

# 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<sub>2</sub>, 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 EDCF 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<sub>2</sub>; TP-receptor

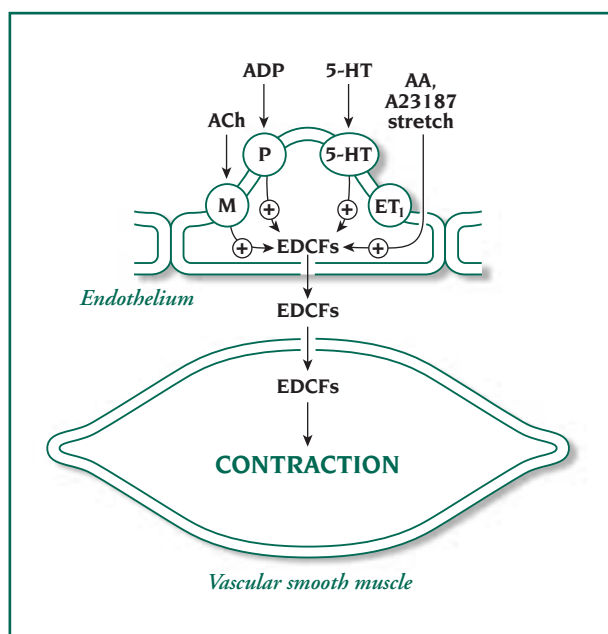
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**A**lmost a quarter of a century ago, Furchgott and Zawadzki<sup>1</sup> described the obligatory role of the endothelial cells in the relaxation of isolated arteries by acetylcholine, at least in the absence of sympathetic tone when the prejunctional (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.<sup>5</sup> 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

<b>COX</b>	cyclooxygenase
<b>EDCF</b>	endothelium-derived contracting factor
<b>EDRF</b>	endothelium-derived relaxing factor
<b>eNOS</b>	endothelium nitric oxide synthase
<b>NO</b>	nitric oxide
<b>SHR</b>	spontaneously hypertensive rat
<b>TP</b>	thromboxane-prostanoid (receptor)
<b>WKY</b>	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<sup>2+</sup> 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.

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,<sup>11</sup> endothelium-dependent contractions could theoretically appear in response to a sudden surge in the production of endogenous NOS inhibitors, such as asymmetric dimethylarginine (ADMA).

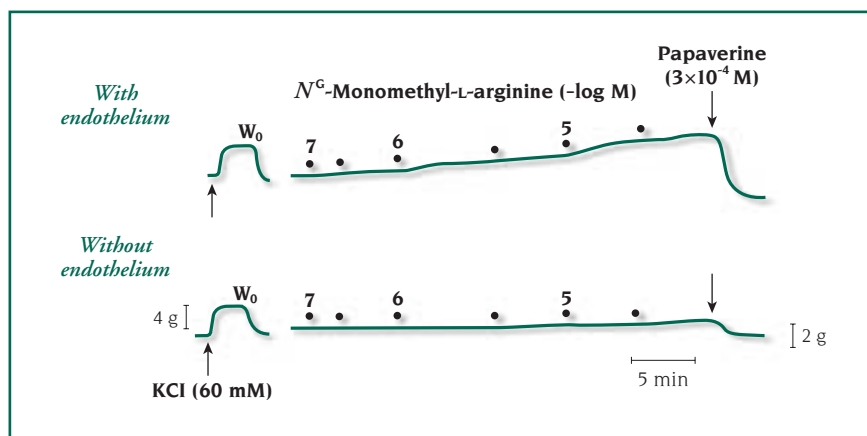
derived free radical production and arachidonic acid metabolism, and which are ultimately due to the activation of thromboxane-prostanoid (TP) receptors.

