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DIALOGUES

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Endothelium-Dependent Contractions

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Expert Answers to Three Key Questions

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Endothelium-dependent contractions: from superoxide anions to TP-receptor agonists

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Besides causing relaxation of the underlying smooth muscle through the release of endothelium-derived relaxing factors (EDRFs), the endothelial cells of certain blood vessels, under given circumstances, can also trigger the contraction (constriction) of these muscle cells. Such acute, endothelium-dependent, increases in contractile tone can be due to the suppression of nitric oxide production (constitutive or stimulated), or to the production of vasoconstrictor peptides (angiotensin II or endothelin-1) or oxygen-derived free radicals (superoxide anions) and/or vasoconstrictor products of arachidonic acid metabolism (endoperoxides, thromboxane A_2, and possibly isoprostanes). The latter have been termed endothelium-derived contracting factors (EDCFs) as they can contribute to moment-to-moment changes in contractile activity of the vascular smooth muscle cells that surround the endothelium from which they originate. EDCF-mediated responses are most pronounced in large cerebral arteries, and are enhanced by aging, spontaneous hypertension, and diabetes. They contribute to the blunting of endothelium-dependent vasodilations in aged subjects and subjects with essential hypertension. Since EDCFs cause contraction of vascular smooth muscle by activation of thromboxane-prostanoid (TP) receptors, selective antagonists at these receptors are able to prevent endothelium-dependent contractions, thus opening up prospects for potential therapeutic implications.

Keywords: arachidonic acid; endoperoxide; endothelin; hypertension; isoprostane; nitric oxide; superoxide anion; thromboxane A_2; TP-receptor
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Almost a quarter of a century ago, Furchgott and Zawadzki[1] described the obligatory role of the endothelial cells in the relaxation of isolated arteries by acetylcholine, at least in the absence of sympathetic tone when the prejunctional (presynaptic) effect of the cholinergic transmitter can contribute. This major discovery not only eventually brought to light the pivotal role of nitric oxide (NO), but also initiated the quest for other endothelium-derived relaxing factors (EDRFs; see references 2-4). However, soon after the discovery of the endothelium-dependency of the response to acetylcholine (and other vasodilators), it became obvious that, in certain blood vessels, under given circumstances, the endothelial cells could trigger contractions, rather than relaxations, of the underlying vascular smooth muscle.[5] These endothelium-dependent contractions were attributed to the release of one or several diffusible factor(s), termed "endothelium-derived contracting factor(s)" (EDCF; *Figure 1, page 212*). This paper summarizes current knowledge on endothelium-dependent contractions, as derived mainly from work in the author's laboratory. After briefly mentioning the suppression of NO release or that of the production of vasoconstrictor peptides as potential causes for such responses, attention will focus on acute endothelium-dependent increases in vascular smooth muscle tone, which involve oxygen-

SELECTED ABBREVIATIONS AND ACRONYMS

COX	cyclooxygenase
EDCF	endothelium-derived contracting factor
EDRF	endothelium-derived relaxing factor
eNOS	endothelium nitric oxide synthase
NO	nitric oxide
SHR	spontaneously hypertensive rat
TP	thromboxane-prostanoid (receptor)
WKY	Wistar-Kyoto (rat)

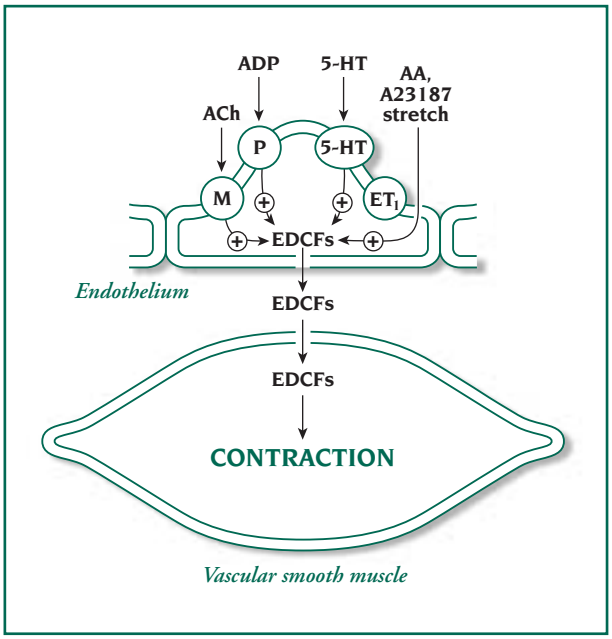

Figure 1. *In certain blood vessels, under given circumstances, the endothelial cells, when activated by neurohumoral mediators, subjected to sudden stretch, or exposed to the Ca^{2+} ionophore A23187, release (a) vasoconstrictor substance(s), called endothelium-derived contracting factor(s), which diffuse(s) to the underlying vascular smooth muscle and initiate(s) its contraction.*

Abbreviations: *AA, arachidonic acid; ACh, acetylcholine; ADP, adenosine diphosphate, EDCF, endothelium-derived contracting factor; ET, endothelin; 5-HT, 5-hydroxytryptamine (serotonin); M, muscarinic receptor; P, purinoceptor.*

derived free radical production and arachidonic acid metabolism, and which are ultimately due to the activation of thromboxane-prostanoid (TP) receptors.

SUPPRESSION OF NITRIC OXIDE RELEASE

Endothelial nitric oxide synthase (eNOS), the physiological source of NO in the blood vessel wall, is a constitutive enzyme, the activity of which is governed by the intracellular calcium concentration. As the latter increases (eg, if shear stress augments or if endothelial cells are exposed to endothelium-dependent vasodilators such as acetylcholine or bradykinin), more NO is produced and a greater inhibition of the contractile apparatus of the underlying smooth muscle ensues. Thus, if vascular smooth muscle cells exhibit myogenic tone, or are stimulated by vasoconstrictor agents, a sudden reduction in NO production can result in acute endothelium-dependent contractions in vitro *(Figure 2),* or acute increases in peripheral resistance and arterial blood pressure in vivo (eg, reference 6). In the latter case, a substantial part of the response should be attributed to the suppression of the inhibitory effect of NO on the production of vasoconstrictor peptides, rather than to a direct relaxing effect of the endothelial mediator on vascular smooth muscle (see reference 7). Possible causes of endothelium-dependent contractions due to reduction in NO release include anoxia, particularly in arteries previously exposed to ischemia-reperfusion injury (eg, references 8-11). In the intact organism, besides hypoxia,[11] endothelium-dependent contractions could theoretically appear in response to a sudden surge in the production of endogenous NOS inhibitors, such a asymmetric dimethylarginine (ADMA).

RELEASE OF VASOCONSTRICTOR PEPTIDES

Since endothelial cells are the major source of converting enzyme, it is conceivable that angiotensin II formed at their surface could diffuse to the underlying vascular smooth muscle cells and cause acute "endothelium-dependent" contractions. Likewise, endothelial cells can generate and release the vasoconstrictor peptide endothelin-1 (see references 7, 12-14; and Faraci and Heistad, in this issue). However, there is little

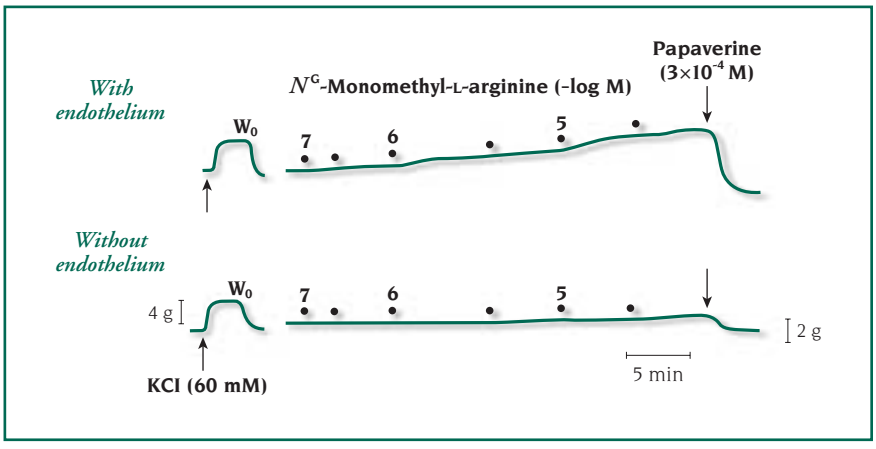

Figure 2. *In the isolated canine basilar artery, the arterial smooth muscle cells possess a relatively high degree of myogenic tone, which is tempered by the constitutive release of nitric oxide (NO) by the endothelial cells, Hence, progressive inhibition of endothelial NO synthase (eNOS) by increasing concentrations of the competitive NOS inhibitor N^G-monomethyl-L-arginine (LNMMA) causes a major contraction in the arterial ring with endothelium* ***(upper curve)*** *and only minimal increases in tension in the ring where most of the endothelial cells have been removed by gentle rubbing* ***(lower curve).***

Courtesy of Dr Z. S. Katusic.

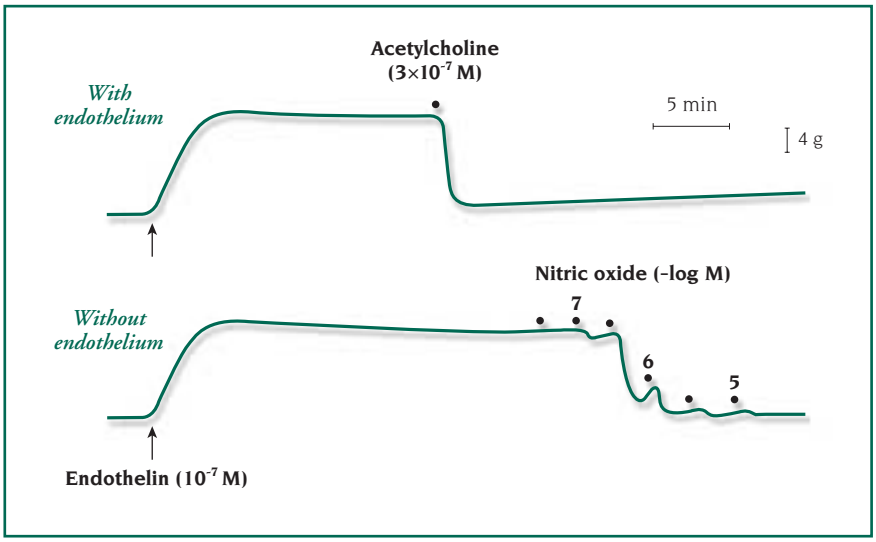

***Figure 3.** In the canine coronary artery, whether with (**upper curve**) or without (**lower curve**) endothelium, endothelin-1 causes a strong, sustained contraction, which is abolished by nitric oxide (NO) whether given exogenously by the investigator (**lower curve**) or released from the endothelium by acetylcholine (**upper curve**). This experiment implies that as long as endothelial cells release sufficient NO, endothelin-1 cannot exert its vasoconstrictor activity.*

***Data from reference 16:** Miller VM, Komori K, Burnett JC Jr, Vanhoutte PM. Differential sensitivity to endothelin in canine arteries and veins.* Am J Physiol. *1989;257: H1127-H1131. Copyright © 1989, American Physiological Society.*

evidence suggesting that acute changes in the release of endothelin-1 contribute to moment-to-moment, endothelium-dependent contractions.[15] In particular, as long as endothelial cells continue to produce NO, the latter's combined inhibitory action on the release and action *(Figure 3)*[16] of endothelin-1 is bound to prevent any major role of the endothelin-1 in local vasomotor control.[7]

CYCLOOXYGENASE-DEPENDENT ENDOTHELIUM-DEPENDENT CONTRACTIONS

The phenomenon

The original observations of the occurrence of endothelium-dependent contractions were made in canine veins while investigating the heterogeneity in endothelium-dependent responsiveness along the vascular tree.[5] Indeed, in these veins, mediators such as arachidonic acid and thrombin, which are endothelium-dependent relaxants in isolated arteries, augmented rather than reduced the contractions evoked by α-adrenergic agonists *(Figure 4)*.[5] Acetylcholine and the calcium ionophore A23187, which cause endothelium-dependent relaxations in most arteries, failed to do so in the canine basilar artery, although, for example, bradykinin was the expected potent endothelium-dependent dilator in this artery preparation.[17] When acetylcholine or A23187 was applied to quiescent basilar arteries, clear-cut endothelium-dependent contractions were obtained *(Figure 5, left, next page)*.[17] Similar EDCF-mediated increases in tension were evidenced in the aorta of spontaneously hypertensive rats (SHR)

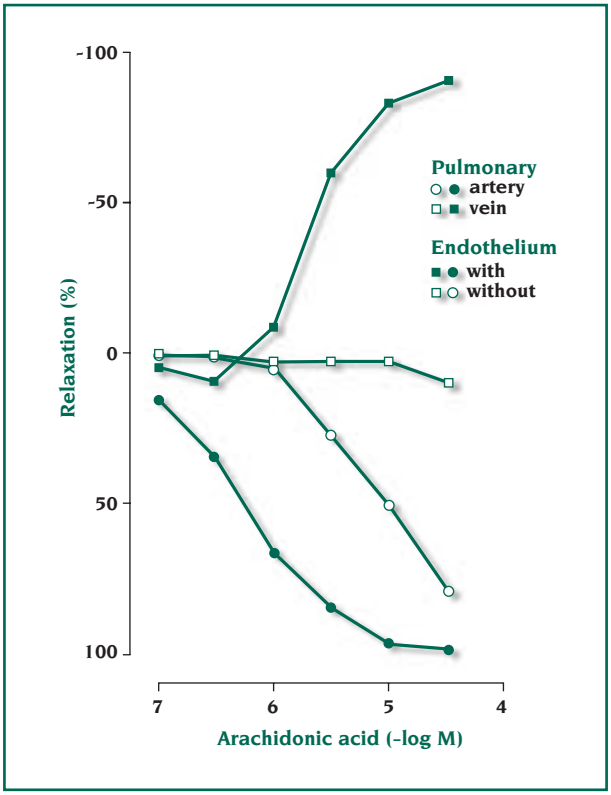

***Figure 4.** Effect of increasing concentrations of arachidonic acid on sustained contractions to norepinephrine in rings, with and without endothelium, prepared from the main pulmonary artery and one pulmonary vein of the same donor animal. In the artery, arachidonic acid causes relaxations that are amplified by the presence of endothelial cells. In the vein, arachidonic acid causes contractions that are strictly endothelium-dependent. These experiments imply that in the pulmonary venous endothelium arachidonic acid causes the release of, or is transformed into, a vasoconstrictor substance (endothelium-derived contracting factor [EDCF]) that activates the underlying smooth muscle.*

***Data from reference 5:** De Mey JG, Vanhoutte PM. Heterogeneous behavior of the canine arterial and venous wall: importance of the endo-thelium.* Circ Res. *1982;51:439-447. Copyright © 1982, American Heart Association.*

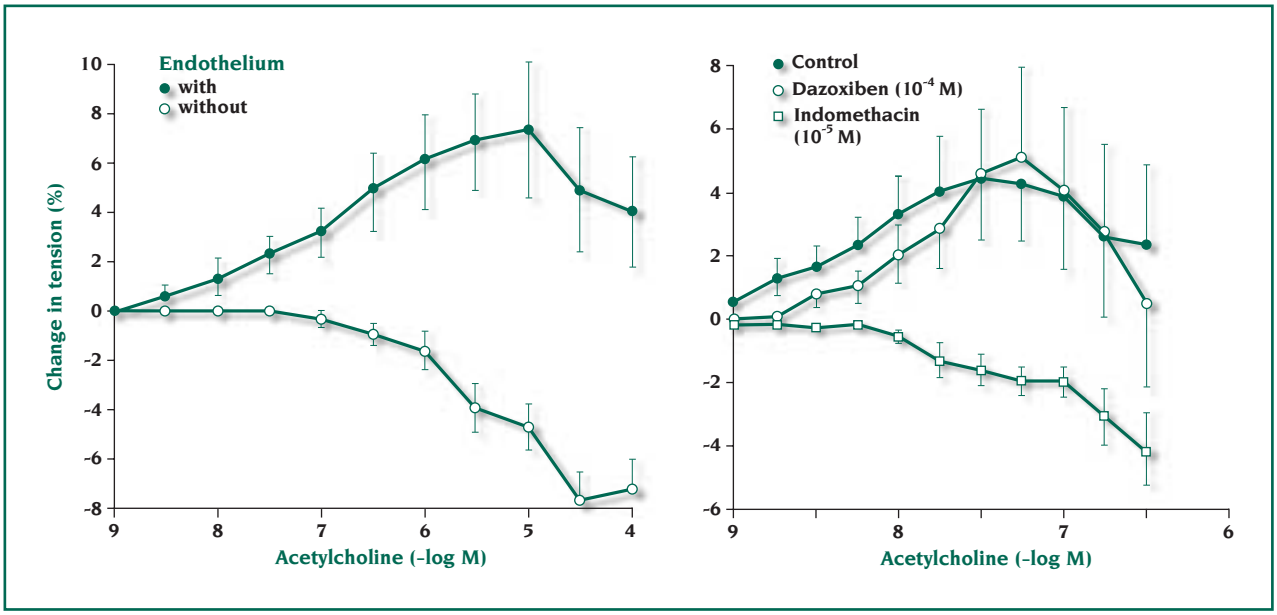

Figure 5. Left panel: *In rings without endothelium of canine basilar arteries, which are not exposed to a vasoconstrictor agent, but possess myogenic tone, acetylcholine has a direct inhibitory effect on the vascular smooth muscle cells. By contrast, if the rings contain endothelium, they contract when exposed to the muscarinic agonist. These experiments demonstrate the existence of endothelium-dependent contractions in this preparation.* ***Right panel:*** *In basilar arteries with endothelium, the contractions evoked by acetylcholine are not affected by dazoxiben (an inhibitor of thromboxane synthase), but abolished by indomethacin (a nonselective inhibitor of cyclooxygenases). These experiments suggest that thromboxane A_2 is not involved in the endothelium-dependent contraction caused by acetylcholine.*

Data from reference 17: *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. Copyright © 1988, Lippincott Williams & Wilkins.*

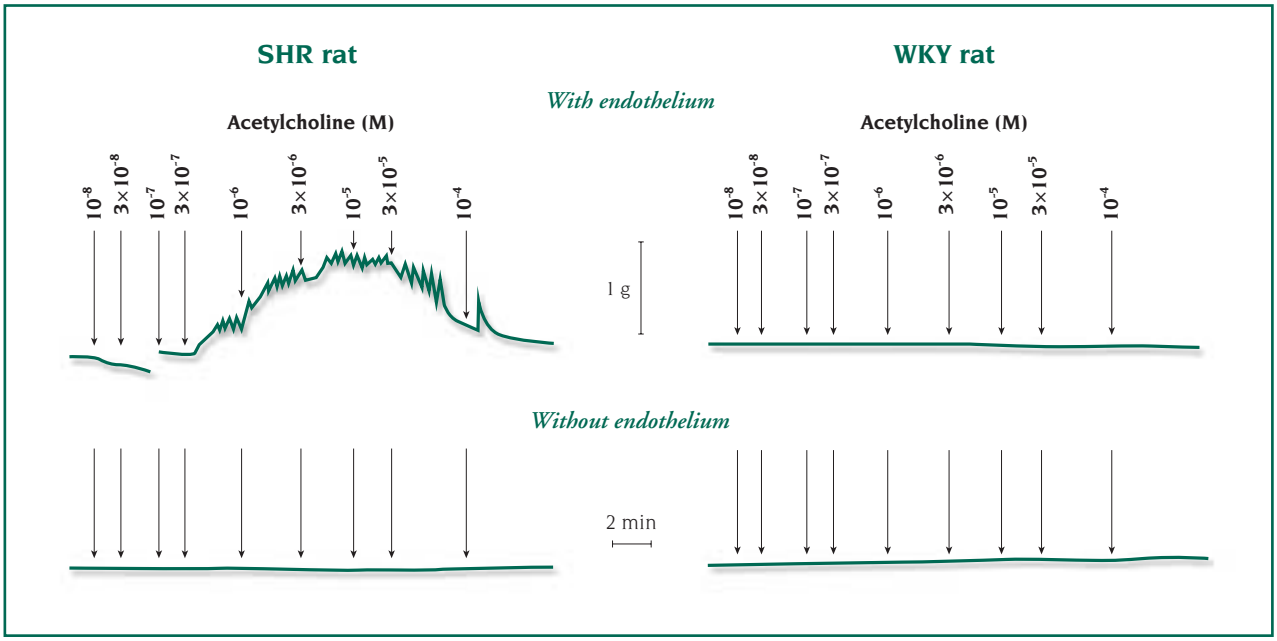

Figure 6. *In quiescent rings without endothelium (**lower panel**) of aortas of spontaneously hypertensive rats (SHR, **left**) and normotensive control Wistar-Kyoto rats (WKY, **right**) increasing concentrations of acetylcholine have no effect. In the presence of endothelium (**upper panel**), the muscarinic agonist causes concentration-dependent, rhythmic contractions only in arteries from the hypertensive strain. These experiments demonstrate that spontaneous hypertension favors the occurrence of endothelium-derived contracting factor (EDCF)-mediated contractions.*

Data from reference 19: *Lüscher TF, Vanhoutte PM. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat.* Hypertension. *1986;8:344-348. Copyright © 1986, Lippincott Williams & Wilkins.*

Figure 6,[17] and in carotid arteries of hypertensive Dahl rats.[18] Thus, the concomitant existence of a relaxing and a contracting component explained the blunted endothelium-dependent relaxation to acetylcholine in blood vessels of these hypertensive animals.[18-20]

Role of arachidonic acid metabolites

The finding that, in canine veins, arachidonic acid augmented rather than depressed contractions *(Figure 4)*[5] pointed to a role of the metabolism of this fatty acid in the divergent endothelium-dependent response. As expected, the endothelium-dependent augmentations were prevented by inhibitors of the cyclooxygenases (COX).[21] The same turned out to be the case in the canine basilar artery *(Figure 5, right),*[17,19] as well as in the SHR aorta, and indomethacin was shown to normalize endothelium-dependent relaxations in the latter *(Figure 7).*[19] Thus, the concept emerged that endothelium-dependent contractions were mediated by one or several COX metabolites. When it became known that two isoforms (COX_1 and COX_2) of the enzyme existed, an obvious question was to define which isoform was involved in the cyclooxygenase-dependent, endothelium-dependent contractions. Although it was intuitively anticipated that COX_2 (the inducible isoform) would be involved in the blood vessels of animals with hypertensive disease, the data in SHR aortas demonstrated that the preferential inhibitors of COX_1 rather than those of COX_2 annulled the response *(Figure 8).*[20,22] Furthermore, when expression of COX_1 was compared in the aorta of adult SHRs and normotensive controls (Wistar-Kyoto rats, WKY), it was found to be significantly greater in the former *(Figure 9, right, next page).*[22,23] Thus, the unavoidable conclusion is that the constitutive isoform of cyclooxygenase is responsible for the blunted endothelium-dependent relaxation to acetylcholine, at least in the SHR aorta.

