H: Endothelium-dependent relaxation in resistance vessels from the spontaneously hypertensive rat. *J Hypertens* 1989;7:661–666
36. Tesfamariam B, Halpern W: Endothelium-dependent and endothelium-independent vasodilation in resistance arteries from hypertensive rats. *Hypertension* 1988;11:440–444
37. Andrews HE, Bruckdorfer KR, Dunn RC, Jacobs M: Low-density lipoproteins inhibit endothelium-dependent relaxation in rabbit aorta. *Nature* 1987;327:237–239
38. Shimokawa H, Vanhoutte PM: Impaired endothelium-dependent relaxation to aggregating platelets and related vasoactive substances in porcine coronary arteries in hypercholesteremia and atherosclerosis. *Circ Res* 1989;64:900–914

KEY WORDS • endothelium • prostaglandin synthase • acetylcholine

(*Hypertension* 1990;15:482–485)