### 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

### 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



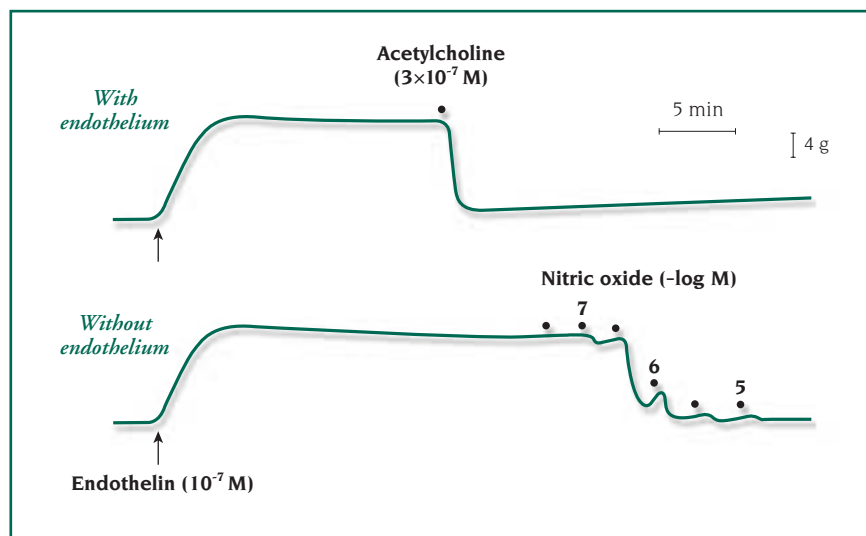
**Figure 2.** In the isolated canine basilar artery, the arterial smooth muscle cells possess a relatively high degree of myogenic tone, which is tempered by the constitutive release of nitric oxide (NO) by the endothelial cells. Hence, progressive inhibition of endothelial NO synthase (eNOS) by increasing concentrations of the competitive NOS inhibitor N<sup>G</sup>-monomethyl-L-arginine (LNMA) 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.<sup>15</sup> In particular, as long as endothelial cells continue to produce NO, the latter's combined inhibitory action on the release and action (Figure 3)<sup>16</sup> of endothelin-1 is bound to prevent any major role of the endothelin-1 in local vasomotor control.<sup>7</sup>

### CYCLOOXYGENASE-DEPENDENT ENDOTHELIUM-DEPENDENT CONTRACTIONS

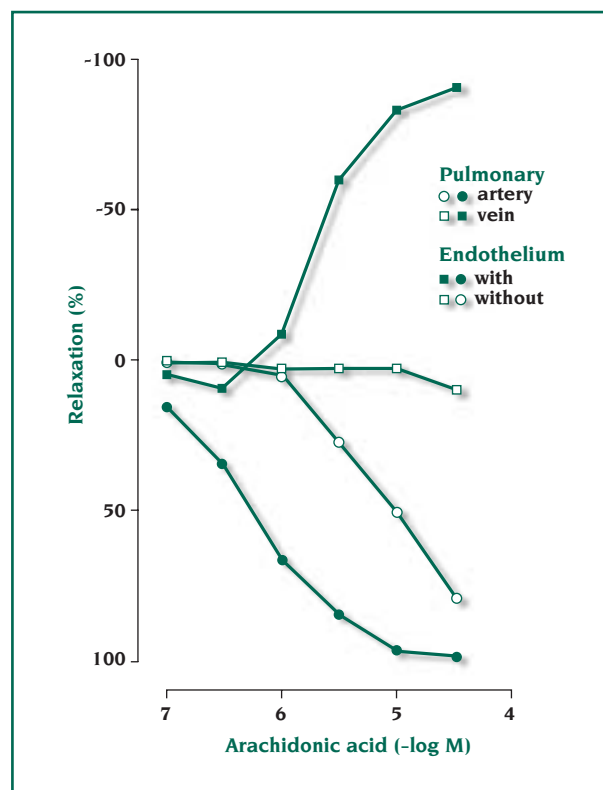
#### The phenomenon

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.<sup>5</sup> 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  $\alpha$ -adrenergic agonists (Figure 4).<sup>5</sup> Acetylcholine and the calcium ionophore A23187, which cause endothe-