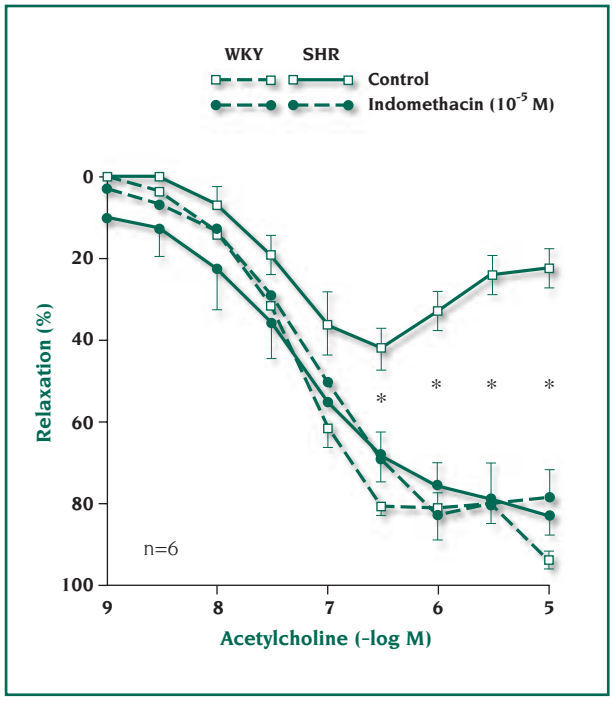

Figure 7. *In contracted rings with endothelium of aortas of normotensive (Wistar-Kyoto rats, WKY) and spontaneously hypertensive rats (SHR), acetylcholine causes concentration-dependent relaxations, which are blunted in blood vessels of the hypertensive strain compared with the controls. Indomethacin, the nonselective inhibitor of cyclooxygenases, does not affect the response to acetylcholine in the WKY aorta, but normalizes that in SHR arteries. These experiments indicate that endothelium-derived contracting factor (EDCF) is responsible for the blunting of the dilator response to acetylcholine in the SHR.*

Data from reference 19: *Lüscher TF, Vanhoutte PM. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat.* Hypertension. *1986;8:344-348. Copyright © 1986, Lippincott Williams & Wilkins.*

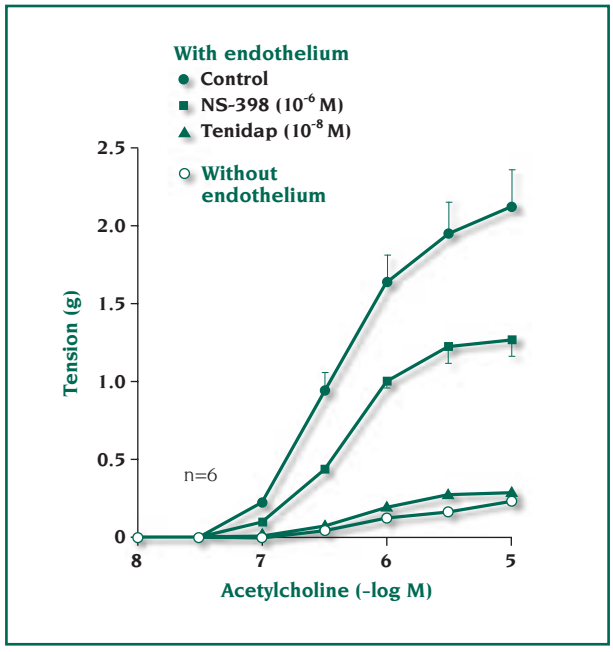

Figure 8. *Contractions to increasing concentrations of acetylcholine in aortas of 35-week-old spontaneously hypertensive rats (SHR), incubated with nitro-L-arginine to prevent the formation of nitric oxide (NO), and potentiate the response. The contraction is endothelium-dependent, slightly reduced by the preferential cyclooxygenase (COX_2)-inhibitor NS-396, and abolished by the preferential COX_1-inhibitor tenidap. These experiments indicate that COX_1, rather than COX_2, is involved in endothelium-derived contracting factor (EDCF)-mediated contractions, and that selective COX_2-inhibitors may not prevent this type of endothelium-dependent response.*

Data from reference 22: *Ge T, Hughes H, Junquero DC, Wu KK, Vanhoutte PM, Boulanger CM. Endothelium-dependent contractions are associated with both augmented expression of prostaglandin H synthase-1 and hypersensitivity to prostaglandin H_2 in the SHR aorta.* Circ Res. *1995;76:1003-1010. Copyright © 1995, American Heart Association.*

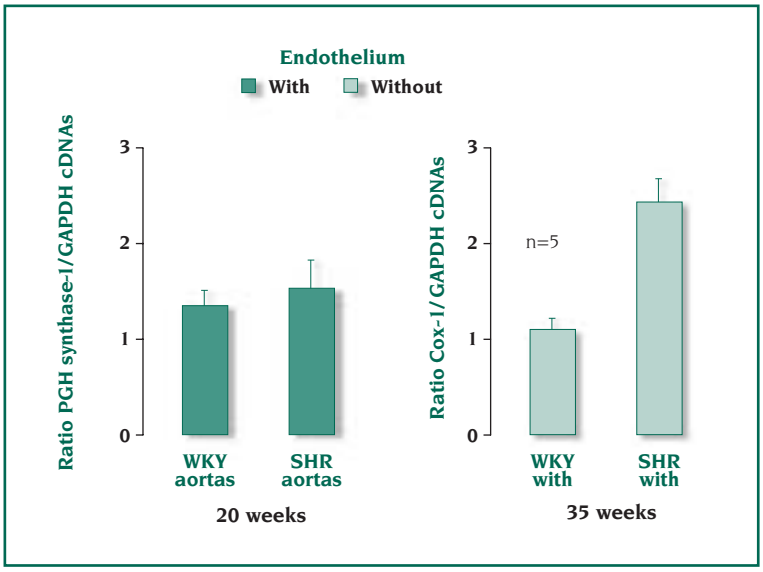

Figure 9. *Expression of the mRNA of prostaglandin H synthase 1 (COX_1), measured by reverse transcriptase polymerase chain reaction (RT-PCR) in aortas with endothelium of normotensive (Wistar-Kyoto rats, WKY) and spontaneously hypertensive rats (SHR) aged 20 (**left**) and 35 (**right**) weeks. The expression is significantly larger only in the older rats, implying that the overexpression of the enzyme is a consequence, rather than a cause of the hypertensive process.*

Abbreviations: *PGH, prostaglandin H; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.*

Data from reference 22: *Ge T, Hughes H, Junquero DC, Wu KK, Vanhoutte PM, Boulanger CM. Endothelium-dependent contractions are associated with both augmented expression of prostaglandin H synthase-1 and hypersensitivity to prostaglandin H_2 in the SHR aorta.* Circ Res. *1995;76: 1003-1010. Copyright © 1995, American Heart Association;* ***and from reference 23:*** *Ge T, Vanhoutte PM, Boulanger CM. Increased response to prostaglandin H_2 precedes changes in PGF-synthase 1 expression in the SHR aorta.* Acta Pharmacol Sinica. *1999;20:1087-1092. Copyright © 1999, Zhongguo Yao Li Xue Bao, Beijing.*

In the canine basilar artery[19] and in the SHR aorta,[18] dazoxiben, an inhibitor of thromboxane synthase, does not reduce the endothelium-dependent contractions evoked by acetylcholine *(Figure 5, right).* This then rules out a major role for thromboxane A_2 in the phenomenon. However, the indomethacin-sensitive, endothelium-dependent contractions that are not influenced by dazoxiben are abolished by antagonists of TP receptors *(Figure 10).*[20,24-26]

These observations imply that an endogenous agonist at TP receptors other than thromboxane A_2 mediates the response. An exception may be when endothelial cells release EDCF upon exposure to endothelins.[27,28] The most likely candidates are endoperoxides, the precursors of thromboxane A_2, which also activate TP receptors. This interpretation is reinforced by the profound inhibitory effect of indomethacin, which strongly suggests a major role for cyclooxygenase, the source of endoperoxides, and by the demonstration that acetylcholine does indeed cause a significant release of endoperoxides in the aorta of the SHR, but not in that of the WKY rat *(Figure 11).*[22] However, this is tempered by the possibility that indomethacin also reduces the production of isoprostanes, the nonenzymatic breakdown products of arachidonic acid, which are also potent agonists at TP receptors.

An obvious question is: where do the metabolites of arachidonic acid originate from? The measurement of COX_1 expression in the SHR aorta yielded no difference between preparations with and without endothelium.[22] This lack of difference has fueled the suggestion that an undefined EDCF is released from the endothelium

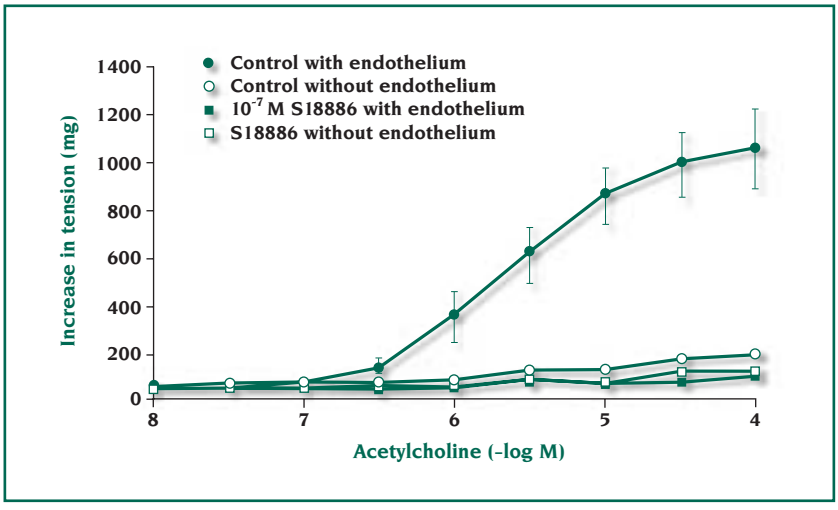

Figure 10. *In the aorta of the spontaneously hypertensive rat, studied in the presence of nitro-L-arginine, acetylcholine causes endothelium-dependent contractions, which are abolished by S18886, a selective antagonist at thromboxane-prostanoid (TP)-receptors. These experiments demonstrate that activation of TP-receptors is a key event in EDCF-mediated responses.*

Data from reference 19: *Yang D, Félétou M, Boulanger CM, et al. Oxygen-derived free radicals mediate endothelium-dependent contractions to acetylcholine in aortas from spontaneously hypertensive rats.* Br J Pharmacol. *2002;136:104-110. Copyright © 2002, Macmillan Specialist Journals.*

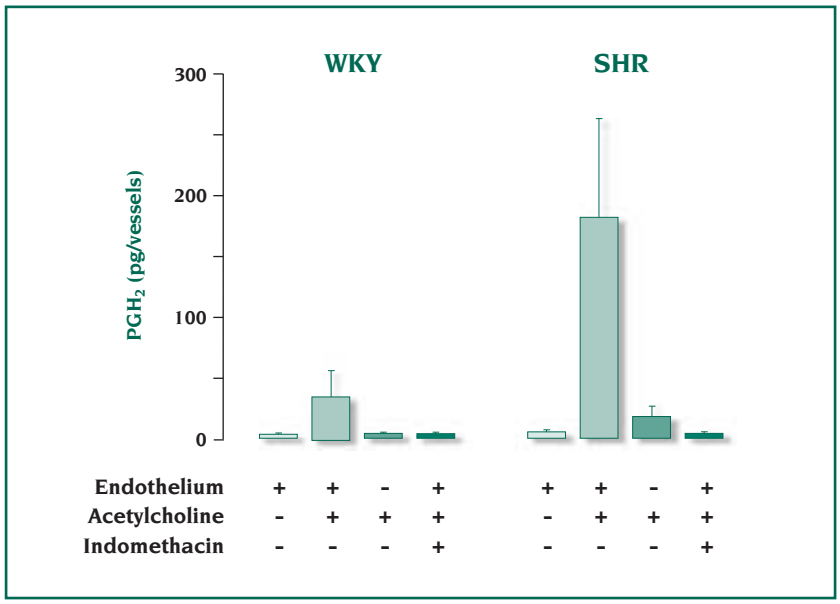

Figure 11. *Release of endoperoxides (prostaglandin H_2, PGH_2) in aortas of normotensive (Wistar-Kyoto rats [WKY]) and spontaneously hypertensive rats (SHR). Effect of presence of endothelial cells, of administration of acetylcholine, and of incubation with indomethacin, a nonselective inhibitor of cyclooxygenases. A significant release in endoperoxides was observed only in aortas with endothelium of the SHR when exposed to acetylcholine in the absence of indomethacin. These experiments strengthen the hypothesis that endoperoxides mediate cyclooxygenase-dependent, endothelium-dependent contractions.*

Data from reference 22: *Ge T, Hughes H, Junquero DC, Wu KK, Vanhoutte PM, Boulanger CM. Endothelium-dependent contractions are associated with both augmented expression of prostaglandin H synthase-1 and hypersensitivity to prostaglandin H_2 in the SHR aorta.* Circ Res. *1995;76: 1003-1010. Copyright © 1995, American Heart Association.*

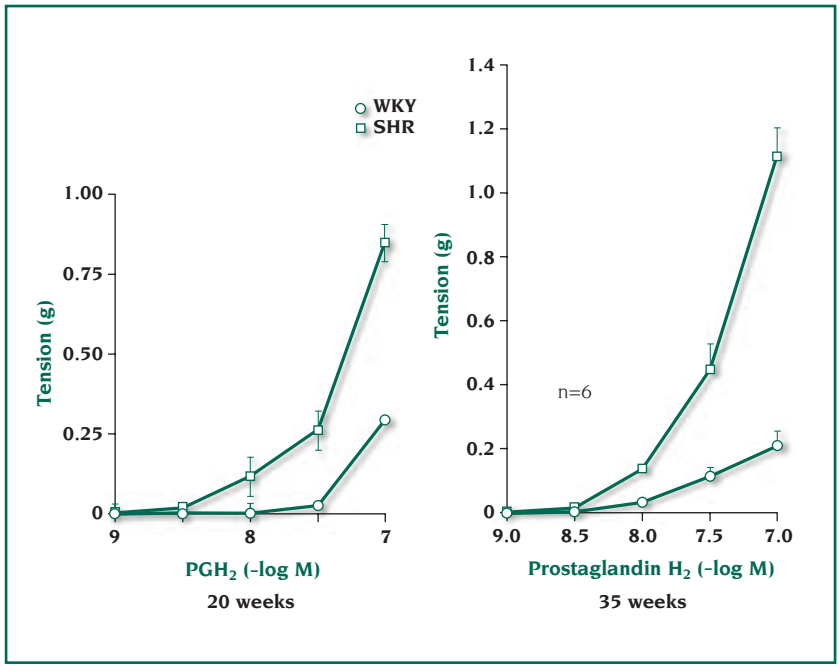

Figure 12. *Contractions evoked by increasing concentrations of exogenous endoperoxides (prostaglandin H_2, PGH_2) in rings without endothelium of aortas from 20-week **(left)** and 35-week-old **(right)** normotensive (Wistar-Kyoto rats [WKY]) and spontaneously hypertensive rats (SHR). In both age groups, the responsiveness to the endoperoxides is significantly greater in the aortas from the hypertensive strain. These experiments suggest that the augmented responsiveness of the vascular smooth muscle of the SHR to thromboxane-prostanoid (TP)-receptor activation is not a consequence of the chronic exposure to an increased arterial blood pressure.*

Data from reference 22: *Ge T, Hughes H, Junquero DC, Wu KK, Vanhoutte PM, Boulanger CM. Endothelium-dependent contractions are associated with both augmented expression of prostaglandin H synthase-1 and hypersensitivity to prostaglandin H_2 in the SHR aorta.* Circ Res. *1995;76: 1003-1010. Copyright © 1995, American Heart Association;* ***and from reference 23:*** *Ge T, Vanhoutte PM, Boulanger CM. Increased response to prostaglandin H_2 precedes changes in PGF-synthase 1 expression in the SHR aorta.* Acta Pharmacol Sinica. *1999;20:1087-1092. Copyright © 1999, Zhongguo Yao Li Xue Bao, Beijing.*

and stimulates the overexpressed COX_1 of the vascular smooth muscle to produce endogenous agonists at TP receptors.[29] However, this interpretation is difficult to maintain in view of the studies demonstrating that the augmented release of endoperoxides caused by acetylcholine is seen only in SHR aortas with endothelium *(Figure 11).*[22] In addition, when the diffusible EDCF is bioassayed, COX_1 inhibitors reduce the response only when administered to the donor endothelial cells, not to the recipient bioassay tissues.[26] The bioassay studies just mentioned demonstrate beyond doubt that activation of the TP receptors on the vascular smooth muscle cells is the ultimate link in the events leading to endothelium-dependent contractions.[26] Hence, a greater than normal release of endoperoxides (and/or isoprostanes) may not be the sole explanation for the occurrence of endothelium-dependent contractions to acetylcholine in isolated blood vessels taken from SHR. Indeed, the aortic smooth muscle of the SHR is more responsive to activation by endoperoxides than that of normotensive control WKY rats *(Figure 12, right).*[22]

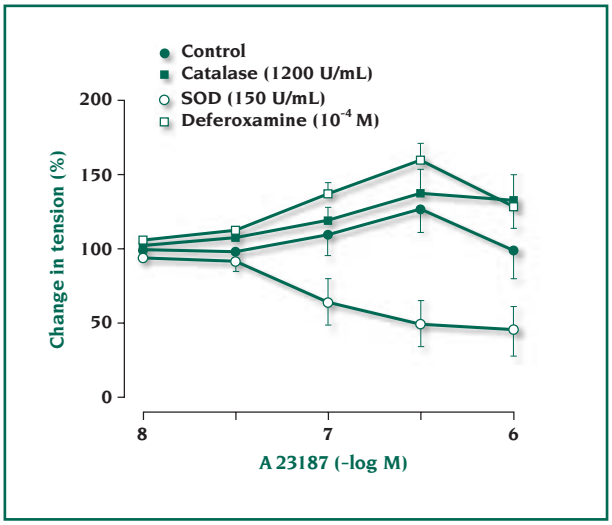

Figure 13. *In rings with endothelium of canine basilar arteries, increasing concentrations of the calcium ionophore A23187 cause contractions [not observed in preparations without endothelium [Katusic et al, 1988]), which are not affected by catalase (which removes hydrogen peroxide) or deferoxamine (which removes hydroxyl radicals), but are significantly reduced by superoxide dismutase (SOD, which dismutates superoxide anions). These experiments demonstrate that superoxide anions themselves play a key role in the endothelium-dependent contraction to acetylcholine in this preparation.*

Data from reference 30: *Katusic ZS, Vanhoutte PM. Superoxide anion is an endothelium-derived contracting factor.* Am J Physiol. *1989;257: H33-H37. Copyright © 1989, American Physiological Society.*

Contribution of oxygen-derived free radicals

In the canine basilar artery, endothelium-dependent contractions are prevented by superoxide dismutase, which dismutates superoxide anions to hydrogen peroxide, but not by catalase or deferoxamine, which scavenge hydrogen peroxide and hydroxyl radicals, respectively *(Figure 13).*[30] This pointed to the crucial role of superoxide anions in the response and led to the conclusion that the free radical in cerebral arteries is EDCF.[30-32] However, this hypothesis makes it hard to reconcile the obvious biological role of superoxide anions as intercellular messengers and their well established extremely short physicochemical half-life.

Whereas in diabetic blood vessels the involvement of oxygen-derived free radicals in endothelium-dependent contractions is clear-cut (see Cohen, in this issue), this has been more difficult to demonstrate in SHR arteries. The generation of oxygen-derived free radicals in the extracellular fluid bathing SHR aortas without endothelium yielded augmented contractions, compared with WKY arteries, which were inhibited by superoxide dismutase, indomethacin, and antagonists at TP receptors.[33] However, scavengers of these radicals, in particular superoxide dismutase, were without effect on endothelium-dependent contractions evoked by acetylcholine. Superoxide dismutase does not, or only partially, permeate cells, but should scavenge superoxide anions present in the intercellular space. Thus, its lack of effect does not favor the attribution of a role for superoxide anions as intercellular messengers of EDCF-mediated responses. More recent findings demonstrate that intracellularly acting scavengers of the free radical inhibit endothelium-dependent contractions to acetylcholine in the SHR aorta *(Figure 14),*[20] and that chronic in vivo depletion of superoxide anions also unmasks

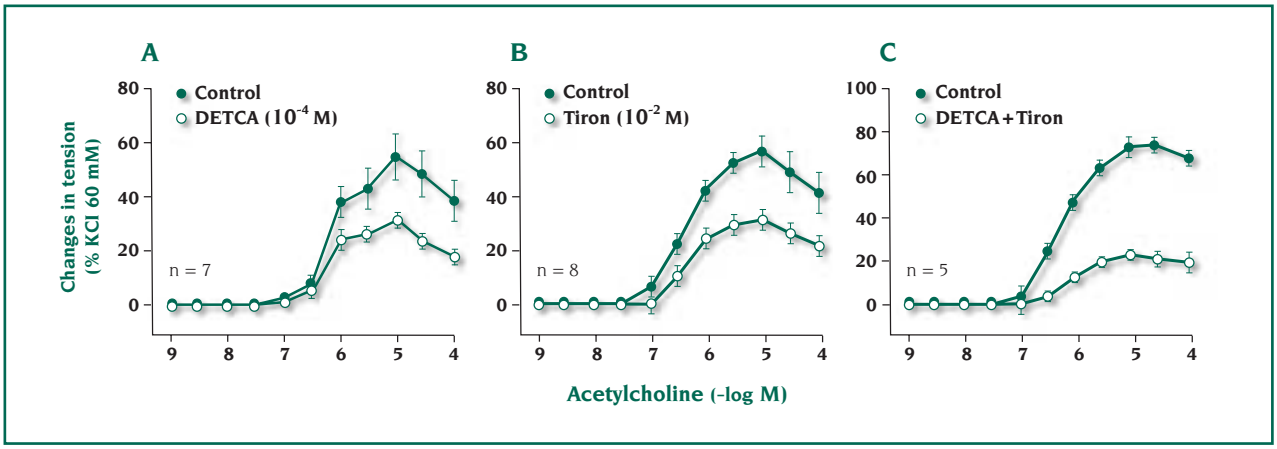

Figure 14. *Contractions to increasing concentrations of acetylcholine (in the presence of nitro-L-arginine) in rings of spontaneously hypertensive rat (SHR) aortas with endothelium. Comparison of the effect of two scavengers [diethyldithiocarbamic acid (DETCA) and 4,5-dihydroxy-1,3-benzene disulfonic acid (Tiron)] of superoxide anions which permeate inside cells. The scavengers were given alone or in combination. Both scavengers given alone (**A and B**) significantly inhibit the contraction to acetylcholine. Given together (**C**), they nearly abolish it. These findings imply that the intracellular production of superoxide anions is a key step in the endothelium-derived contracting factor (EDCF)-mediated response of the SHR aorta.*

Data from reference 20: *Yang D, Félétou M, Boulanger CM, et al. Oxygen-derived free radicals mediate endothelium-dependent contractions to acetylcholine in aortas from spontaneously hypertensive rats.* Br J Pharmacol. *2002;136:104-110. Copyright © 2002, Macmillan Specialist Journals.*

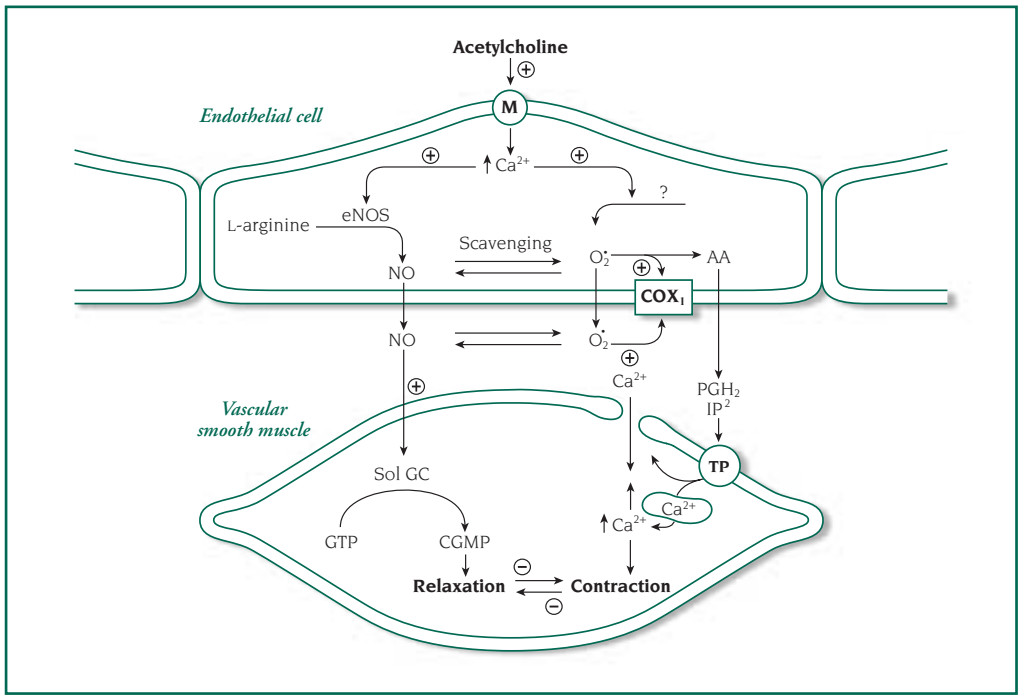

Figure 15.
Unifying hypothesis explaining cyclo-oxygenase-dependent endothelium-dependent contractions to acetylcholine.

Abbreviations: *AA, arachidonic acid; cGMP, cyclic guanosine monophosphate; COX_1, cyclooxygenase 1; eNOS, endothelial nitric oxide synthase; IP, isoprostanes; M, muscarinic receptor; NO, nitric oxide; O_2, superoxide anions; PGH_2, endoperoxides (prostaglandin H_2); Sol GC, soluble guanylate cyclase; SR, sarcoplasmic reticulum; TP, thromboxane prostanoid receptors; +, activation; -, inhibition; ?, unknown site of formation.*

an inhibitory effect of superoxide anions on these responses.[20] Conversely, the exogenous generation of free radicals in the extracellular space exacerbates the endothelium-dependent contractions to acetylcholine in the SHR aorta.[34] Hence, the presence of superoxide anions in the intercellular space must contribute to, or at least facilitate, EDCF-mediated contractions. Again, it is not easy to reconcile this conclusion, based on pharmacological experiments, with the extremely short half-life of the radical.

From superoxide anions to TP receptors

The experiments summarized above make it possible to suggest an unifying concept to explain EDCF-mediated responses, at least in blood vessels from SHR *(Figure 15).* Acetylcholine activates muscarinic receptors on the endothelial cell membrane, resulting in an increased intracellular concentration of calcium (which can be mimicked by the calcium ionophore A23187). The activation of the muscarinic receptors leads to two parallel events.[35] NOS increases its activity, yielding more NO, which diffuses to the smooth muscle, where it stimulates soluble guanylate cyclase to produce more cyclic guanosine monophosphate (cGMP). The augmented endothelial intracellular calcium concentration also stimulates the production of superoxide anions from an undefined source. Depending on the amount of NO being produced, which scavenges superoxide anions,[36] more or fewer superoxide anions can diffuse outside the endothelial cells. The intracellular, but also in part the extracellular superoxide anions stimulate the overexpressed, presumably membrane-bound, COX_1 to transform arachidonic acid into endoperoxides, and possibly isoprostanes. The latter could also be formed by the direct action of the free radical on the fatty acid. Endoperoxides and/or isoprostanes diffuse to the vascular smooth muscle to activate the hyperresponsive TP receptors. To observe endothelium-dependent contractions, both the greater activity of COX_1 and the hyperresponsiveness of the TP receptors are required.[22,23] A reduction in the release or bioavailability of NO greatly augments the amplitude of the endothelium-dependent contractions, as does an increased production of oxygen-derived free radicals; conversely, scavenging or depletion of superoxide anions, mainly inside the cells, depresses the phenomenon.[20,26,37]

Role in health and disease

In large cerebral arteries, acute stretch causes cyclo-oxygenase-dependent, endothelium-dependent contractions, a response that may contribute to the physiological autoregulation of the diameter of those blood vessels when exposed to sudden increases in arterial blood pressure.[38] However, it is more likely that the occurrence of such responses reflects a pathological process. Indeed, they are prominent not only in blood vessels of hypertensive rats, but also in diabetic arter-

ies (see Cohen, in this issue). In the latter case, they are clearly the consequence of the hyperglycemic state. In the case of spontaneous hypertension, the augmented response to endoperoxides, unlike the overexpression of COX_1, is already present in the aorta of younger SHR, at a time when they are not yet overtly hypertensive *(Figures 9 and 12, left).*[22,23,39,40] This hyperreactivity may constitute an important genetic basis for the disease. By contrast, the overexpression of cyclooxygenase and the resulting overproduction of endoperoxides (and possibly isoprostanes) must be viewed as an adjustment to the hypertensive process. The most likely explanation is that chronic essential hypertension results in premature aging of the endothelial cells, with, as one of the consequences, the overexpression and hyperactivity of COX_1. This interpretation is strengthened by the finding that endothelium-dependent contractions indeed appear progressively in the arteries of aging normotensive animals.[41-43] Likewise, in porcine coronary arteries covered with regenerated, senescent endothelial cells, an EDCF-mediated response can be observed.[28,44] Finally, this interpretation is in line with the results obtained in aging and hypertensive humans (see Taddei et al, in this issue). Thus, EDCF contributes to the endothelial dysfunction, which is expressed in particular by a reduced protective role against platelet aggregation,[45,46] which eventually initiates the atherosclerotic process, the most feared complication of both hypertension and diabetes.[47] This conclusion is considerably reinforced by the fact that the TP-receptor antagonist S18886 is able to inhibit the atherosclerotic process.[48-50]

THREE KEY QUESTIONS

"Endothelium-Dependent Contractions" is the topic of this, the third and last issue of a cycle devoted to the "endothelium" and its implications in cardiac disease (see *Dialogues Cardiovasc Med.* 1998;3:No. 4—Endothelium; and 2001;6:No. 4—Kinin Receptors and Endothelium-Dependent Responses). After recalling Furchgott and Zawadzki's groundbreaking discovery, reported in 1980, of the endothelium's essential role in the *relaxation* of arteries through the release of endothelium-dependent relaxing factors (EDRFs), the preceding pages endeavored to highlight the current understanding and applications of the intriguing, so-to-speak "mirror-image" discovery, only two years later, that the endothelium also played a pivotal role in eliciting *contractions* of the vascular smooth muscle, through factors, which, predictably enough, were dubbed endothelium-dependent contracting factors (EDCFs). In the following section, as usual, three experts in their respective fields single out salient aspects broached by the Lead Article. First come two chapters looking at experimental findings. Richard A. Cohen, making the point that EDCFs seem to be players in diabetic vascular disease, asks: **"Does EDCF contribute to diabetic endothelial cell dysfunction?"** Frank M. Faraci and Donald D. Heistad, noting that EDCFs also have functional effects in the cerebral circulation, reflect on the possible contribution of these factors to cerebral ischemia, vasospasm, and stroke, ask: **"Does EDCF play a role in the regulation of cerebral vascular tone?"** Stefano Taddei, Agostino Virdis, Lorenzo Ghiadoni, Daniele Versari, and Antonio Salvetti, building on these findings from the laboratory, turn their attention to their implications in humans, with a particular focus on the relevance of cyclooxygenase-dependent EDCFs in the control of vascular reactivity, and echo the clinician's preoccupations, by posing the question: **"Does EDCF play an important role in humans?"** All three experts are, of course, quick to point out the exciting therapeutic prospects opened by the current deeper understanding of endothelial function and EDCF.

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Does EDCF contribute to diabetic endothelial cell dysfunction?

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Diabetic vascular disease is accompanied by decreased formation of the vasodilators nitric oxide and prostacyclin and increased formation of vasoconstrictor eicosanoids. The alteration in function is caused by exposure of endothelial cells to elevated glucose and its effect to increase the formation of oxygen-derived free radicals. These in turn cause nitric oxide synthase to produce peroxynitrite, a damaging molecule. This inactivates prostacyclin synthase, leading to the accumulation of eicosanoids. These endothelium-derived constrictor factors (EDCF) also increase endothelial cell adhesion molecules and atherosclerotic lesions. The abnormalities can be prevented by adequate scavenging of oxygen-derived free radicals or by blocking the actions of the eicosanoids at thromboxane-prostanoid (TP) receptors.

Keywords: EDCF; diabetes; TP receptor; oxidant stress; endothelium; nitric oxide; prostacyclin

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The rate of progression of cardiovascular disease and the incidence of clinical vascular complications are dramatically increased in diabetes mellitus.[1] Blood vessels in the heart, kidney, eyes, and limbs are affected, leading to thrombotic and arteriosclerotic occlusive vascular disease. Based primarily on studies in diabetic experimental animals, endothelial dysfunction is thought to underlie alterations in vascular permeability, thrombogenicity, and vasomotor dysfunction.[2] The presence of abnormal endothelium-dependent vasodilatation in diabetic patients correlates with the clinical severity of the disease as well as the presence of complications related to it, including neuropathy and nephropathy.[3] In normal man as well as in experimental animals, endothelial cell dysfunction occurs following exposure for a few hours of blood vessels to elevated levels of glucose or fatty acids that mimic the levels found in the blood of diabetic patients.[4,5] This suggests that the metabolic manifestations of diabetes to which the endothelial cell is exposed lead directly to endothelial cell dysfunction. When endothelial cell dysfunction is present in diabetic patients, it carries with it an increased risk of coronary artery events and stroke, as well as increased risk of morbidity accompanying surgery.[6] Thus, it is important to understand the pathogenetic mechanisms that link the initial endothelial dysfunction that occurs when endothelial cells are exposed to elevated glucose and lipids with the accelerated progression of vascular disease. Endothelium-derived contracting factor(s) (EDCF) may represent such a link.

EDCF reflects an alteration in endothelial cell vasomotor function characterized by impaired vasodilatation and enhanced vasoconstriction. This alteration is thought to result from an imbalance of vasodilator and vasoconstrictor factors released by endothelial cells *(Figure 1, page 226).* Evidence exists that such an imbalance does exist in diabetes, and that it is important in

SELECTED ABBREVIATIONS AND ACRONYMS

EDCF	endothelium-derived contracting factor
HETE	hydroxyeicosatetraenoic (acid)
NO	nitric oxide
PG	prostaglandin
PGH_2	prostaglandin endoperoxide
PGI_2	prostacyclin
SOD	superoxide dismutase
TP	thromboxane-prostanoid (receptor)
TxA_2	thromboxane A_2

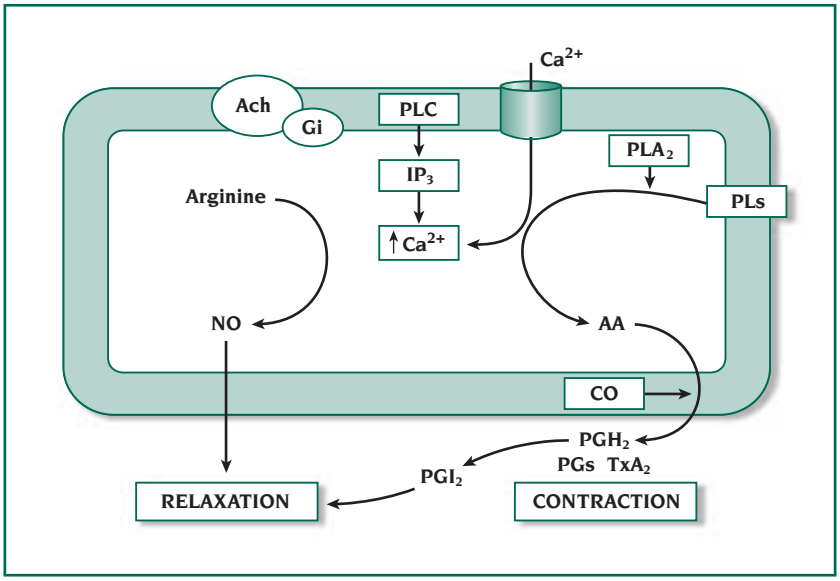

Figure 1. *Elaboration of vascular relaxing and contracting factors by vascular endothelium. Receptor-dependent agonists, like acetylcholine (Ach) stimulate phospholipase C (PLC) and inositol 1,4,5-trisphosphate (IP_3) via G-proteins (Gi) to increase intracellular calcium (Ca^{2+}). Ca^{2+} stimulates nitric oxide synthase to convert arginine to nitric oxide (NO) to relax vascular smooth muscle. Ca^{2+} also stimulates phospholipase A_2 (PLA_2) to release arachidonic acid (AA) from membrane phospholipids (PLs), which via cyclooxygenase is converted to prostaglandin endoperoxide (PGH_2). PGH_2 is precursor to vasodilating prostacyclin (PGI_2) and vasoconstrictor prostaglandins (PGs) including thromboxane A_2 (TxA_2).*

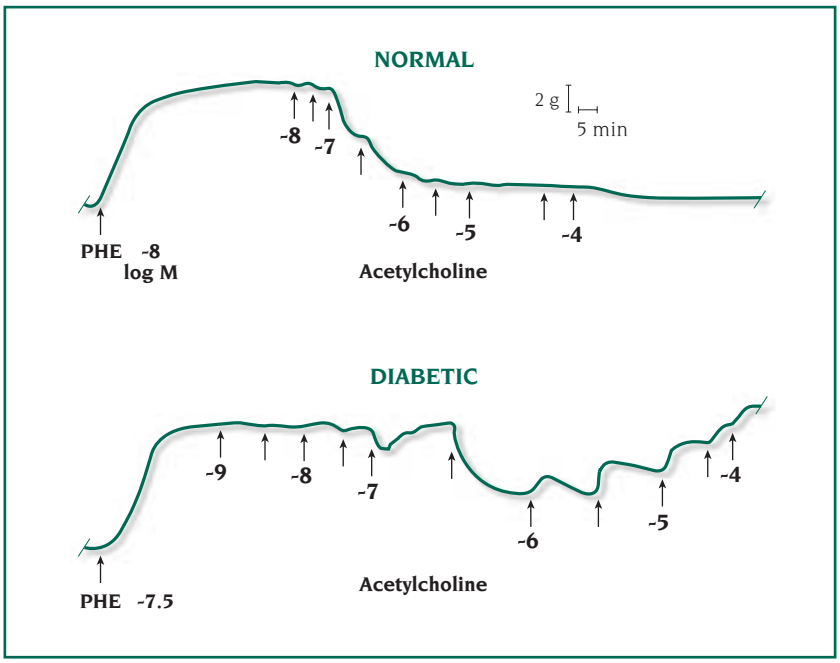

Figure 2. *Endothelial cell dysfunction in diabetic rabbit aorta. Rabbits were treated with alloxan or vehicle to produce diabetes for 6 weeks. The aorta was removed to measure contractions to phenylephrine (PHE) and relaxations to acetylcholine (arrows indicate half-log increments in concentration). The lower aortic ring from a diabetic rabbit contracts normally to phenylephrine, but does not relax normally to acetylcholine. Note the contractions caused by acetylcholine.*

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mediating changes in vascular function. In addition, it appears that EDCF arises as a result of vascular oxidant stress caused by elevated glucose. Finally, EDCF may be not only a marker of endothelial dysfunction, but may also accelerate vascular disease in diabetes. Just as endothelial dysfunction associated with other cardiovascular risk factors represents a predictor of future vascular complications, EDCF may serve as a marker for the increased propensity of diabetic patients to suffer vascular complications.[6]

IMPAIRED ENDOTHELIUM-DEPENDENT VASODILATATION IN DIABETES

Diverse cultured cell and animal studies, as well as studies of physiological measurements of vascular function in vivo or on isolated arteries from diabetic patients, indicate that impaired endothelium-dependent vasodilatation is a characteristic of both type 1 and type 2 diabetes.[2] Because the vasodilator functions of the endothelium are mediated primarily by nitric oxide (NO) and prostacyclin, abnormal vasodilatation in diabetes can result from impaired synthesis and release, increased destruction, or decreased response to these vasodilators. Thus, for NO, decreased expression of NO synthase has been noted in the skin of diabetic patients,[3] although, despite impaired vasodilatation, increased expression of the synthase is a characteristic of short-term cell and animal models.[7] Destruction of NO by superoxide anion may be increased, because many studies have found that superoxide dismutase, which specifically scavenges the superoxide radical, can ameliorate diabetic endothelial dysfunction.[8] Despite this finding, vasodilatation to NO donors such as sodium nitroprusside may be nor-

mal, likely because these donors release NO intracellularly or in a form that is not reactive with superoxide anion. Decreased levels of prostacyclin are also a feature of diabetic vascular disease.