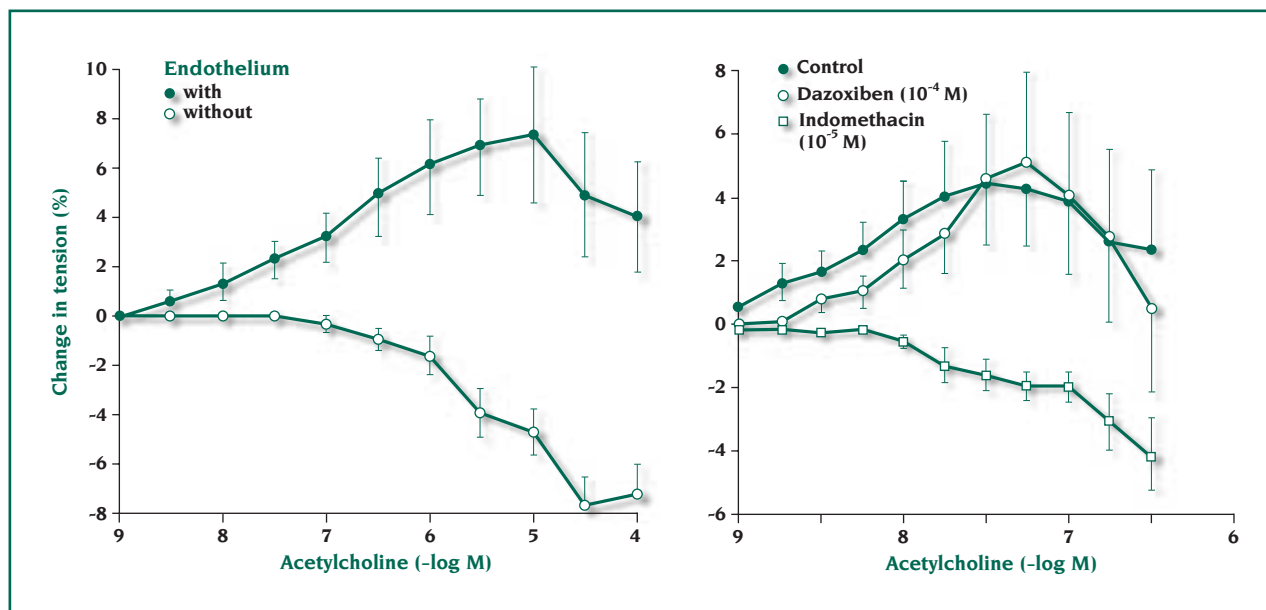
**Figure 4.** Effect of increasing concentrations of arachidonic acid on sustained contractions to norepinephrine in rings, with and without endothelium, prepared from the main pulmonary artery and one pulmonary vein of the same donor animal. In the artery, arachidonic acid causes relaxations that are 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 endothelium. *Circ Res.* 1982;51:439-447. Copyright © 1982, American Heart Association.

lium-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.<sup>17</sup> When acetylcholine or A23187 was applied to quiescent basilar arteries, clear-cut endothelium-dependent contractions were obtained (Figure 5, left, next page).<sup>17</sup> Similar EDCF-mediated increases in tension were evidenced in the aorta of spontaneously hypertensive rats (SHR)

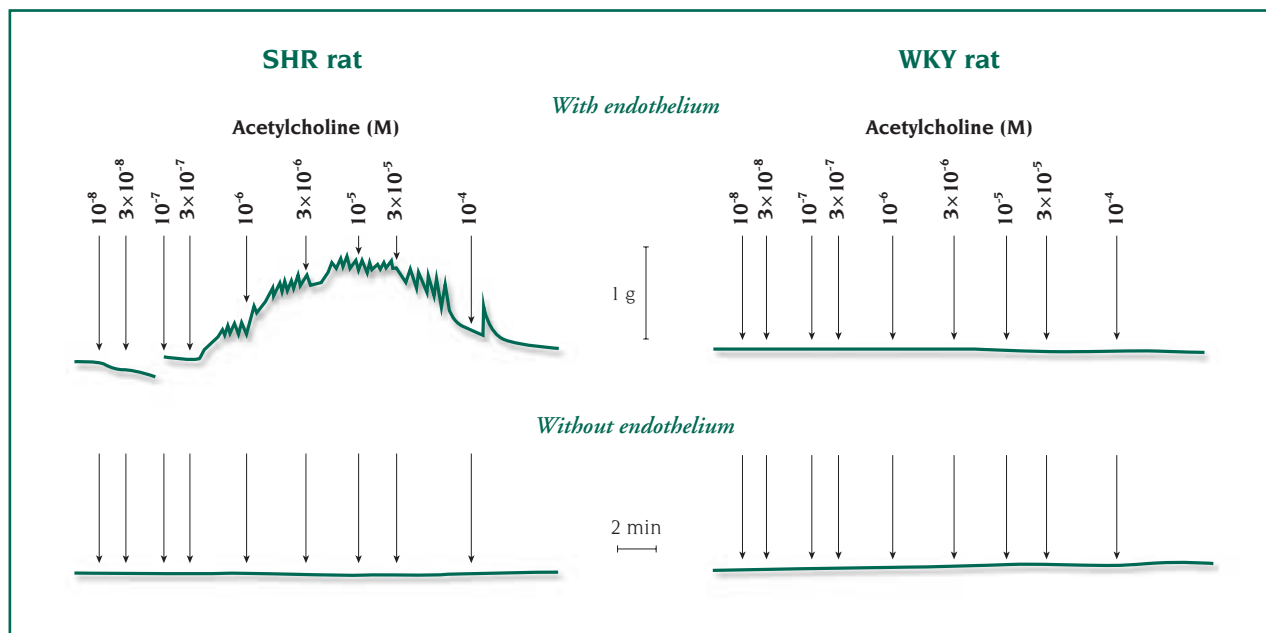


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**Figure 5.** Left panel: In rings without endothelium of canine basilar arteries, which are not exposed to a vasoconstrictor agent, but possess myogenic tone, acetylcholine has a direct inhibitory effect on the vascular smooth muscle cells. By contrast, 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<sub>2</sub> 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.

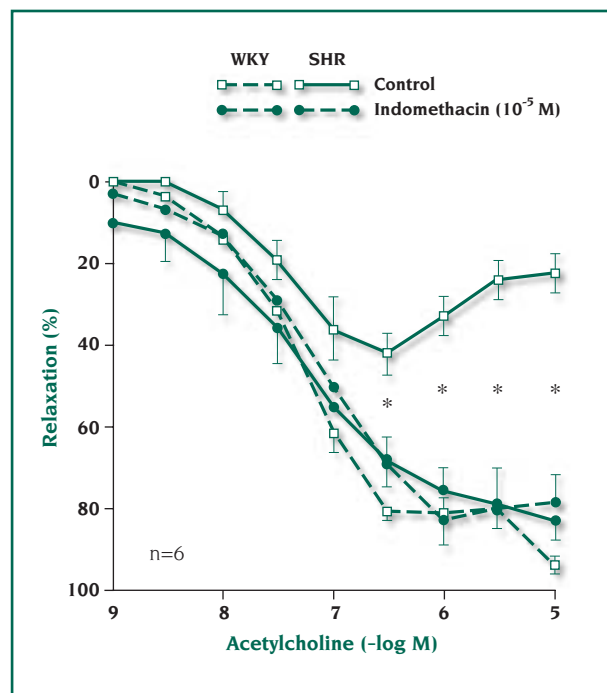


Figure 6,<sup>17</sup> and in carotid arteries of hypertensive Dahl rats.<sup>18</sup> Thus, the concomitant existence of a relaxing and a contracting component explained the blunted endothelium-dependent relaxation to acetylcholine in blood vessels of these hypertensive animals.<sup>18-20</sup>