EDCF IN DIABETES

In addition to impaired vasodilator mechanisms, augmented endothelium-derived vasoconstrictors may exist in diabetic macrovasculature and microvasculature. Results from the diabetic rabbit model shown in *Figure 2*,[9] demonstrate that the endothelium-dependent vasodilator produces the abnormal endothelial function *(Figure 3)*.[11] In contrast, a similarly hyperosmolar concentration of a nonmetabolized sugar like mannose has no effect.[12]

EDCF has been implicated in this and other studies of diabetic animal and human arteries by the finding that thromboxane A_2 (TxA_2)–prostanoid receptor (TP) antagonists may normalize the response of the diabetic artery, while leaving that of the normal artery unaffected.[10,12] Shown in *Figure 3* is a normal endothelium-dependent vasodilator response to acetylcholine in a rabbit aorta that was exposed to elevated glucose, but treated with a TP-receptor antagonist, SQ29548. In contrast to the beneficial effect of the TP-receptor antagonist, thromboxane synthase inhibitors have no effect.[10] This suggests that eicosanoids other than TxA_2 mediate the EDCF-mediated vasoconstriction. Because exogenous prostaglandin endoperoxide, the prostaglandin precursor, and 15-HETE (hydroxyeicosatetraenoic [acid]) mimic the effect of diabetes on endothelial function in normal arteries, and their production is increased in diabetes, these mediators may be implicated as EDCFs.[13,14]

OXIDANT STRESS, DIABETIC ENDOTHELIAL CELLS, AND EDCF

Another feature of diabetic endothelial dysfunction is oxidant stress or increased oxygen-derived free radicals. This is indicated by the fact that several scavengers of oxygen-derived free radicals can prevent or reverse the affect of exposure to elevated glucose or diabetes. *Figure 3* shows that a rabbit artery treated with superoxide dismutase does not develop abnormal vasodilatation when exposed to elevated glucose. Superoxide dismutase also reverses the abnormal endothelium-dependent relaxations in vessels of diabetic animals, such as those shown

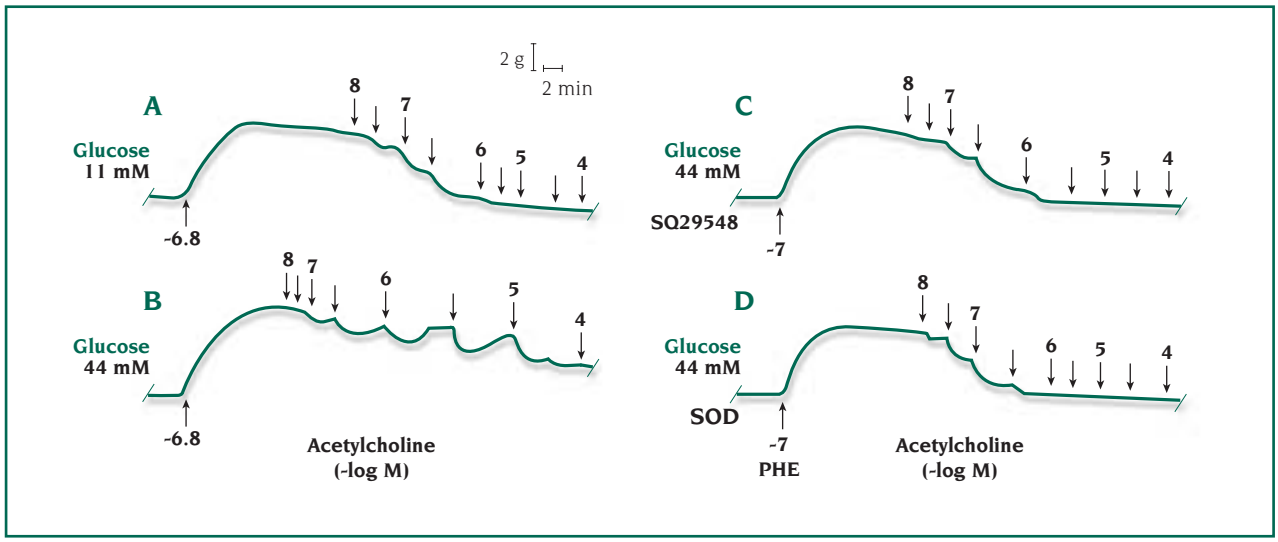

Figure 3. *Endothelial cell dysfunction caused by exposure to elevated glucose. Force recordings of isolated normal rabbit aortic rings showing the effects of exposure for 6 hours to an elevated glucose concentration of 44 mmol/L (mM) on endothelium-dependent relaxation to acetylcholine. Compared with the control ring* ***(A)****, the relaxation of the ring exposed to elevated glucose is reduced* ***(B)****. Treatment with either a thromboxane A_2 receptor antagonist (SQ29548, 1 µmol/L), or superoxide dismutase (SOD, 150 units/mL) prevented the abnormal response in the rings exposed to elevated glucose. Arrows with numbers indicate half-log increases in concentration. Data are from references 10 and 15.*

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in *Figure 2.*[15] These findings, confirmed by several investigators, indicate that superoxide anion, or several oxidant derivatives of it, are responsible for either decreased NO function, and/or the increased vasoconstriction observed. Such a free radical–dependent mechanism may also apply to diabetic patients as suggested by the finding that high doses of vitamins C or E can acutely reverse abnormal endothelial function in diabetic patients[16] or that which exists in normal subjects after short-term exposure of their forearm vasculature to glucose infusions.[5]

Studies of endothelial cells exposed to elevated glucose concentrations in culture also indicate that a free radical mechanism is involved in the reduced production of both NO and prostacyclin in diabetes. When human[17] or bovine[7] aortic endothelial cells are exposed to elevated glucose concentrations for 3 days, cyclic GMP levels are much lower, indicative of reduced NO activity. The decreased NO activity is accompanied by a marked increase in the release of superoxide anion by the cells. The increased superoxide anion production is blocked by inhibitors of NO synthase *(Figure 4A),*[7] indicating that under these conditions the NO synthase enzyme is functionally uncoupled. This decreases NO and increases superoxide anion production. Similar uncoupling of NO synthase has been observed in a number of pathological models in addition to diabetes, and may be due to reduced levels of the cofactor tetrahydrobiopterin[18] and/or oxidative modification of the synthase protein itself.[7] Thus, in endothelial cells exposed to elevated glucose, NO synthase can become a generator of superoxide anion in addition to NO. Importantly, these two radicals react together rapidly to form peroxynitrite ($ONOO^-$), which

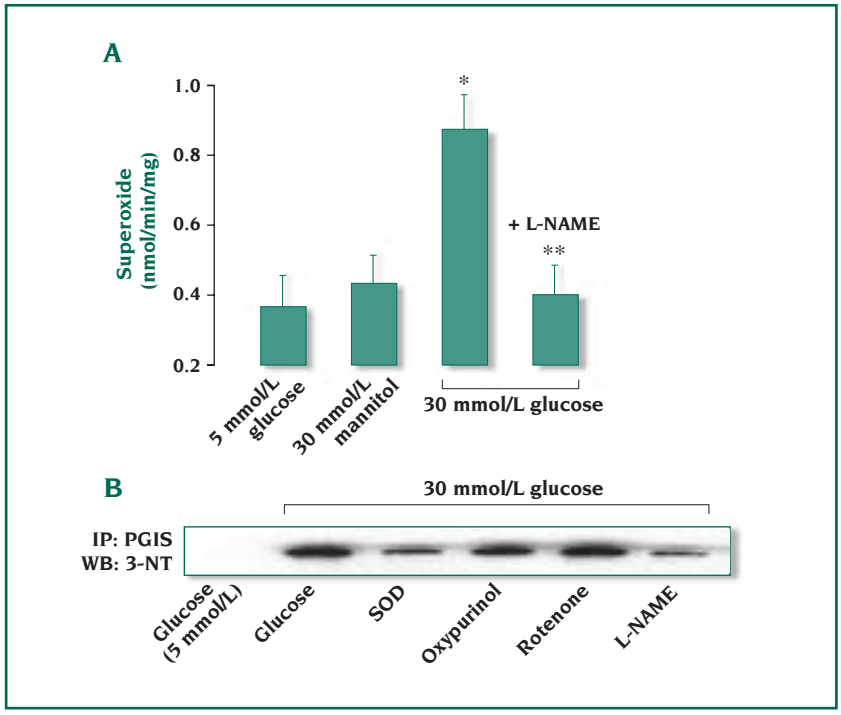

Figure 4. *Endothelial nitric oxide synthase releases superoxide anion in endothelial cells exposed to elevated glucose. Bovine aortic endothelial cells were exposed for 3 days to control (5 mmol/L) glucose, elevated glucose (30 mmol/L), or elevated mannitol (25 mmol/L plus 5 mmol/L glucose) as an osmotic control. Superoxide anion production was measured by cytochrome c reduction* ***(A).*** *Elevated glucose, but not mannitol, caused a more than threefold increase in superoxide anion production that was prevented in cells treated with a nitric oxide synthase inhibitor, L-nitro-arginine methyl ester (L-NAME). Evidence that superoxide anion and nitric oxide combine to form peroxynitrite is demonstrated by the tyrosine nitration of the endothelial cell protein prostacyclin synthase* ***(B).*** *Prostacyclin synthase (PGIS) was immunoprecipitated (IP) from lysates of endothelial cells exposed to elevated glucose and western blotted (WB) for nitrotyrosine (3-NT). Elevated glucose increased tyrosine-nitrated prostacyclin synthase, a process that was attenuated by superoxide dismutase (SOD) or L-NAME, but not oxypurinol or rotenone, inhibitors of superoxide anion production by xanthine oxidase and mitochondria.*

Reproduced from reference *7: Zou MH, Shi C, Cohen RA. Oxidation of the zinc-thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite.* J Clin Invest. *2002;109:817-826. Copyright © 2002, American Society for Clinical Investigation.*

itself is an extremely reactive oxidant destructive to lipids, proteins, and DNA *(Figure 5).*

Production of $ONOO^-$ in cells exposed to elevated glucose is suggested by the finding that prostacyclin synthesis in the cells is reduced dramatically. This is because the prostacyclin synthase enzyme is exquisitely sensitive to inactivation by $ONOO^-$ by a mechanism involving nitration of the tyrosine amino acids in the protein.[17] In endothelial cells exposed to elevated glucose for 3 days, prostacyclin synthase undergoes tyrosine nitration and prostacyclin production is inhibited.[17] This alteration of protein structure and function is prevented by either superoxide dismutase or by inhibiting NO synthase *(Figure 4).* Similar endothelial NO synthase and prostacyclin synthase dysfunction is found in tissues of diabetic mice.[7] The sensitivity to oxidants of prostacyclin synthase is likely to be critically important to the imbalance of vasodilators and vasoconstrictors seen in diabetes. When prostacyclin synthase in isolated arteries is intentionally inactivated, prostacyclin-dependent vasodilatation is replaced by prostaglandin-dependent

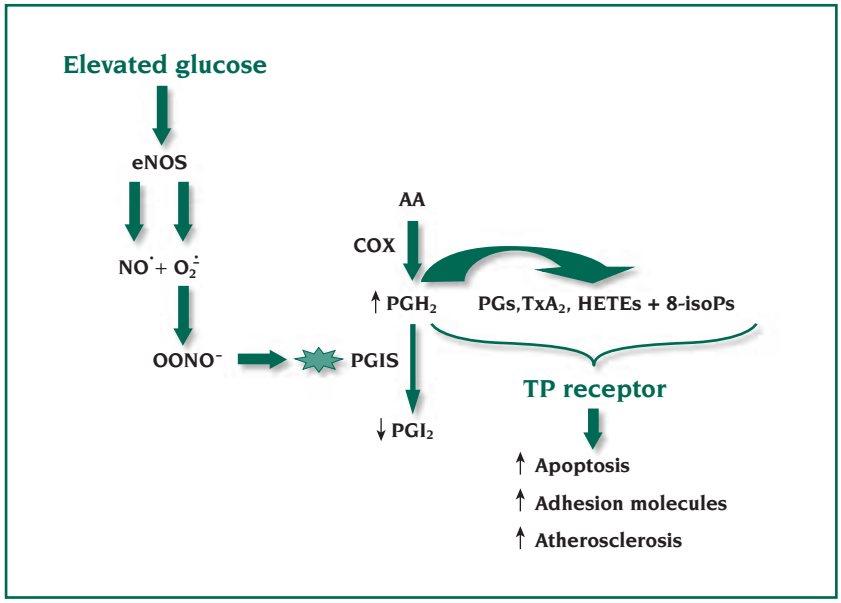

Figure 5. *Hypothetical mechanism of how oxidants inactivate prostacyclin synthase and lead to thromboxane-prostanoid (TP) receptor–mediated events in endothelial cells exposed to elevated glucose. Exposure of endothelial cells to elevated glucose leads to functional uncoupling of nitric oxide synthase (eNOS), which then produces superoxide anion ($O_2^{\cdot-}$) in addition to nitric oxide (NO). These react to form peroxynitrite ($OONO^-$). $OONO^-$ can oxidatively inactivate prostacyclin synthase and reduce the formation of prostacyclin (PGI_2). In addition, inactivation of prostacyclin synthase redirects arachidonic acid (AA) metabolism such that the cyclooxygenase product prostaglandin endoperoxide (PGH_2) accumulates. This leads to increased production of thromboxane A_2 (TxA_2), hydroxyeicosatetraenoic acids (HETEs), and 8-isoprostanes (8-isoPs). These TP receptor agonists are implicated in increased endothelial cell apoptosis and adhesion molecules, as well as atherosclerotic lesions. COX, cytochrome c oxidase; PGs, prostaglandins.*

vasoconstriction.[19] This is thought to occur as a result of arachidonic acid metabolism being redirected. As a result of inactivation of the prostacyclin synthase enzyme, the prostacyclin precursor prostaglandin endoperoxide (PGH_2), which is itself a potent vasoconstrictor, accumulates and also leads to increases in other eicosanoids, including TxA_2 and 15-HETE. These eicosanoids all vasoconstrict by stimulating TP receptors.[13,14] In the presence of oxidants, there is also accumulation of 8-isoprostanes, oxidized arachidonic acid derivatives that also vasoconstrict via TP receptors. Thus, the endothelial cell dysfunction in diabetes is due to several factors and represents both a reduction in the vasodilators, NO, and prostacyclin, but also an increase in eicosanoid vasoconstrictors that stimulate TP receptors.

EDCF AND VASCULAR DISEASE IN DIABETES

There are several indications that the endothelial cell dysfunction in diabetes contributes to acceleration of vascular disease. For one, the decrease in NO and prostacyclin, which are both known to have vascular protective actions, is expected to adversely affect the development of vascular disease. This has been directly shown in studies demonstrating that atherosclerosis is increased in animals when NO synthase[20] or prostacyclin receptors have been genetically deleted.[21] Of equal importance to the loss of the effects of these vasodilator and vascular protective factors, the vasoconstrictor actions of EDCF in diabetes may also be accompanied by TP-receptor–mediated vascular abnormalities that promote vascular disease.

As an example, in endothelial cells exposed to elevated glucose, there is increased expression of adhesion molecules that are responsible for the influx of macrophages that constitute the atherosclerotic plaque.[17] The increase in adhesion molecule expression parallels the inactivation of prostacyclin synthase and is prevented by either inhibiting NO synthase or scavenging superoxide anion, indicating that superoxide anion derived from endothelial NO synthase is involved. Furthermore, although the reduced prostacyclin production is not affected, blocking TP receptors prevents the increase in adhesion molecules.[17] By inference, the inactivation of prostacyclin synthase is related to the production of one or more eicosanoids that stimulate TP receptors, which, in turn, stimulate the expression of adhesion molecules. Similar studies have shown that endothelial cells exposed to elevated glucose also undergo programmed cell death, or apoptosis, which has been suggested as being important in promoting vascular disease.[17] In endothelial cells exposed to elevated glucose, blocking TP receptors also prevents the increased apoptosis that occurs.

Stimulation of TP receptors by endogenous vascular eicosanoids has also been suggested to play a role in atherogenesis and neointimal proliferation. Blockade of TP receptors with a potent TP receptor antagonist, S18886, was shown to significantly decrease atherosclerosis in mice.[22] Because aspirin, which significantly diminished platelet production of TxA_2, had no effect on atherogenesis, these studies pointed to other vascular eicosanoids, whose production was not blocked by aspirin, as important mediators.[22]

Mimicking the increased vascular disease seen in human diabetic patients, diabetic animals demonstrate

accelerated atherogenesis.[7] Recent studies indicate that the TP-receptor antagonist S18886 prevents this acceleration of atherosclerosis caused by diabetes, suggesting that mechanisms exist in vivo that are similar to those that increase adhesion molecules and apoptosis in endothelial cells exposed in culture to elevated glucose.[23] There are, in addition, reports that blocking TP receptors can slow vascular and renal complications in diabetic patients.[24] Although further studies are needed to investigate the relative role of TxA_2 released from platelets compared with that of other vascular eicosanoids, the studies discussed above strongly suggest that vascular eicosanoids play a significant role. If diabetes increases PGH_2 production in endothelial cells, this EDCF would likely promote platelet aggregation by stimulating platelet TP receptors and by serving as substrate to produce more platelet TxA_2. Thus, abnormal platelet-endothelial cell interactions and increased thrombosis in diabetes might be explained.

SUMMARY

EDCF in diabetes is evidenced by reduction in the vasodilators NO and prostacyclin and increase in TP-receptor–stimulating eicosanoids. This imbalance is due to oxidant stress that arises in endothelial cells exposed to elevated glucose, which partially inactivates NO synthase and prostacyclin synthase. The eicosanoids that arise as a result of the oxidant-induced inactivation of prostacyclin synthase stimulate TP receptors to increase endothelial adhesion molecules and cell death, a process that accelerates vascular disease. Because clinical control of diabetic metabolic status is often suboptimal, future adjunct therapies will likely depend upon preventing the oxidant effects of elevated glucose and/or blocking the actions of the eicosanoids produced.

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Does EDCF play a role in the regulation of cerebral vascular tone?

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Several endothelium-derived contracting factors (EDCFs) are produced that have functional effects in the cerebral circulation. Although most studies suggest that EDCFs do not affect resting tone of cerebral blood vessels under normal conditions, recent findings indicate that EDCFs contribute importantly to vascular dysfunction in disease states. This review will focus on the nature and influence of EDCFs produced under pathophysiological conditions and that may potentially contribute to cerebral ischemia, vasospasm, or stroke.

Keywords: endothelium; EDCF; reactive oxygen species; endothelin; cerebral circulation

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Endothelium-derived relaxing factor (EDRF) was first described in the now classic work by Furchgott and Zawadzki using rabbit aorta.[1] This work was quickly followed by Lee's demonstration of endothelium-dependent relaxation in cerebral arteries.[2] Many studies over the past two decades have shown that endothelium exerts a powerful influence on vascular tone in brain. This influence is mediated by production and release of a diverse group of EDRFs and endothelium-derived contracting factors (EDCFs).[3-4]

Among the relaxing factors, nitric oxide (NO), produced by the endothelial isoform of NO synthase, is the major EDRF. Other EDRFs include products of cyclooxygenases (COX-1 and COX-2) and endothelium-derived hyperpolarizing factor(s), which may be important under some conditions or for select stimuli.[3-6] The purpose of this review is to summarize the nature and influence of EDCFs that appear to contribute to vascular dysfunction in brain. The focus will be on three groups of EDCFs—endothelin, products of COX, and reactive oxygen species.

ENDOTHELIN

The EDCF that has received the most attention is endothelin, a peptide originally isolated from aortic endothelium. There are three isopeptides of endothelin, all of which are products of separate genes (ET-1, ET-2, and ET-3).[7] Endothelins are synthesized as larger preproforms, which are then converted to propeptides. For example, prepro-endothelin-1 is cleaved to big-endothelin-1, which is then converted to endothelin-1 (ET-1) by endothelin-converting enzyme (ECE).[7] Of the three isopeptides, endothelium is thought to only produce ET-1.[4,7]

Endothelins produce effects in blood vessels through activation of two groups of receptors, endothelin-A (ET_A) and endothelin-B (ET_B) receptors. In general, ET_A receptors are expressed in vascular muscle and mediate contraction to endothelin. The effect of activation of ET_B receptors on vascular tone depends on localization of the receptor. ET_B receptors are expressed in smooth

SELECTED ABBREVIATIONS AND ACRONYMS

COX	cyclooxygenase
EDCF	endothelium-derived contracting factor
ECE	endothelin-converting enzyme
EDRF	endothelium-derived relaxing factor
ET	endothelin
NO	nitric oxide
SOD	superoxide dismutase

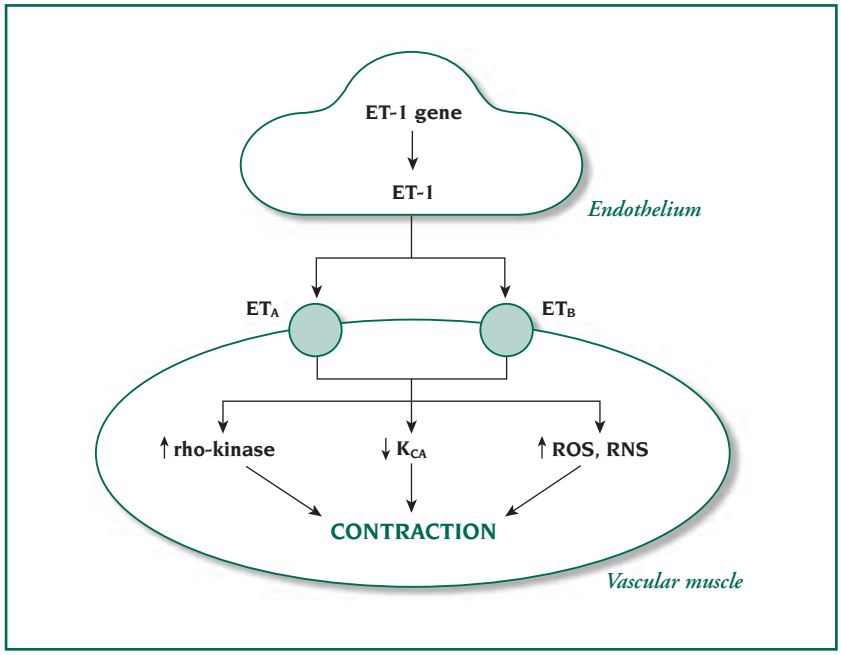

Figure 1. *Summary of synthesis of endothelin-1 (ET-1), an important endothelium-derived contracting factor (EDCF) in the cerebral circulation, in endothelium, and mechanisms by which ET-1 produces contraction of vascular muscle. Gene transcription results in formation of ET-1. ET-1 can activate both ET_A and ET_B receptors on vascular muscle and cause contraction. Contraction of cerebral vascular muscle is mediated by ET_A receptors in normal vessels, but can be mediated by ET_B receptors in some disease states* (see text for details). *Contraction of smooth muscle in response to activation of ET_A and/or ET_B receptors may be mediated by multiple mechanisms including activation of rho-kinase, decreased activity of calcium activated potassium channels (K_{ca}), and stimulation of formation of reactive oxygen and reactive nitrogen species (ROS and RNS, respectively).*

muscle in some blood vessels or under some pathophysiological conditions (see below) where they mediate contraction *(Figure 1).* In contrast, activation of ET_B receptors on endothelium normally produces endothelium-dependent relaxation.