### Role of arachidonic acid metabolites

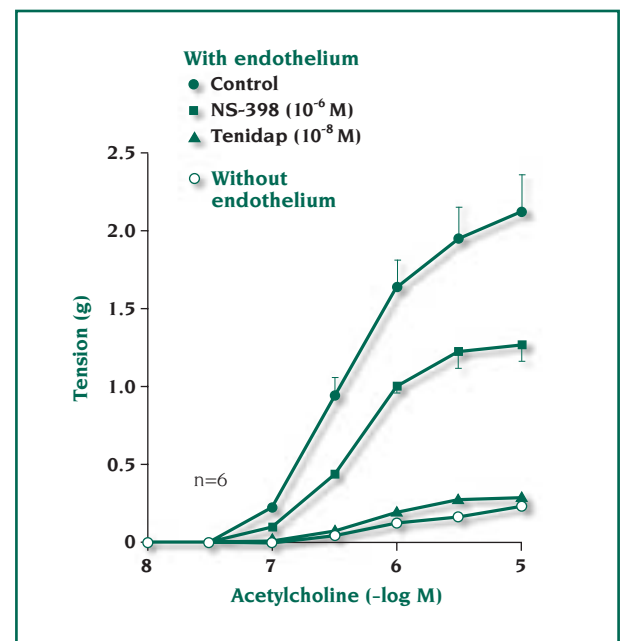
The finding that, in canine veins, arachidonic acid augmented rather than depressed contractions (Figure 4)<sup>5</sup> pointed to a role of the metabolism of this fatty acid in the divergent endothelium-dependent response. As expected, the endothelium-dependent augmentations were prevented by inhibitors of the cyclooxygenases (COX).<sup>21</sup> The same turned out to be the case in the canine basilar artery (Figure 5, right),<sup>17,19</sup> as well as in the SHR aorta, and indomethacin was shown to normalize endothelium-dependent relaxations in the latter (Figure 7).<sup>19</sup> Thus, the concept emerged that endothe-

lium-dependent contractions were mediated by one or several COX metabolites. When it became known that two isoforms (COX<sub>1</sub> and COX<sub>2</sub>) 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<sub>2</sub> (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<sub>1</sub> rather than those of COX<sub>2</sub> annulled the response (Figure 8).<sup>20,22</sup> Furthermore, when expression of COX<sub>1</sub> 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).<sup>22,23</sup> 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.

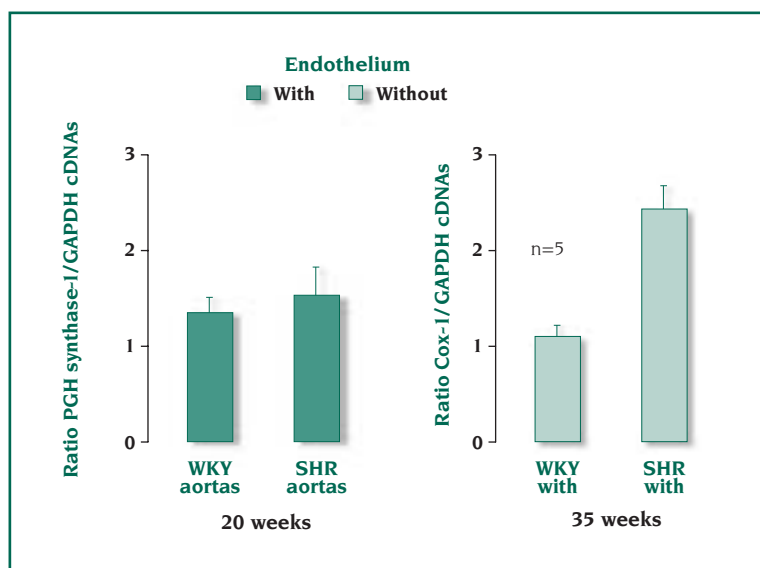
**Data from reference 19:** Lüscher TF, Vanhoutte PM. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. Hypertension. 1986;8:344-348. Copyright © 1986, Lippincott Williams & Wilkins.



**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<sub>2</sub>)-inhibitor NS-396, and abolished by the preferential COX<sub>1</sub>-inhibitor tenidap. These experiments indicate that COX<sub>1</sub>, rather than COX<sub>2</sub>, is involved in endothelium-derived contracting factor (EDCF)-mediated contractions, and that selective COX<sub>2</sub>-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<sub>2</sub> in the SHR aorta. Circ Res. 1995;76:1003-1010. Copyright © 1995, American Heart Association.

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**Figure 9.** Expression of the mRNA of prostaglandin H synthase 1 (COX<sub>1</sub>), 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.