Endothelin gene expression in normal brain endothelium appears to be low or absent, but can be enhanced by several factors including thrombin, transforming growth factor–β, hemoglobin, tumor necrosis factor–α, and reactive oxygen species.[4,7] Conversely, endothelin gene expression can be inhibited by NO and/or cyclic guanosine monophosphate (GMP).[7]

ET-1 produces potent and long-lasting contraction of cerebral vessels both in vivo and vitro.[4] Vasoconstriction in response to ET-1 is dependent on extracellular calcium and may be mediated by several mechanisms including activation of protein kinase C and/or the Rho kinase pathway.[4,8] ET-1 can also produce contraction of cerebral vessels by inhibition of calcium-activated potassium channels, as well as formation of reactive oxygen and nitrogen species *(Figure 1).*[9,10]

The predominant mechanism of vasoconstriction in response to endothelin in cerebral blood vessels is by activation of ET_A receptors.[4] Many studies have suggested that endothelin does not contribute to regulation of cerebral vascular tone under normal conditions, as application of inhibitors of ET_A, or both ET_A and ET_B, receptors has no effect on tone of cerebral vessels.[4] In contrast, a recent study reported that inhibition of ET_A receptors increases sensitivity of the middle cerebral artery to NO in intact, but not endothelium-denuded, vessels.[11] These results suggested that endothelium-derived endothelin affects tone of normal vessels by altering sensitivity to NO.[11] In contrast to its apparently limited role in normal blood vessels, endothelin may have major effects on vascular tone in brain under pathophysiological conditions.

Levels of endothelin are increased in the basilar artery and in cerebrospinal fluid following subarachnoid hemorrhage.[4,12] Evidence that these changes are functionally important is provided by the findings that vasospasm following experimental subarachnoid hemorrhage can be significantly attenuated by antagonists of ET_A receptors, combined ET_A/ET_B receptor antagonists, inhibitors of ECE, or antisense oligonucleotides for preproendothelin-1 mRNA.[4,12-15]

As noted above, contraction of cerebral arteries to endothelin is usually mediated by ET_A receptors. Interestingly, increased expression of ET_B receptors in vascular muscle may contribute to vasoconstriction after subarachnoid hemorrhage and ischemia *(Figure 1).*[16,17] Ischemia produces increased levels of endothelin-B receptor mRNA in vascular muscle, and ET_B-mediated increases in vascular tone can be detected following cerebral ischemia.[17] Thus, although ET_B receptors produce relaxation of cerebral vessels normally (via the endothelium),[4] expression of ET_B receptors in vascular muscle can occur during disease states and may contribute to vascular dysfunction and increases in cerebral vascular resistance.

Although most studies of the role of endothelin in cerebral vessels have focused on ischemia and subarachnoid hemorrhage, endothelin

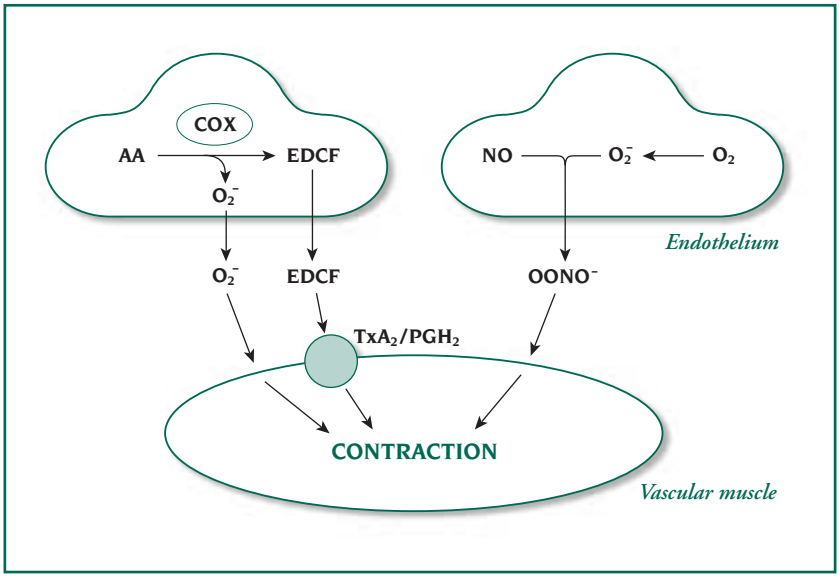

Figure 2. *Two mechanisms of endothelial dysfunction in cerebral vessels. A cyclooxygenase (COX)-derived endothelium-derived contracting factor (EDCF) may be formed from arachidonic acid (AA). This EDCF can diffuse to vascular muscle and cause contraction by activation of thromboxane A_2/prostaglandin H_2 receptors (TxA_2/PGH_2). Superoxide (O_2^-), produced by the COX pathway, may also produce contraction of vascular muscle. Superoxide can also react with nitric oxide (NO), inactivating NO and resulting in formation of peroxynitrite ($ONOO^-$). $ONOO^-$ can produce contraction of vascular muscle* (see text for details).

may influence the cerebral circulation in other disease states. For example, responses of cerebral arteries to NO are impaired in a rat model of heart failure and are restored to normal by an ET_A receptor antagonist.[18] Experiments utilizing chronic treatment of spontaneously hypertensive rats with bosentan, an ET_A and ET_B receptor antagonist, suggest that endothelin contributes to the development of hypertrophy, but not remodeling or changes in distensibility, of cerebral arterioles.[19] These findings suggest that endogenous endothelin contributes to cerebral vascular dysfunction in several disease states.

A CYCLOOXYGENASE-DEPENDENT EDCF

In cerebral arteries, such as the canine basilar artery, and in some pathophysiological conditions, COX-derived contracting substances may be produced by endothelium *(Figure 2).*[3] These EDCFs are products of arachidonic acid metabolism and produce contraction by activation of PGH_2/thromboxane A_2 receptors *(Figure 2).*[3,4,20] COX-mediated endothelium-dependent contraction occurs in response to diverse stimuli including acetylcholine, A23187, arachidonic acid, mechanical stretch, anoxia, purines, nicotine, angiotensin, norepinephrine, and serotonin.[3,4] The relative importance of the two COX isoforms, COX-1 vs COX-2, as sources of EDCF in the cerebral circulation is not yet known.

It is noteworthy that most of the studies that suggest that a COX-derived EDCF has functional effects in normal cerebral arteries have been performed using the canine basilar artery.[3] To what extent the production of EDCF observed in this artery is representative of that of other species is not known.

Endothelial function is altered in several pathophysiological states and with aging. For some of these disease states, impairment of endothelial function may include production of COX-derived EDCF(s) *(Figure 2).* For example, endothelial function in the cerebral circulation is altered in the spontaneously hypertensive rat and in a rat model of type 1 diabetes.[4,21,22] In cerebral arterioles, indomethacin or inhibition of prostaglandin H_2/thromboxane A_2 receptors restores endothelial function to normal, suggesting that endothelial dysfunction is mediated by a COX-derived EDCF.[21,22] Similarly, recent evidence suggests that impaired endothelial function in a novel murine model of hypertension (transgenic mice that express human renin and human angiotensinogen) involves production of a COX-derived contracting factor.[23] Inhibitors of COX or prostaglandin H_2/thromboxane A_2 receptors have no effect on resting tone or responses to endothelium-dependent stimuli in control animals, indicating that the same EDCF has no influence on vascular tone under normal conditions.[4,21-23] The chemical nature of the COX-dependent EDCF in brain is not clear. One recent hypothesis is that isoprostanes, which activate thromboxane A_2 receptors, may function as one type of EDCF.[24] Isoprostanes are novel arachidonic acid metabolites that may be generated by the interaction of superoxide with arachidonic acid (independent of COX or other enzyme activity). At least one isoprostane (8-*iso* $PGF_{2\alpha}$) is a COX product, however.[24] In addition, superoxide generated during COX activity could interact with arachidonic acid to form isoprostanes.

REACTIVE OXYGEN AND REACTIVE NITROGEN SPECIES

Reactive oxygen species (superoxide, hydrogen peroxide, hydroxyl radical) and reactive nitrogen species

(including peroxynitrite) can be generated by all of the major cell types within the vessel wall including endothelium. In different species and vessels, both relaxation and contraction in response to superoxide (or other reactive oxygen species) have been described in cerebral blood vessels.[4,5,20,25-28] Under some conditions, for example, reactive oxygen and nitrogen species produce contraction of cerebral arteries. This contractile response may be due to several mechanisms, including inactivation of basal NO, inhibition of potassium channels, and/or direct activation of vasoconstrictor pathways *(Figure 2).*

A recent study suggested that the direct effect of peroxynitrite on cerebral vascular muscle is contraction and that this contractile effect is due to inhibition of calcium-dependent potassium channels *(Figure 2).*[29] Responses to peroxynitrite are diverse, however, as the reactive nitrogen species produces dilatation of cerebral arterioles in vivo.[25] In large cerebral arteries, increases in flow or NADPH (which presumably activate an NAD(P)H oxidase) can produce superoxide-mediated contraction.[26,27] In a model of inflammation (local treatment with magnesium silicate in vivo), sustained spasm of the basilar artery can be prevented by exogenous application of the CuZn isoform of superoxide dismutase (SOD).[30] Overexpression of CuZn-SOD or extracellular SOD (EC-SOD) in genetically altered mice[31,32] or increased expression of EC-SOD in meninges and cerebral arteries by gene transfer[33] each inhibit vasospasm following subarachnoid hemorrhage, which suggests that superoxide is a key mediator of vasospasm.

It is unclear why reactive oxygen and nitrogen species have such divergent effects on tone in different cerebral arteries and in vessels from different species. In relation to vasoconstrictor effects of reactive oxygen species, a recent study suggested that low levels of superoxide produce relaxation and high concentrations of superoxide produce contraction of cerebral arteries.[26] Similarly, in cerebral arterioles in vivo, a superoxide-generating system (acetaldehyde + xanthine oxidase) produced dilatation at low substrate concentrations and a biphasic response (constriction followed by dilatation) at higher substrate concentrations.[28]

It is noteworthy that there may be interactions between different EDCFs. For example, endothelin can cause production of superoxide in brain[34] and in vascular cells.[10] Endothelin-derived superoxide can be produced by cyclooxygenase and NAD(P)H oxidase.[10,34] Conversely, reactive oxygen species can stimulate formation of endothelin.[35] Such interactions may help to explain why vasospasm after subarachnoid hemorrhage can be attenuated both by inhibitors of endothelin formation (or receptors) and scavengers of superoxide.

CONCLUSION

A "family" of EDCFs are produced that have functional effects on the cerebral circulation. Under several pathophysiological conditions, impaired endothelial function may be mediated, at least in part, by EDCFs. Increased EDCF formation may shift the balance of vascular tone toward constriction, thus contributing to reductions in cerebral blood flow, vasospasm, and ischemia.

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Does EDCF play an important role in humans?

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Endothelial cells produce cyclooxygenase-dependent vasoconstrictor substances, such as prostanoids and oxygen free radicals. Cyclooxygenase-dependent endothelium-derived contracting factors (EDCFs) were at first identified as responsible for impaired endothelium-dependent vasodilation in patients with essential hypertension. However, production of EDCFs is a phenomenon specific to aging, with essential hypertension merely causing an earlier onset and worsening of this alteration. Furthermore, both in aging and in hypertension, the appearance of cyclooxygenase-derived contracting factors is associated with a parallel decrease in nitric oxide (NO) availability, suggesting that these substances could be oxygen free radicals. Other clinical conditions characterized by production of cyclooxygenase-dependent EDCFs are acute estrogen deprivation and heart failure. By reducing NO availability, EDCFs could conceivably act as promoters of atherosclerosis and cardiovascular events.

Keywords: endothelium; nitric oxide; cyclooxygenase; oxidative stress; essential hypertension; aging

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Soon after the initial discovery by Furchgott and Zawadzki[1] of the obligatory role of endothelial cells in relaxations of rabbit isolated arteries to acetylcholine, De Mey and Vanhoutte[2] found that the endothelium could also induce contractions of isolated canine arteries and veins.

A large number of agents and physical stimuli produce such contractions, and depending on the agent, stimulus, and anatomic origin of the blood vessel, several different endothelium-derived contracting factors (EDCFs) have been described, including cyclooxygenase-dependent EDCFs, endothelin and angiotensin II.[3] Although relaxing factors play a primary role in circulatory regulation in physiological conditions, available experimental evidence indicates that contracting factors may become important regulators of vascular tone and structure in aging or in pathological conditions such as hypertension, diabetes, vasospasm, and reperfusion injury.[4]

Among the different pathways leading to endothelium-dependent contractions, cyclooxygenase plays a primary role. Arachidonic acid induces endothelium-dependent contractions in arteries and veins, which can be inhibited by cyclooxygenase blockers.[4] Moreover, cyclooxygenase-dependent EDCFs can also be induced by acetylcholine and the calcium ionophore A23187 in different vessels in experimental models of hypertension or diabetes.[4]

So far, in animal vessels, two kinds of mediators of cyclooxygenase-dependent EDCFs have been identified, including oxygen free radicals (mainly superoxide anions), generated by hydroperoxidase activity of the enzyme, and prostanoids, such as thromboxane A_2 or prostaglandin H_2 *(Figure 1, page 238).*[3,4]

While prostanoids act exclusively as direct vasoconstrictors, oxygen free radicals can either directly constrict vascular smooth muscle, possibly by acting on prostaglandin H_2 receptors, or indirectly, since they are potent inducers of NO breakdown *(Figure 1).*

This review discusses the relevance of cyclooxygenase-dependent EDCFs in the control of vascular reactivity in humans.

SELECTED ABBREVIATIONS AND ACRONYMS

EDCF	endothelium-derived contracting factor
ERT	estrogen replacement therapy
L-NMMA	N^G-monomethyl-L-arginine
NO	nitric oxide

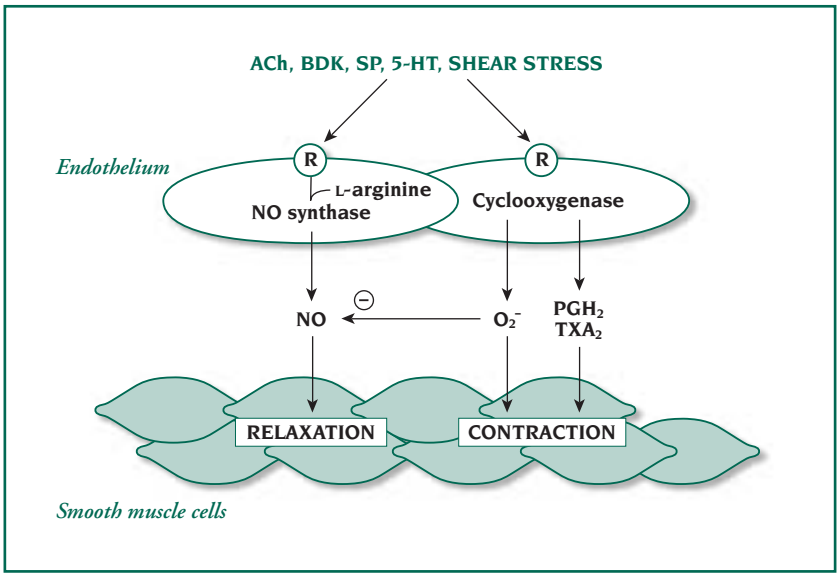

Figure 1. *Schematic diagram showing endothelium-derived nitric oxide (NO) and cyclooxygenase-dependent contracting factors in the vessel wall. In certain conditions, including advancing age or essential hypertension, endothelial stimulation can activate not only the L-arginine–NO pathway, but also cyclooxygenase, inducing production and secretion of prostanoids such as thromboxane A_2 (TXA_2) or prostaglandin H_2 (PGH_2) and oxygen free radicals, which cause vasoconstriction. Oxygen free radicals are also potent inducers of NO breakdown. ACh, acetylcholine; BDK, bradykinin; 5-HT, serotonin; R, receptor; SP, substance P.*

IDENTIFICATION OF CYCLOOXYGENASE-DEPENDENT EDCFs IN HUMANS

Studies in humans have been mainly conducted in the peripheral vasculature by means of the perfused forearm technique. Specific agonists and antagonists are directly infused into the brachial artery at systemically ineffective rates so as to induce adequate local vascular changes without systemic effects and consequent reflexogenic hemodynamic modifications. With this technique, the increase or decrease in forearm blood flow, measured by strain gauge venous plethysmography, is an index of local vasodilation or vasoconstriction.

To assess the possible production of cyclooxygenase-dependent EDCFs in humans, studies have been conducted using indomethacin, a cyclooxygenase inhibitor. In healthy subjects, the response to the endothelium-dependent vasodilator acetylcholine remains unchanged by intrabrachial indomethacin infusion, indicating that cyclooxygenase products do not play a major role in the regulation of endothelial responses in healthy conditions.[5] In contrast, in patients with essential hypertension, who typically show a blunted response to acetylcholine compared with normotensive controls,[5-7] the cyclooxygenase inhibitor increases, and almost normalizes, the vasodilating effect of the muscarinic agonist.[5] This finding clearly indicates the production of cyclooxygenase-dependent EDCFs that contribute to the pathogenesis of endothelial dysfunction in human primary hypertension. Patients with hypertension secondary to primary aldosteronism or renovascular disease are also characterized by curtailed endothelium-dependent vasodilation.[5] However, in these models of secondary hypertension, indomethacin does not improve the response to acetylcholine, indicating that EDCFs play no role in determin-

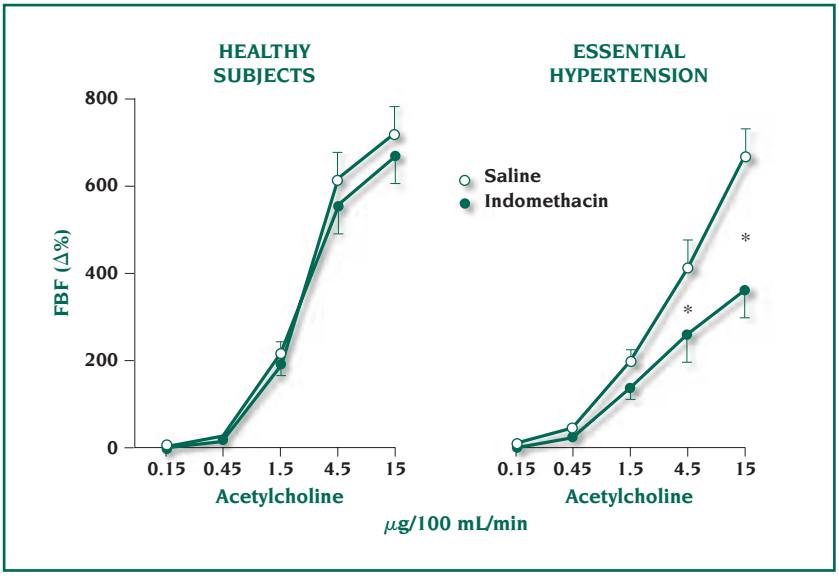

Figure 2. *Acetylcholine-induced increase in forearm blood flow (FBF) in the presence of saline (0.2 mL/min) or indomethacin at 50 μg/100 mL forearm tissue/min in normotensive subjects and patients with essential hypertension. Data are shown as means ± SD and expressed as FBF % increase above baseline. * Denotes a significant difference between infusion in control conditions and in the presence of different infusion rates of indomethacin (P<0.05 or less).*

Adapted from reference 5: *Taddei S, Virdis A, Mattei P, Salvetti A. Vasodilation to acetylcholine in primary and secondary forms of human hypertension.* Hypertension. *1993;21(6, pt 2):929-933.*

ing endothelial dysfunction in human secondary hypertension. These results, in perfect agreement with experimental findings,[4] demonstrate that production of cyclooxygenase-dependent EDCFs is not a mere consequence of blood pressure increase, but rather a specific pathological characteristic of genetic hypertension. Despite this, it must be noted that cyclooxygenase-dependent EDCF production has so far been identified only during endothelium stimulation by acetylcholine, while no evidence is available for other specific receptor-operated endothelial agonists such as bradykinin, substance P, serotonin, etc. Moreover, no findings are available on the role of EDCF in modulating endothelial responses activated by physical stimuli such as flow increase in large arteries.

The effect of indomethacin is achieved with a high infusion rate, 50 μg/100 mL forearm tissue/min, given to induce a local plasma drug concentration of around 10^{-5} M. Although this is the indomethacin concentration employed in animal experiments,[8] at these doses the drug may no longer be selective. Moreover, a 10 times lower indomethacin infusion rate, 5 μg/100 mL forearm tissue/min, did not change the vascular response to acetylcholine *(Figure 2)*.[5,9] It is therefore crucial to employ this compound at an adequate local concentration in order to be able to demonstrate the possible presence of cyclooxygenase-dependent EDCFs, at least in patients with essential hypertension, since lower concentrations such as those obtained by systemic administration would be devoid of effectiveness.

Finally, an important finding is that intrabrachial infusion of indomethacin does not change local basal flow. This indicates that cyclooxygenase-dependent EDCFs are not tonically produced and therefore do not contribute to the control of basal vascular tone. Only receptor-mediated endothelial activation can induce the production of these substances.

CYCLOOXYGENASE-DEPENDENT EDCFs IN AGING AND ESSENTIAL HYPERTENSION

Is the presence of cyclooxygenase-dependent EDCFs specific to and/or related to the pathogenesis of essential hypertension? This possibility seems to be excluded by the results obtained in young normotensive offspring of essential hypertensive patients. In such subjects, despite the presence of an impaired response to acetylcholine as compared with matched offspring of normotensive subjects, indomethacin fails to improve endothelium-dependent vasodilation.[10] It therefore appears that the genetic predisposition to develop hypertension can be associated with endothelial dysfunction, but not with production of cyclooxygenase-dependent EDCF. Thus, EDCFs cannot universally participate in endothelial dysfunction in essential hypertension.

On the other hand, endothelial dysfunction itself is not specific to essential hypertension, but more generally is a common characteristic of cardiovascular risk. The nonspecific association of hypertension and endothelial dysfunction is confirmed by experimental results in several animal models of genetic hypertension, which suggest that impaired endothelium-dependent vasodilation is an acceleration of an endothelial dysfunction that is characteristic of aging.[11] In humans, convincing evidence demonstrates that aging is one of the main determinants of endothelial dysfunction. The effect of aging can be detected both in the microcirculation and macrocirculation of forearm[12] and coronary vessels,[13] and is so marked that, in the forearm microcirculation, its negative impact can be detected even in the presence of essential hypertension.[12]

During exploration of the mechanisms responsible for age-related endothelial dysfunction, it was observed that, in normotensive subjects, the principal mechanism responsible for this alteration was a primary defect in the L-arginine–NO pathway, since the inhibiting effect of N^G-monomethyl-L-arginine (L-NMMA) on vasodilation to acetylcholine progressively declines in parallel with increasing age. In contrast, in subjects younger than 60 years, despite a significant age-related reduction in the response to acetylcholine, indomethacin infusion does not change the vasodilating effect of the muscarinic agonist, ruling out a contribution of EDCF in the pathogenesis of endothelial dysfunction.[14] However, in subjects older than 60 years, the infusion of the cyclooxygenase inhibitor potentiates the vasodilation to acetylcholine.[14] Thus, after this age, EDCF production starts to be detected and relevant,[14] and production of cyclooxygenase-dependent factors is associated with a further and parallel impairment in the L-arginine–NO pathway.[14] In patients with essential hypertension, on the other hand, the mechanisms involved in age-related endothelial dysfunction are similar to those found in healthy individuals, except that they are characterized by earlier onset. Thus, production of cyclo-oxygenase-dependent EDCFs starts to be detectable in an age-range of 31 to 45 years, and, in patients older than 45 years, the potentiating effect of indomethacin is augmented in parallel with increasing age.[14] Taken

together, this series of results supports the possibility that cyclo-oxygenase-dependent EDCF production is a phenomenon characteristic of aging, with essential hypertension merely causing earlier onset of this endothelial alteration.

addition, oxygen free radicals can also cause NO breakdown, thereby reducing its availability. It is significant that, in patients with essential hypertension, agonist-evoked vasodilation is resistant to inhibition induced by L-NMMA, a specific

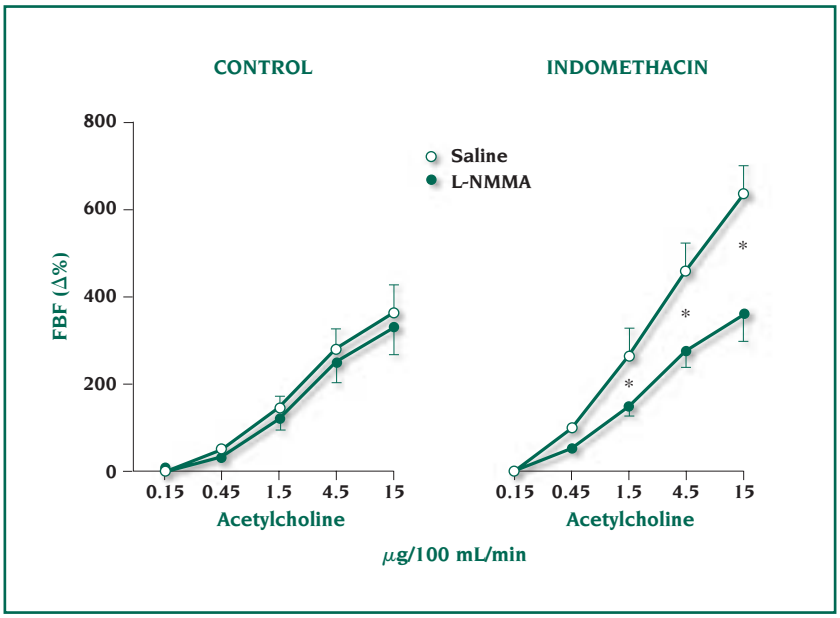

Figure 3. *Acetylcholine-induced increase in forearm blood flow (FBF) in the absence (**left**) and presence (**right**) of indomethacin (50 μg/100 mL forearm tissue/min) under control conditions (saline, 0.2 mL/min) and in the presence of* N^G*-mono-methyl-L-arginine (L-NMMA, 100 μg/100 mL forearm tissue/min) in patients with essential hypertension. Data are shown as means ± SD and expressed as FBF % increase above baseline. *Denotes a significant difference between infusion with and without L-NMMA (P<0.05 or less)*

Reproduced from reference 15: *Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A. Cyclooxygenase inhibition restores nitric oxide activity in essential hypertension.* Hypertension. *1997;29 (1, pt 2):274-279. Copyright © 1997, Lippincott Wiliams & Wilkins.*

NATURE OF CYCLOOXYGENASE-DEPENDENT EDCFs IN AGING AND ESSENTIAL HYPERTENSION

Experimental evidence has identified different cyclooxygenase-dependent EDCFs, including prostanoids such as thromboxane A_2 or prostaglandin H_2 or oxygen free radicals (mainly superoxide anions).[4] Such substances can counterbalance the relaxing activity of NO, the net effect being the algebraic sum between the degree of relaxation versus the degree of constriction. In

NO-synthase inhibitor.[9] This finding supports the presence of a pathological pathway leading to impairment of NO availability in essential hypertension. Note, however, that, in these patients, intrabrachial indomethacin not only increases the vasodilating response to acetylcholine, as previously described, but also restores the inhibiting effect of L-NMMA *(Figure 3),*[15] strongly indicating that cyclooxygenase activity produces substances that reduce NO availability. These substances are probably oxygen free radicals, since intrabrachial infusion of the scavenger vitamin C not only increases the response to acetylcholine, but also restores the inhibiting effect of L-NMMA.[9] Moreover, when simultaneously tested in the same study population, vitamin C and indomethacin show a similar degree of potentiation of the response to acetylcholine *(Figure 4)*[9]*;* in addition, the association of both compounds causes no further improvement in endothelium-dependent vasodilation *(Figure 5).*[9,14] Finally, similarly to the results obtained with indomethacin, vitamin C can improve vasodilation to acetylcholine in normotensive subjects and patients with essential hypertension older than 60 and 30 years, respectively.[16]

This cumulative evidence seems to indicate that, in aging and essential hypertension, EDCFs could be identified as oxygen free radicals produced by cyclooxygenase activity. However, whether these oxidative substances are directly produced by cyclooxygenase or indirectly by thromboxane A_2 or prostaglandin H_2 activity still needs to be established. Only future experiments with specific inhibitors of thromboxane synthase or selective antagonists for thromboxane A_2 or prostaglandin H_2 receptors, which at the present time are not available for clinical utilization, will give adequate information on the possible contribution of these substances.

OTHER CLINICAL CONDITIONS CHARACTERIZED BY CYCLOOXYGENASE-DEPENDENT EDCFs: ACUTE ESTROGEN DEFICENCY AND HEART FAILURE

Estrogen can protect the vessel wall by acting on endothelial responses.[17] Quite logically, menopause is characterized by endothelial dysfunction in both normotensive and hypertensive females.[18]

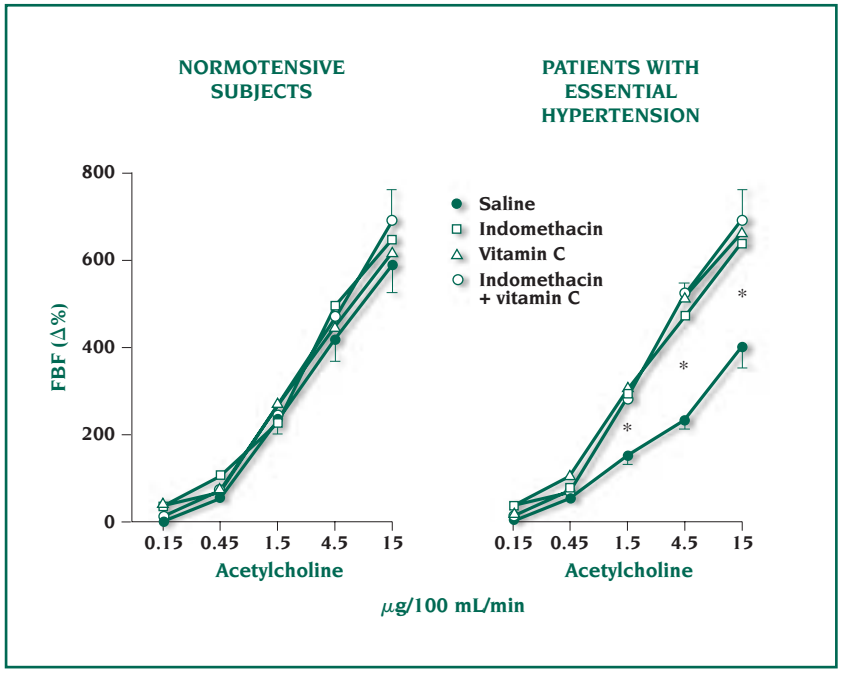

Figure 4. *Acetylcholine-induced increase in forearm blood flow (FBF) in the presence of saline (0.2 mL/min); indomethacin (50 μg/100 mL forearm tissue/min); vitamin C (8 mg/100 mL forearm tissue/min), and simultaneous indomethacin and vitamin C in patients with essential hypertension. Data are shown as means ± SD and expressed as FBF% increase above baseline. *Denotes a significant difference between infusion in control conditions and in the presence of vitamin C, indomethacin or vitamin C plus indomethacin (P<0.05 or less).*

Reproduced from reference 9: *Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A. Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension.* Circulation. *1998;97:2222-2229. Copyright © 1998, American Heart Association.*

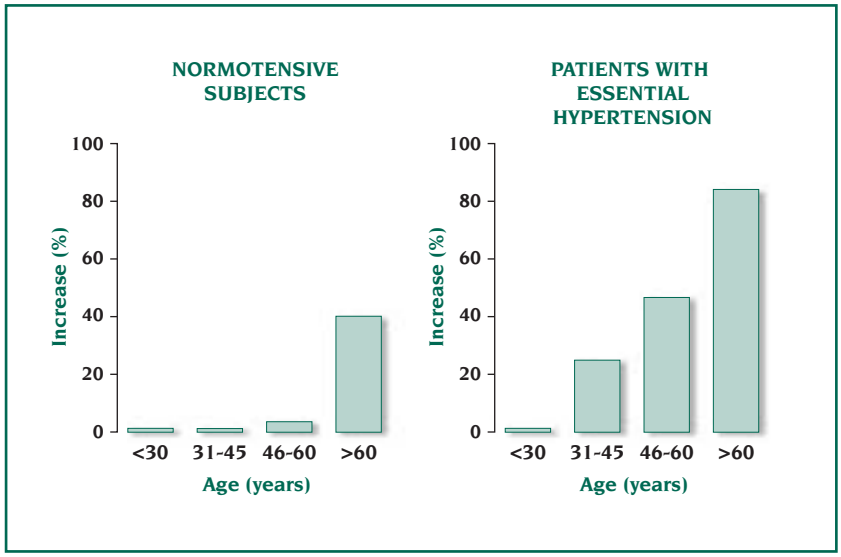

Figure 5. *Bars show the potentiating effect induced by indomethacin infusion (50 μg/100 mL forearm tissue/min) on the vasodilating response to acetylcholine in normotensive subjects and patients with essential hypertension divided into subgroups according to age.*

Reproduced from reference 14: *Taddei S, Virdis A, Mattei P, et al. Hypertension causes premature aging of endothelial function in humans.* Hypertension. *1997;97:29:736-743. Copyright © 1997, Lippincott Williams & Wilkins.*

A good model to evaluate the role of acute estrogen deprivation in modulating endothelial responses is provided by normotensive females who undergo ovariectomy and hysterectomy to remove a uterine leiomyoma. In these subjects, acute estrogen deprivation consequent to ovariectomy was associated with a decrease in response to acetylcholine, but not to sodium nitroprusside,[19] while normal endothelium-dependent vasodilation was restored by estrogen replacement therapy (ERT).[19] These results confirm that estrogen can preserve endothelial function. In keeping with this interpretation, prior to ovariectomy, the response to acetylcholine remained unchanged with indomethacin, but was blunted by L-NMMA.[19] Following ovariectomy, indomethacin-induced facilitation of the response to acetylcholine was observed, while the inhibiting effect of L-NMMA disappeared, suggesting that acute endogenous estrogen deprivation leads to EDCF production and a parallel drastic decrease in NO availability.[19] When endothelial responses were tested again after ERT, vasodilation to acetylcholine was sensitive to L-NMMA and no longer affected by indomethacin. EDCFs produced after estrogen deprivation are probably oxygen free radicals, since vitamin C exerted an effect on endothelial responses superimposable to that observed with indomethacin.[19] Taken together, and in line with experimental evidence,[20] these results suggest that estrogen protects endothelial function by inhibiting the production of cyclooxygenase-dependent EDCFs, which again are very likely to be oxygen free radicals.

Finally, in patients with congestive heart failure (New York Heart Association functional class II-III), systemic cyclooxygenase inhibition with oral indomethacin (50 mg) in-

duced a slight (+39%), but statistically significant, increase in the blunted vasodilating response to acetylcholine.[21] However, no information is available on the characterization of the cyclooxygenase-dependent vasoconstrictor substances released in response to acetylcholine in patients with heart failure. Whether cyclooxygenase-dependent EDCFs are responsible for the impaired endothelium-dependent vasodilation in other cardiovascular risk factors has not yet been evaluated.

ARE EDCFs IMPORTANT IN HUMANS?

In humans, endothelial dysfunction is the common clinical characteristic of different cardiovascular risk factors including not only aging and hypertension, but also postmenopause, hypercholesterolemia, diabetes mellitus, smoking, and hyperhomocysteinemia.[22] Furthermore, the simultaneous presence of several cardiovascular risk factors induces a further deterioration of endothelium-dependent vasodilatation.

The main alteration ascribable to endothelial dysfunction is reduced (or absent) NO availability. It should be borne in mind that, in addition to its relaxing activity, this substance can also inhibit platelet aggregation, vascular smooth cell proliferation and migration, monocyte adhesion, adhesion molecule expression, and endothelin production, thus protecting the vessel wall from the development of atherosclerosis and thrombosis.[22] As a consequence of the reduction in NO bioactivity, the dysfunctional endothelium becomes a promoter of atherosclerotic lesions in coronary and carotid arteries. Even more importantly, it is an independent predictor of cardiovascular events.[23] Given the crucial role of NO in the pathogenesis of cardiovascular disease, it cannot be overlooked that the one of the principal mechanisms leading to impaired NO availability, at least in aging or hypertension, is the production of cyclooxygenase-dependent EDCFs. Moreover, available evidence indicates that other pathways potentially leading to oxidative stress in essential hypertension, such as xanthine oxidase or angiotensin II (which is a potent stimulator of membrane NADH/NADPH oxidases), have no role. The possible contribution of xanthine oxidase has been excluded by evidence that the specific inhibitor allopurinol does not improve the blunted vasodilating effect of acetylcholine in the forearm microcirculation of patients with essential hypertension.[24] Moreover, the role of angiotensin II in essential hypertension seems to be excluded by several studies demonstrating that treatment with angiotensin-converting enzyme (ACE) inhibitors or AT_1 receptor antagonists has no effect on endothelial dysfunction.[25] Thus, to date, cyclooxygenase-dependent EDCF production represents the main mechanism identified that leads to impairment in NO availability, at least in aging and hypertension. Therefore, EDCFs, by inducing endothelial dysfunction, are important promoters of cardiovascular disease, playing their role in the very early phase of the pathogenetic process. Considering that EDCF inhibition can achieve complete restoration of NO availability, these substances could represent an important target for the prevention of cardiovascular disease. Unfortunately, cyclooxygenase inhibitors cannot be used, since it is not possible to reach the very high concentration needed to block endothelial cyclooxygenase by the oral route of administration. Moreover, the lack of identification of the exact molecular nature of such EDCFs does not allow the utilization of specific antagonists.

CONCLUSIONS

It is well documented that endothelial cells are capable of producing contracting factors, including cyclooxygenase derivatives, which, up to now, have been identified in aging, essential hypertension, acute estrogen deprivation, or heart failure. Although the exact nature of these substances has not yet been identified, EDCFs seem to be implicated in the production of, oxygen free radicals, which reduce NO availability. Since NO has a protective effect on the vessel wall, it is conceivable that, in pathological conditions, production of cyclooxygenase-dependent EDCFs can lead to impaired NO effectiveness, thereby promoting atherosclerosis and cardiovascular events. In future, cyclooxygenase EDCFs could therefore become an important therapeutic target for the prevention of cardiovascular disease.

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Dialogues Cardiovasc Med. 2002;7:245

A Lexicon of the Heart

Stunning

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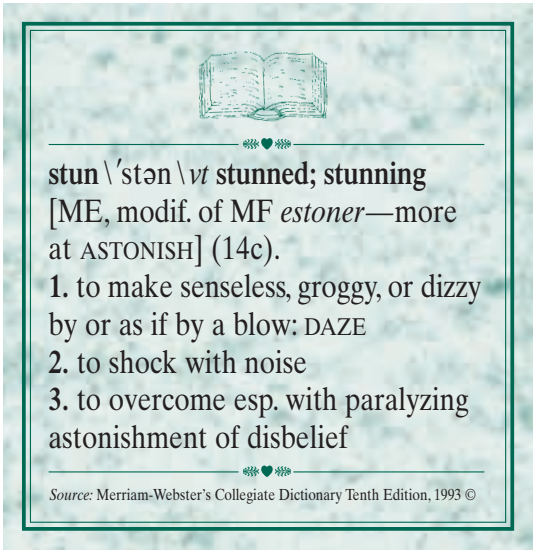

Myocardium ceases contracting a few seconds after the onset of ischemia. Thereafter, the affected myocardium stretches rather than shortens with each systole. This contractile deficit does not disappear if one salvages the damaged tissue by timely reperfusion. Rather, it persists for a few or many hours depending on the severity of the injury. The failure of the tissue to resume contraction after reperfusion was totally unexpected when it first was observed some 27 years ago,[1] but the fact that it occurs has been confirmed by many investigators studying the effects of ischemia and reperfusion in both animal and human hearts. Moreover, this regional contractile failure may be very important because it can cause enough of a decrease in cardiac output to exacerbate preexisting congestive cardiac failure or may be sufficient to cause the development of congestive failure in a previously asymptomatic patient.

This unusual failure of living myocardium to contract after an episode of ischemia and reperfusion was first described in dogs in Stephen Vatner's laboratory in 1975 by Guy Heyndrickx, et al[1] in experiments in which a coronary artery was occluded briefly followed by reperfusion. They showed that the myocardium that had been ischemic did not contract for a substantial period of time after it was reperfused with oxygenated blood even though this acontractile tissue was alive and otherwise appeared to be functioning normally.

These experiments were done in conscious dogs with implanted measurement devices. The results, though dramatic, were largely ignored until 1982, when Braunwald and Kloner,[2] in an editorial in *Circulation,* gave the phenomenon the striking name *"stunning."*

> Stunning is defined as the temporary failure of myocardium reversibly injured by ischemia to contract after it is reperfused with arterial blood.

The editorial was written to emphasize that monuments to an antecedent episode of ischemia persist for substantial periods of time in reversibly injured myocardium. In other words, this tissue, which I considered to be normal when I first defined *reversible myocardial injury,*[3] is quite different from virgin myocardium. One of these differences is *"stunning."* Another is *"preconditioning."* A third monument involves the adenine nucleotide pool. As shown in *Figure 1,*[4] the ATP of severely ischemic canine myocardium decreases by about 75% over a 15-minute period of ischemia. After successful reperfusion, this deficit in ATP persists for hours or days because adenine nucleotides are resynthesized slowly in myocardium. Thus, in living myocardium, a depressed tissue ATP is a monument to an episode of ischemic injury. Note that this monument varies in severity as a function of the duration of the episode of ischemia. Furthermore, most other monuments to an episode of ischemia, including stunning, are similar to the changes in ATP in the sense that the changes, whether beneficial or deleterious, are virtually always more marked after a long than after a short episode of ischemia.

BIOLOGY OF STUNNING

Our understanding of the biology of stunning comes primarily from studies in experimental animals.[5] This is be-

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cause it is much easier to control the numerous variables involved in ischemia and reperfusion in the animal heart. These studies have established that reperfusion of ischemic myocardium is required to induce stunning and, in fact, that stunning is a form of *reperfusion injury.* Moreover, the severity of the contractile failure increases with the duration of the initial ischemic insult. The longer the period of ischemia used to induce it, the more marked the deficit and vice versa. In the dog heart, the maximal degree of stunning results from 15 minutes of severe ischemia, which is the longest period of ischemia tolerated by myocytes without the appearance of some irreversible injury. On the other hand, a few minutes of ischemia results in barely detectable stunning. In severe stunning, the contractile deficit persists for 24 hours or more while in mild stunning the deficit is minimal and persists only for minutes or a few hours.

It is of interest that one can maintain a persistent stunned state by exposing the myocardium to multiple brief episodes of ischemia such as might be induced by repetitive episodes of angina in the human heart.[5] It has been proposed that this may be the cause of many cases of *hibernation,*[6] which is another condition in which living myocardium does not contract efficiently.

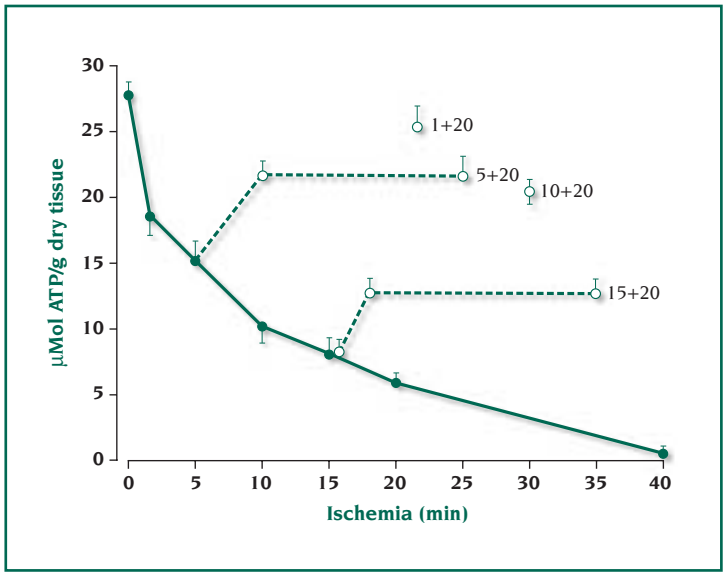

Figure 1. *ATP as a monument to ischemia. The solid line on this graph shows the decline in ATP found in acute ischemia as a function of the duration of severe ischemia. ATP decreases in the ischemic tissue because the demand of the tissue for high-energy phosphate (HEP) exceeds the supply. The dashed lines show the effect of reperfusion after 1, 5, 10, or 15 minutes of ischemia on net tissue ATP. Reperfusion stops the decline in ATP because oxidative energy release resumes after about 30 seconds of reflow. Note, however, that reperfusion does not restore ATP to control levels. This is because some of the adenine nucleotide pool is destroyed during ischemia. Nevertheless, resumption of oxidative metabolism restores 6 to 8 µmoles of ATP via rephosphorylation of the excess AMP and ADP formed in the tissue during ischemia. Note that ATP does not rise further after 20 minutes or even many hours of reperfusion because* de novo *synthesis of adenine nucleotides is slow. After 15 minutes of severe ischemia, more than 4 days of reperfusion are required to restore tissue ATP to the levels found in virgin heart. Thus, a decreased tissue ATP is a monument to the severity of the initial insult.*

Adapted from reference 4: *Jennings RB, Reimer KA, Steenbergen C Jr, Murry CE. Energy metabolism in myocardial ischemia. In: Dhalla NS, Innes IR, Beamish RE, eds.* Myocardial Ischemia. *Boston, Mass: Martinus Nijhoff Publisher; 1987:185-198.*

The last important bit of biology is the fact that you can restore the contractile function of stunned myocardium by systemic administration of a catecholamine such as dobutamine.

MECHANISMS OF STUNNING

Most of the stunning effect clearly is due to the release of O_2-derived free radicals at the time of reperfusion. The credit for this discovery belongs to Roberto Bolli, who, in a series of brilliant experiments, showed that oxygen-derived free radicals released at the time of reperfusion caused most of the stunning effect.[7-9] The data shown in *Figure 2 (next page)*[7] trace the production of O_2-derived free radicals in the coronary sinus blood during an episode of reversible ischemia followed by reperfusion. Note that free radical production begins at the onset of reperfusion and peaks at 5 minutes. Thereafter, the rate of production of free radicals slows greatly until they largely disappear 30 to 60 minutes after the onset. It is of great interest that one can prevent most of the free radical production by infusing a free radical scavenger immediately prior to the onset of reperfusion, while infusion of these scavengers after 5 minutes of reflow has virtually no effect.

In the experiments shown in *Figure 2,*[7] mercaptopropionylglycine (MPG), a water-soluble scavenger that enters the myocyte, was used to prevent stunning. MPG is an important scavenger because it scavenges the hydroxyl radical that is produced in the tissue from superoxide via the Haber-Weiss and Fenton reactions. Both of these reactions are catalyzed by Fe. The beneficial effects of MPG plus the observation that much of the stunning effect can be prevented by the administration of agents that chelate Fe and thereby prevent or reduce the formation of hydroxyl radicals, establishes that hydroxyl radicals are critical mediators

of acute stunning. Thus, free radicals produced at the time of reperfusion induce a deleterious effect on contraction. The cellular components with which the free radicals react to induce the contractile deficit have not been identified. It seems clear that the response to calcium is involved either at the level of entry of Ca^{2+} into the myocyte or at the level of the myofibrils themselves. The fact that one can overcome much of the contractile deficit with catecholamines suggests that the contractile system is intact, but less sensitive.

CLINICAL RELEVANCE OF STUNNING

Since all mammalian hearts so far tested demonstrate stunning, I have no doubt that it occurs in man. By definition, the circumstances in which it will develop involve any condition in which the heart is exposed to 3 to 5 minutes of normothermic severe ischemia. In fact, using ultrasonic techniques, it has been demonstrated in the human heart after 5 minutes of ischemia induced by balloon inflation at the time of angioplasty.[10] This brief episode of ischemia was followed by depressed function in some damaged left ventricular segments that persisted for as long as 24 hours.

Regional stunning occurs after thrombolytic therapy in the myocytes salvaged by reperfusion with arterial blood. It also almost certainly occurs after coronary vasospasm, in unstable angina, and in demand ischemia. The problem of assessing how much the stunning contributes to hemodynamic problems in any given patient is complicated by the fact that the coronary artery disease that leads to stunning is often associated with some myocytic necrosis or with scarring. Since necrotic myocytes, fibrous tissue, and stunned tissue all are acontractile, it is difficult to isolate the impact of stunning from other causes of contractile failure.

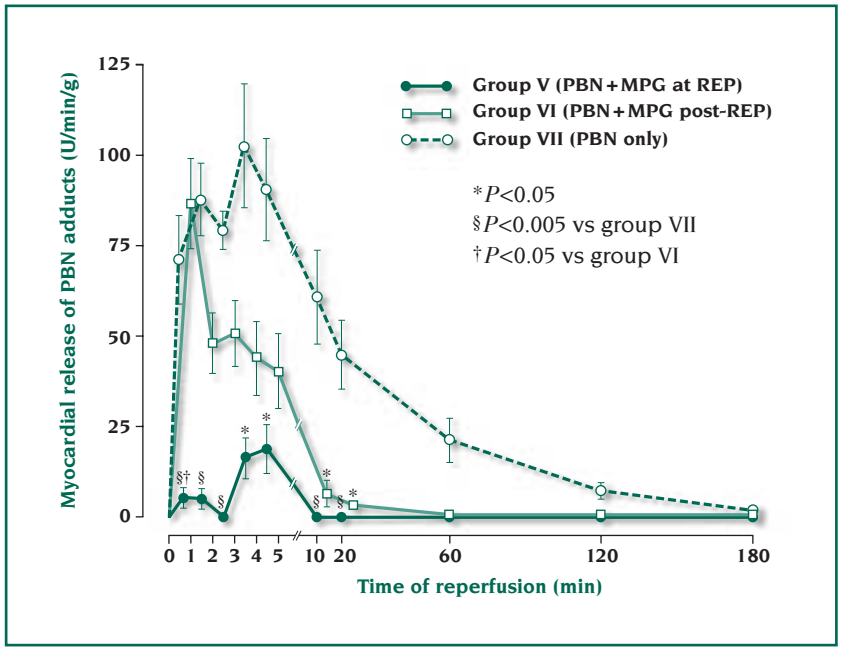

Figure 2. *Direct demonstration of the release of hydroxyl radicals into the coronary venous blood at the time of reperfusion of the anterior descending bed of the canine heart after 15 minutes of severe ischemia, a period of ischemic injury that induces maximum stunning. Since free radicals are very short-lived, they were detected in this study by the spin trap technique using an infusion of alpha-phenyl-*N*-tert-butylnitrone (PBN) into the coronary arterial tree. The top line (Group VII, ○--○) is from untreated hearts and shows that abundant free radicals are produced during the first five minutes of reperfusion while the bottom line (Group V, ●—●) shows that mercaptopropionylglycine (MPG), a hydroxyl radical scavenger, given at the time of reperfusion, greatly reduces hydroxyl radical production. The middle line (Group VI, □—□) shows that administration of MPG after 1 minute of reperfusion reduces hydroxyl radical production, but fails to prevent the massive release of radicals produced during the first minute of ischemia. It also fails to prevent stunning. Post-REP, postreperfusion; REP, reperfusion.*

***Adapted from reference** 7: Bolli R, Jeroudi MO, Patel BS, et al. Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion. Evidence that myocardial "stunning" is a manifestation of reperfusion injury.* Circ Res. *1989;65: 607-622. Copyright © 1989, American Heart Association.*

Stunning also occurs in hearts subjected to ischemic cardiac arrest at the time of open-heart surgery. In this case, the stunning is global, ie, it involves the entire heart. As in regional stunning, function of globally stunned hearts is usually improved by catecholamines. Although prevention of stunning is of potentially great value, so far there has been no trial designed to reduce the severity or to prevent either global or regional stunning by administration of a free radical scavenger.

ACUTE STUNNING

This brief treatise defines our current knowledge of the development and importance of stunning in the virgin heart. Repetitive episodes of ischemia and reperfusion also cause stunning. Here, however, O_2-derived free radicals in the form of peroxynitrite are believed to be the active deleterious agent.

In these hearts, increased nitric oxide (NO) synthase induced by earlier episodes of ischemia and reperfusion leads to marked increases in NO production during the episodes of ischemia and reperfusion. It is this increased tissue NO that forms peroxynitrite from hydroxyl radicals formed at the time of reperfusion (see Bolli[11] for a recent review). The subject will be considered in a later issue of *Dialogues* under the title *"Late preconditioning."*

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Heterogeneous behavior of the canine arterial and venous wall. Importance of the endothelium

J. G. De Mey, P. M. Vanhoutte

Circ Res. 1982;51:439-447

During the period when the regulatory functions of the endothelium and its role in pathophysiological conditions such as hypertension and atherosclerosis were being discovered, this paper was the first to precisely characterize the heterogeneous response of vessels of various origins to vasoactive substances.

In their experiments, Vanhoutte's group used dissected rings of canine femoral, pulmonary, saphenous, and splenic arteries and veins to carry out isometric tension recordings. While emphasis was put on keeping the endothelium intact, other vessels were mechanically denuded from the endothelium to determine its contribution to vasomotion. The status of the endothelium was carefully assessed by light and electron microcopy, followed by further fixation with osmium tetroxide and a polychromatic staining, since other methods, apart from the effect of acetylcholine, to determine the functional integrity of the endothelium were not yet established.

Preconstriction of the vessel segments was achieved by norepinephrine or electrical field stimulation. The maximal response ranged from 2.7 to 17.8 g in arteries and from 2.2 to 20.3 g in veins. Both arteries and veins from the pulmonary circulation exhibited the lowest contractile response, while femoral and saphenous vessels displayed rather strong contractile forces. While adenosine led to relaxation in preconstricted arteries and veins, isoproterenol, in contrast, induced relaxation in preconstricted veins. The incubation of precontracted arteries with thrombin led to dose-dependent relaxations, which were absent in deendothelialized preparations, and precontracted veins reacted with an increase in tension. All veins contracted when exposed to acetylcholine, but only in pulmonary veins did removal of the endothelium augment the contraction, due to the lack of nitric oxide, as was discovered several years later. For the same reason, in precontracted arteries, acetylcholine caused concentration-dependent relaxations that could not be observed in preparations without endothelium. In a further set of experiments with precontracted arteries, arachidonic acid induced comparable concentration-dependent relaxations by increasing the synthesis of prostaglandins and nitric oxide, as determined in subsequent studies.

Finally, the effect of anoxia on contractile responses to norepinephrine was investigated. Anoxia, induced for 10 minutes during contractile responses to norepinephrine, led to further vasoconstriction in all vascular beds and was significantly more pronounced in preparations with endothelium. A further relevant aspect was that, in arteries, the endothelial cells had predominantly inhibitory effects, while, in veins, excitatory effects dominated.

The main conclusion of the study was that arteries from different regions react homogenously, while, in contrast, veins display a rather individual reaction. This study certainly demonstrated the importance of the endothelium in the regulation of vascular tone and its local variations. Although the study was mainly descriptive, it provided a good basis for further subsequent studies, leading to a major advancement in the understanding of the regulation of vascular tone.

1982

Pope John Paul II canonizes the reverend Maximilian Kolbe who volunteered to die in place of another inmate at Auschwitz; the English ship *Marie Rose,* which sank during an engagement with France in 1545, is raised at Portsmouth; and Helmut Kohl is elected the new German Chancellor replacing Helmut Schmidt

Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat

T. F. Lüscher, P. M. Vanhoutte

Hypertension. 1986;8:344-348

Six years following the discovery of the endothelium-derived relaxing factor (EDRF), and even before it was demonstrated that nitric oxide was one among several EDRF candidates by Moncada's group in 1987, this paper hypothesized the existence of endothelium-derived vasoconstricting factors (EDCFs).

In a model using rings of thoracic aorta from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY), isometric tension recordings were performed on rings suspended in organ chambers. In some rings, the endothelium was mechanically denuded. Under basal conditions, the endothelium-dependent vasodilator acetylcholine (ACh) had no significant effect on rings from WKY with or without endothelium or on rings from SHR without endothelium. In contrast, in rings from SHR with endothelium, higher concentrations of ACh caused quite marked contractions. The dose-response curve could be shifted to the right by coincubation with atropine, while blocking the nicotinic receptor with hexamethonium was without effect, suggesting an involvement of the muscarinic receptor in mediating this endothelium-dependent constriction. Neither inhibitors of leukotriene synthase, prostacyclin synthase, or thromboxane synthase affected these contractions. In rings with endothelium, endothelium-dependent relaxations in response to ACh were depressed in vessels from SHR, in contrast to WKY.

In rings without endothelium, experiments with prostaglandins D_2, E_1, E_2, $F_{2\alpha}$, and I_2, as well as prostacyclin, resulted in similar dose-dependent contractile responses. However, only low concentrations of prostaglandin $F_{2\alpha}$ had a moderately stronger effect on aortic rings from hypertensive rats. These findings led the authors to correctly conclude that the source of the ACh-induced contraction was the vascular endothelium. The most likely candidate substance at that time appeared to be a product of arachidonic acid metabolism, synthesized through the cyclooxygenase pathway, because the phospholipase A_2 inhibitor quinacrine abolished the contractions evoked by acetylcholine. Since both indomethacin and meclofenamate abolished acetylcholine-induced contraction, the substance could be pinpointed to be a product of the cyclooxygenase pathway. For its part, prostacyclin could be ruled out due to a lack of effect of tranylcypromine and imidazole. A further candidate, prostaglandin $F_{2\alpha}$, could also be ruled out, since the rings of normotensive rats reacted with significant contractions to prostaglandin $F_{2\alpha}$ as well.

This article is remarkable in that it was the first to describe the release of endothelium-dependent constricting agents in response to the endothelium-dependent vasodilator ACh, and that endothelial dysfunction may also, at least in part, be due to the release of vasoconstricting substances. Although it took several years to identify the precise nature of the substances involved, the significant contribution of endoperoxides such as prostaglandin H_2 in this phenomenon was clearly described.

1986

French existentialist writer Simone de Beauvoir, author of *The Second Sex* and lover of the late philosopher Jean-Paul Sartre, dies in Paris, aged 78; the worst peace-time nuclear disaster occurs in Chernobyl when a reactor goes into meltdown, releasing clouds of radioactive material into the atmosphere; and US basketball player Michael Johnson sets an NBA playoff record with 63 points in a game

A novel potent vasoconstrictor peptide produced by vascular endothelial cells

M. Yanagisawa, H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto, T. Masaki

Nature. 1988;332:411-415

This landmark study was the first to describe a new vasoconstrictor peptide whose existence had been previously postulated by other groups. The search was on for protease-sensitive vasoconstrictor activity depending on extracellular Ca^{2+} and not associated with established vasoactive mechanisms such as adrenergic or cholinergic transmitters.

Yanagisawa et al isolated a 21-aminoacid peptide from the supernatant of cultured endothelial cells. Analysis of the aminoacid structure by means of gas-phase peptide sequencing revealed that there were four cysteine residues forming two intrachain disulfide bonds within the peptide, resulting in a unique structure. These findings led to subsequent identification of a new family of peptides, the endothelins, which exhibited a strong structural similarity (regional homologies) to a group of neurotoxins. This made it possible to elucidate their mechanism of action, which later studies showed to involve activation of Ca^{2+} channels via several endothelin-specific receptors.

The initially isolated peptide, endothelin-1, leads to a contraction of vascular strips characterized by the fact that it is slow in onset, but long-lasting. Dose-response curves revealed that endothelin-1 was the most powerful existing mammalian vasoconstrictor, being more potent by almost one magnitude than vasoconstrictors such as angiotensin II or vasopressin. Screening of a cDNA library constructed from porcine endothelial cell mRNA demonstrated that porcine endothelin was encoded by a single copy gene. As confirmed in later studies, it was concluded that the active peptide is cleaved out of a 203-aminoacid–containing prepro-protein by means of a dibasic pair-specific endopeptidase, leading to a precursor termed big-endothelin, which contains 39 amino acids and already has some vasoconstrictive activity. A second enzyme, called endothelin-converting enzyme, leads, by site-specific cleavage, to the release of the active peptide. Synthetic endothelin-1, prepared by liquid phase chemistry, had complete biological activity and could be used for the first in vivo experiments. These experiments showed that intravenous bolus injection of endothelin-1 caused a sustained rise in arterial pressure in rats, and that up to 60 minutes was required for a return to baseline levels. Separate in vitro studies revealed that the effect could be completely inhibited by Ca^{2+}-free bathing solution, and that attenuation of the vasoconstriction could also be achieved by coincubation with Ca^{2+} channel blockers. To determine the mechanism whereby endothelin-1 synthesis regulation is achieved, mRNA concentrations were measured, revealing that thrombin, epinephrine, and the Ca^{2+} ionophore A23187 were potent stimuli of endothelin-1 synthesis. As later experiments would demonstrate, nitric oxide is a strong suppressor of endothelin-1 synthesis, and endothelin-1 synthesis is modulated by complex effects on the endothelin-converting enzyme.

This paper initiated a completely new field of research and led to a wealth of publications in the following years. Subsequent research showed that reactive oxygen species (ROS) had strong stimulatory effects on the expression of the endothelium-derived vasoconstrictor endothelin and that endothelin in turn stimulates vascular ROS production, ultimately leading to a vicious cycle, which may contribute in a decisive way to the phenomenon of endothelial dysfunction. One of the initial ideas, however, of making use of these findings to develop new antihypertensive drugs, has, so far, not proven to be successful, mainly because the substances under investigation still have significant side effects. It was only recently, in 2001, that an endothelin receptor antagonist, bosentan, was licensed for the treatment of pulmonary hypertension.

1988

Graceland, by Paul Simon, wins the Record of the Year trophy at the 30th Grammy Awards; the Pulitzer Prize goes to Toni Morrison for *Beloved;* and the World Ladies' Figure Skating Championship in Budapest is won by Katarina Witt of the German Democratic Republic

Superoxide anion is an endothelium-derived contracting factor

Z. S. Katusic, P. M. Vanhoutte

Am J Physiol. 1989;257(1 pt 2):H33-H37

Katusic and Vanhoutte published one of the first scientific papers, after the discovery of the vasodilatory effects of NO, that clearly demonstrated that endothelium-derived reactive oxygen species (ROS) contributed in a significant way to the regulation of vascular tone. Their study was based on the search for an unidentified endothelium-derived vasoconstrictor product of cyclooxygenase, since both the inhibition of this pathway by the cyclooxygenase inhibitor indomethacin and the removal of the endothelium completely abolished the contractile effect induced by calcium ionophore.

The authors used rings of canine basilar arteries for isometric force recordings in oxygenated organ chambers. Superoxide anion was generated by xanthine oxidase and xanthine in the presence of catalase. Incubation of rings with superoxide anion caused significant contractions, which could be inhibited by superoxide dismutase (SOD) and were abolished when heat-inactivated xanthine oxidase was used, thus confirming that the substance was a free radical. In quiescent preparations, the endothelium-dependent contractions to A23187 were significantly reduced in the presence of SOD or SOD and catalase. In preparations contracted with uridine 5'-triphosphate (UTP), the calcium ionophore A23187 caused a further endothelium-dependent increase in tension. Endothelium-dependent contractions to A23187 were not affected by catalase alone or deferoxamine.

The study therefore confirmed earlier observations that the calcium ionophore A23187 causes endothelium-dependent contractions. The effect of A23187 could be abolished by indomethacin, an inhibitor of cyclooxygenase, and by removal of the endothelium. Furthermore, SOD was shown to prevent endothelium-dependent contractions to A23187.

Four potential explanations may account for the observed endothelium-dependent contractions: (i) chemical protection of NO; (ii) formation of hydrogen peroxide and hydroxyl radicals; (iii) inhibition of the contractile effect of superoxide anions on vascular smooth muscle; and (iv) inhibition of prostaglandin and thromboxane A_2 synthesis. Chemical protection of NO, still termed EDRF at that time, appeared unlikely since the addition of SOD and catalase in the presence of indomethacin did not unmask the relaxation to A23187. Formation of hydrogen peroxide and hydroxyl radicals also appeared unlikely, since catalase and deferoxamine did not affect contractions to A23187. Since the contraction of vascular smooth muscle to A23187 was not affected by coincubation with indomethacin, SOD, and catalase, it also appeared unlikely that either cyclooxygenase inhibitors or radical scavengers were the cause of the effects observed. Therefore, stimulation of prostaglandin and thromboxane A_2 synthesis by A23187 was the interpretation favored by the authors. It was speculated that selective inhibition of prostacyclin synthesis would shift the balance towards thromboxane A_2, thus leading to vasoconstriction.

This study demonstrated for the first time that superoxide anions derived from the endothelium were able to cause endothelium-dependent constrictions. This study provided the basis for a better understanding of the mechanisms underlying endothelial dysfunction in patients with cardiovascular risk factors such as hypertension, hypercholesterolemia, and diabetes mellitus, where the endothelium-dependent superoxide production is markedly increased.

1989

France's bicentennial celebrations reach their climax on July 14th, 200 years after the storming of the Bastille; Peter Koech of Kenya sets a new 3-km steeplechase record (8:05:39 min) in Stockholm; and Javier Sotomayor of Cuba sets a new high jump record (8'0") in San Juan

Role of endothelium-derived nitric oxide in the regulation of blood pressure

D. D. Rees, R. M. Palmer, S. Moncada

Proc Natl Acad Sci U S A. 1989;86:3375-3378

Rees and colleagues were the first to describe increases in blood pressure in response to inhibition of the endothelial nitric oxide synthase (eNOS). The impact of this manuscript was markedly enhanced by the demonstration that the effects of NOS inhibitors on blood pressure were reversed stereospecifically by L- and not by D-arginine, which was used in many subsequent studies to address the contribution of eNOS to the regulation of vascular tone in various vascular beds of experimental animals and patients.

For the experiments, arterial blood pressure was measured in the carotid artery of anesthetized and instrumented rabbits. Since earlier experiments had indicated that L-arginine was the physiological precursor of nitric oxide (NO), the L-arginine analogue N^G-monomethyl-L-arginine (L-NMMA) was used to inhibit the release of NO. In this model, L-NMMA, but not its enantiomer D-NMMA, caused a dose-dependent increase in arterial blood pressure, which could be reversed by the administration of L-arginine, which itself had no vasoactive effect. Acetylcholine and glyceryl trinitrate caused a dose-dependent fall in arterial blood pressure by increasing the vascular NO concentrations in an endothelium-dependent and independent fashion, respectively. The hypothesized mechanism of NO activity was confirmed by the observation that L-NMMA inhibited the acetylcholine-induced hypotension, while it did not affect the hypotension due to glyceryl trinitrate. Neither the cyclooxygenase inhibitor indomethacin nor the α_1-receptor antagonist prazosin or bilateral vagotomy had any significant impact on blood pressure or the effect of L-NMMA, ruling out an interference of established regulatory systems. In a separate set of ex vivo experiments with excised aortas from untreated animals, infusion of acetylcholine caused a release of NO, as detected by cascade bioassay with spiral strips of rabbit aorta or chemiluminescence. In contrast, the release of NO induced by acetylcholine from aortas of animals treated with L-NMMA was significantly inhibited. Under these conditions as well, infusion of L-arginine restored the NO release of aortas pretreated with L-NMMA, while aortas of untreated animals showed no alterations of NO release.

This elegant study was the first to directly demonstrate the in vivo effects of endothelium-derived NO by using the L-arginine analog L-NMMA, which competitively inhibits the physiological substrate of the NO-synthase, L-arginine, as determined in later studies. Having overcome the difficulties due to the extremely short half-life of NO, which is only seconds under in vivo conditions, and the lack of a specific inhibitor, the authors established the important role that NO plays in the regulation of vascular tone. They further confirmed that the radical NO was released by classic enzymatic cleavage from a well-known amino acid, L-arginine. Initial hypotheses that other mechanisms, such as adrenergic or vagal stimuli, could be causal for the vasoactive effects, were definitively ruled out, and concomitant findings such as the alteration of heart rate were correctly attributed to reflex mechanisms.

This paper gave rise to much excitement, since it showed that baseline NO production from arteries and veins decisively contributes to the regulation of blood pressure. The tools described in this study, such as inhibition of NO production by L-NMMA, reversibility of these effects by L- and not D-arginine, as well as the lack of efficacy of L-NMMA in inhibiting the vasodilator effects of glyceryl trinitrate, were used in numerous subsequent studies of endothelial function/dysfunction in experimental animals and patients.

1989

Van Gogh's Portrait of *Doctor Gachet* is sold at auction for $825 million;
Soviet president Gorbachev visits Beijing to meet Chinese Leader Deng Xiaoping for the first Sino-Soviet summit in 30 years; and
Kenya announces worldwide ban on ivory to preserve its elephant herds

Contraction of diabetic rabbit aorta caused by endothelium-derived PGH_2-TxA_2

B. Tesfamariam, J. A. Jakubowski, R. A. Cohen

Am J Physiol. 1989;257(5 pt 2):H1327-H1333

In this paper, Tesfamariam and colleagues provided the first evidence that release of endothelium-derived prostaglandin H_2–thromboxane A_2 (PGH_2-TxA_2) was responsible for endothelial dysfunction and endothelium-dependent constriction in the setting of diabetes mellitus.

In the authors' experiments, rabbits were rendered diabetic by administration of alloxan for 6 weeks. Subsequently, the aortas were isolated, aortic rings were suspended from strain gauges, and isometric forces were recorded. In half of the vessels, the endothelium was denuded, and the presence or absence of endothelium was confirmed by the reaction to concentration-dependent relaxation caused by acetylcholine or A23187. When rings were preconstricted with phenylephrine, the relaxations due to acetylcholine were significantly decreased in aortic rings from diabetic rabbits. At higher concentrations and in aortic rings from diabetic rabbits, acetylcholine even caused contractions. The measurement of protein concentrations by enzyme-linked immunosorbent assay revealed that the synthesis of thromboxane B_2 was significantly increased in the endothelium of aortic rings of diabetic rabbits. Incubation with the cyclooxygenase inhibitor indomethacin or the thromboxane receptor antagonist SQ29548 restored the relaxation due to acetylcholine, while indomethacin or SQ29548 alone had no vasoactive properties.

Prostaglandins such as prostaglandin E_2, prostaglandin I2, or U46619 caused equally intense dose-dependent contractions of resting aortic rings of both normal and diabetic rabbits, ruling out an alteration of the vascular reactivity to prostaglandins. The relaxations in response to the non-receptor-mediated endothelium-dependent vasodilator A23187, as well as to the endothelium-independent vasodilator nitroprusside, were similar in aortas of normal and diabetic animals. In further studies, it could be documented that the source of the increased amounts of thromboxane A_2 was the endothelium, since after denudation of the endothelial layer, vessels of diabetic animals reacted as those from normal animals. The role of prostacyclin appeared less important, since it was significantly less potent, and appeared not to be synthesized in higher amounts in vessels of diabetic rabbits. The mechanism of the increased production of thromboxane A_2 remained unclear, since the metabolism of arachidonic acid could only be incompletely monitored. However, using radiolabeled arachidonic acid, altered metabolism of the exogenous precursor appeared unlikely.

The results of the study clearly demonstrated that a cyclooxygenase product such as prostaglandin H_2–thromboxane A_2 released by endothelial cells contributed in a marked fashion to vascular dysfunction in the setting of diabetes mellitus.

Recently published studies have shed new light on these data, by demonstrating that oxygen-derived free radicals (ROS, reactive oxygen species) stimulate the activity of prostaglandin H synthase, subsequently increasing the synthesis of thromboxane A_2, thereby linking two of the most attractive concepts of vascular dysfunction in the setting of diabetes.

1989

A rare conjunction of Venus, Mars, Uranus, Neptune, Saturn, and the Moon occurs; The communist regime falls in Czechoslovakia in the wake of the Velvet Revolution led by Václav Havel; and Brazil holds its first free presidential election for 29 years

Thromboxane A_2 receptor antagonists inhibit endothelium-dependent contractions

W. Auch-Schwelk, Z. S. Katusic, P. M. Vanhoutte

Hypertension. 1990;15(6 pt 2):699-703

This paper addresses the mechanisms underlying reactive oxygen species (ROS)-induced vasoconstriction, with focus on the cyclooxygenase pathway. Based on their findings, the authors hypothesized that superoxide anions formed during the conversion of prostaglandin G_2 (PGG_2) to prostaglandin H_2 (PGH_2) may ultimately cause endothelium-dependent vasoconstriction via stimulation of thromboxane A_2–prostaglandin H_2 (TXA_2-PGH_2) receptors.

Rings of thoracic aorta from normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were suspended in organ chambers and used for the determination of isometric force. In some of the experiments, the endothelium was removed by rubbing the intimal surface. The synthesis of prostaglandins by aortic segments with or without endothelium was determined by radioimmunoassay. The stimulation of the TXA_2-PGH_2 receptors with the prostaglandin derivative U46619 caused significant contractions of the vessels without endothelium, comparable in size to the magnitude of norepinephrine-induced vasoconstriction. Furthermore, the vasoconstrictive effect of acetylcholine in SHR could be abolished by the TXA_2-PGH_2 receptor antagonists AH23848, SQ29548, and R68070, which were determined as being inert with regard to prostaglandin metabolism. In contrast, dazoxiben, an inhibitor of thromboxane synthase, was without effect under comparable conditions.

In separate experiments, again addressing the biosynthesis of prostaglandins, increased synthesis of the prostaglandins 6-keto-prostaglandin $F_{1\alpha}$, prostaglandin $F_{2\alpha}$, or thromboxane A_2 due to acetylcholine could be ruled out. Since for this and a variety of other reasons a direct stimulation of TXA_2-PGH_2 receptors by prostaglandins appeared unlikely, the focus of research was directed toward the effects of oxygen-derived free radicals: Earlier studies demonstrated that the action of oxygen-derived free radicals could be inhibited by the TXA_2-PGH_2 receptor antagonists AH23848, SQ29548, and R68070. Accordingly, in this study, oxygen-derived free radicals generated by a xanthine–xanthine oxidase system demonstrated a comparable vasoconstriction to that seen with acetylcholine. This effect was also inhibited by the TXA_2-PGH_2 receptor antagonists AH23848 and R68070.

Although the synthesis of oxygen-derived free radicals by the vascular endothelium could not be proven directly, it was ultimately concluded that oxygen-derived free radicals caused contraction by stimulating TXA_2-PGH_2 receptors, a hypothesis that was confirmed by several subsequent studies. The study was remarkable, inasmuch as it helped to understand that vessels exposed to oxidative stress actually cause constriction via formation of ROS in a prostaglandin-dependent fashion, thereby linking two key concepts of endothelium-derived vasoconstriction.

1990

Robert Noyce, coinventor of the semiconductor and founder of Intel Corporation, dies;
Monica Seles defeats Stefi Graff 7-6, 6-4, to win the French Open title;
and NASA reports that the new $1.5 billion Hubble space telescope has major optical defects

Vasodilation to acetylcholine in primary and secondary forms of human hypertension

S. Taddei, A. Virdis, P. Mattei, A. Salvetti

Hypertension. 1993;21(6 pt 2):929-933

In this interesting study performed in patients with various etiologies of hypertension, the authors demonstrated that endothelial dysfunction was markedly improved by indomethacin, suggesting that a cyclooxygenase-dependent vasoconstrictor mechanism participates, at least in part, in the blunting of the vasodilator effects of the endothelium-dependent vasodilator acetylcholine.

In this study, four groups of patients were compared: (i) normotensive subjects; (ii) patients with essential hypertension; (iii) patients with primary aldosteronism, documented by functional parameters and the presence of an adrenal adenoma (5 of these patients underwent resection of an aldosterone-secreting adenoma during the study period); and (iv) patients with renovascular hypertension, documented by significant renal artery stenosis and suppression of renin secretion in the contralateral kidney. Arterial blood pressure was measured through a cannula inserted into the brachial artery, and forearm blood flow was by determined by strain-gauge venous plethysmography. Endothelium-dependent vasodilatation was assessed by recording dose-response curves to intra-arterial application of acetylcholine. The role of cyclooxygenase was determined by additional infusion of indomethacin, and the endothelium-independent vascular reactivity to nitrovasodilators was measured via infusion of sodium nitroprusside. Patients with both primary and secondary hypertension had comparable baseline characteristics in terms of age, sex, lipid parameters, and blood pressure. Forearm acetylcholine-induced vasodilation was significantly reduced in patients with essential hypertension, compared with normotensive controls. Cyclooxygenase inhibition with indomethacin improved flow responses to acetylcholine, while having no effect on the endothelium-dependent relaxations in controls. In patients with primary aldosteronism as well, the vasodilator effect to acetylcholine was reduced, but the response was not affected by concomitant application of indomethacin, indicating a different mechanism of underlying endothelial dysfunction in this particular patient group. However, assessment of endothelium function after surgery revealed a normalized response to acetylcholine. In the group with renovascular hypertension, the vasodilation induced by acetylcholine was blunted, but not modified by indomethacin. In all groups, the vasodilation induced by nitroprusside was of comparable magnitude, indicating that the sensitivity of the smooth muscle to nitric oxide per se was unaffected by hypertension.

This study demonstrated in an elegant way that human hypertension, whether essential or secondary, is characterized by reduced endothelium-dependent vasodilation. Using indomethacin, the authors were able to show that, only in patients with essential hypertension, a cyclooxygenase-dependent vasoconstrictor substance contributed to the endothelial dysfunction. The authors therefore suggested that this endothelium-derived factor was involved in the pathogenesis of essential hypertension itself.

1993

British Nobel prizewinning author
Sir William Golding dies;
US film actress Julia Roberts marries
country singer Lyle Lovett in Marion, Indiana;
and Japanese crown prince Naruhito
marries princess Masako

Endothelium-dependent contractions are associated with both augmented expression of prostaglandin H synthase-1 and hypersensitivity to prostaglandin H_2 in the SHR aorta

T. Ge, H. Hughes, D. C. Junquero, K. K. Wu, P. M. Vanhoutte, C. M. Boulanger

Circ Res. 1995;76:1003-1010

In this comprehensive study, the group of P. M. Vanhoutte carefully investigated the effects of various components of the cyclooxygenase (COX) pathway on vascular tone, in the wake of a previous discovery by the same group demonstrating the importance of prostaglandins in the pathological vasomotion observed in essential hypertension. Furthermore, it was one of the first studies to consider the modulating functions of cytokines in the regulation of vascular tone.

After homogenizing aortic tissue from normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), reverse transcriptase-polymerase chain reaction and western blotting showed that both the cDNA and protein levels of prostaglandin H (PGH) synthase–1—also termed COX-1—were increased in the hypertensive animals. Measurement of isometric force in aortic rings of SHR rats revealed that the response to acetylcholine was caused by activation of prostaglandin H (PGH) synthase–1, since tenidap, an inhibitor of PGH synthase–1, abolished the endothelium-dependent contraction to acetylcholine, whereas the specific inhibition of PGH synthase–2 by NS-398 only minimally impaired the response to acetylcholine.

By investigating the contractile response of aortic rings to acetylcholine of both strains of rats, the authors could further demonstrate that endothelium-dependent contractions to acetylcholine in the aortas of SHR rats were associated with an increased expression of PGH synthase–1, compared with normotensive WKY rats. Surprisingly, as determined by reverse transcriptase-polymerase chain reaction and western blotting, there was no difference in the expression of PGH synthase–1 in preparations with or without endothelium, although endothelium was obligatory for the effect of acetylcholine, thus implying, firstly, a significant contribution of vascular media to the activity of PGH synthase-1, and, secondly, the existence of an "endothelium-derived indirect contracting factor." Prostaglandin $F_{2\alpha}$ and the unstable precursor prostaglandin H_2 were measured by a complex method including solid phase extraction, thin-layer chromatography, and mass spectrometry. Their measurement revealed that both prostaglandin $F_{2\alpha}$ and prostaglandin H_2 were synthesized in significant amounts by the endothelium of SHR following stimulation with acetylcholine, but not by normotensive WKY rats, and that this effect was inhibited by coincubation with indomethacin. Comparison of the effects of prostaglandins $F_{2\alpha}$, H_2, and the thromboxane analog U46619 on the contraction of aortic rings revealed greater contractions to prostaglandins H_2 in vessels with intact endothelium, while the contractions were similar after denudation of the vessels.

By using an armamentarium of techniques, the authors were able not only to elegantly pinpoint the enzymatic source of the vasoconstrictive prostaglandins, PGH synthase–1, but also to demonstrate for the first time the existence of hypersensitivity of vascular smooth muscle cells to endoperoxides in vessels from SHR, thus greatly improving our understanding of the complex nature of vascular dysfunction in essential hypertension.

1995

Hundreds die as India suffers its hottest weather of the last century with temperatures reaching 45°C; Chechen gunmen take 2000 patients and staff hostage in a hospital in the southern Russian town of Budyonnovsk; and Italian pianist Arturo Benedetti Michelangeli dies, aged 75

Oxygen-derived free radicals mediate endothelium-dependent contractions to acetylcholine in aortas from spontaneously hypertensive rats

D. Yang, M. Feletou, C. M. Boulanger, H. F. Wu, N. Levens, J. N. Zhang, P. M. Vanhoutte

Br J Pharmacol. 2002;136:104-110

Building on their earlier observations on the role of prostaglandins in the regulation of vascular tone, this remarkable study by P. M. Vanhoutte's group tested whether reactive oxygen species (ROS) could account for endothelium-dependent contractions in response to acetylcholine.

Experiments were performed with aortic rings from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats, some of which were pretreated in vivo for 10 days prior to the experiments with the radical scavenger dimethylthiourea. The aortic rings were suspended in organ chambers and changes in isometric tension recorded. In some preparations, the endothelium was removed. In contrast to WKY rats, rings from SHR exhibited a pronounced contraction in response to stimulation with acetylcholine. This effect could be partially inhibited by coincubation with the prostaglandin H (PGH) synthase–2 inhibitor NS-398, and completely inhibited by the PGH synthase–1 inhibitor valeryl salicylate or the selective thromboxane A_2 (TP)-receptor antagonist S18886, demonstrating that this effect was specifically due to activation of TP-receptors by endoperoxides, such as prostaglandin H_2. Incubation with oxygen-derived free radicals generated by xanthine–xanthine oxidase caused dose-dependent contractions in rings with or without endothelium of both WKY and SHR rats, but contractions of rings from SHR rats were significantly greater. Coincubation with radical scavengers such as allopurinol, deferoxamine, or superoxide dismutase significantly reduced the contractions in SHR rats. Furthermore, coincubation with valeryl salicylate or NS-398 also significantly reduced the contractions.

In a different set of experiments, chronic in vivo pretreatment of SHR with dimethylthiourea reduced aortic ring contractions to acetylcholine. In these aortic rings, a further reduction of contractions was achieved by coincubation with the radical scavengers superoxide dismutase, catalase, or deferoxamine. A comparable contraction as with acetylcholine w as achieved with xanthine oxidase in aortic rings from SHR, an effect that was diminished by a vivo pretreatment with dimethylthiourea.

In summary, the authors hypothesized that, in aortas of SHR, acetylcholine-induced endothelium-dependent contractions were mediated by the synthesis of superoxide anion and the subsequent dismutation into hydrogen peroxide and hydroxyl radicals. The oxygen-derived radicals subsequently appear to activate cyclooxygenase-1 to produce endoperoxides such as prostaglandin H_2. An essential step in this cascade appears to be the activation of G-protein–coupled TP-receptors.

This study is one of the first to precisely link the effects of prostaglandin derivatives and ROS, thus illustrating the dense interaction between the two groups of substances in the regulation of vascular tone. These observations may have an important impact on further research into vascular biology, since increased endothelial ROS production plays a crucial role in mediating vascular dysfunction in the presence of cardiovascular risk factors such as hypertension, diabetes mellitus, and hypercholesterolemia, as well as in chronic congestive heart failure.

2002

French president Jacques Chirac is reelected after defeating far-right candidate Jean-Marie Le Pen 82% to 18% in the final round of the election; maverick Dutch politician Pim Fortuyn is shot dead as he leaves a radio station in the city of Hilversum; and *Spider-Man* smashes US box-office records, grossing more than $114 million in its first three days of release

Endothelium-Dependent Contractions

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