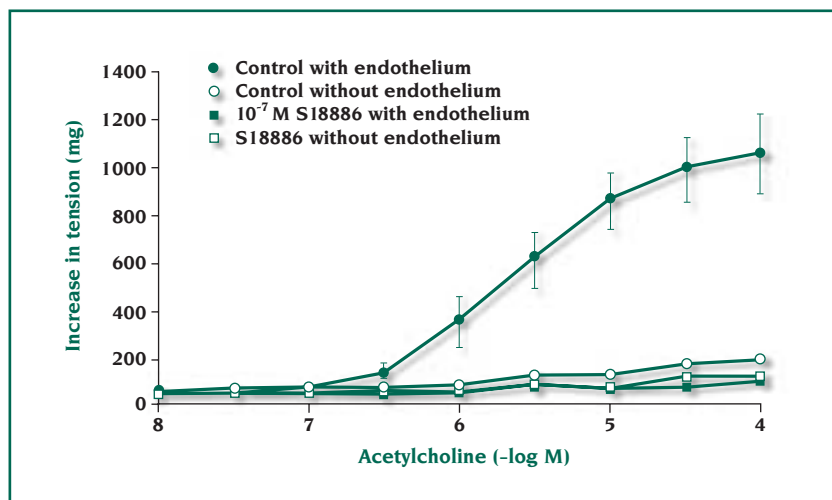
**Data from reference 22:** Ge T, Hughes H, Junquero DC, Wu KK, Vanhoutte PM, Boulanger CM. Endothelium-dependent contractions are associated with both augmented expression of prostaglandin H synthase-1 and hypersensitivity to prostaglandin H<sub>2</sub> in the SHR aorta. *Circ Res.* 1995;76:1003-1010. Copyright © 1995, American Heart Association; **and from reference 23:** Ge T, Vanhoutte PM, Boulanger CM. Increased response to prostaglandin H<sub>2</sub> 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<sup>19</sup> and in the SHR aorta,<sup>18</sup> 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<sub>2</sub> 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).<sup>20,24-26</sup>

These observations imply that an endogenous agonist at TP receptors other than thromboxane A<sub>2</sub> mediates the response. An exception may be when endothelial cells release EDCF upon exposure to endothelins.<sup>27,28</sup> The most likely candidates are endoperoxides, the precursors of thromboxane A<sub>2</sub>, which also activate TP receptors. This interpretation is reinforced by the pro-

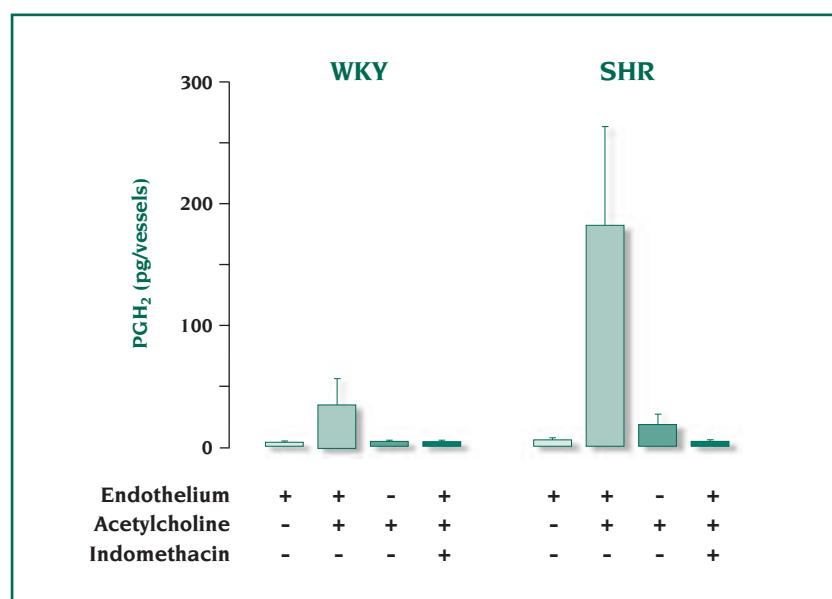
found inhibitory effect of indomethacin, which strongly suggests a major role for cyclooxygenase, the source of endoperoxides, and by the demonstration that acetylcholine does indeed cause a significant release of endoperoxides in the aorta of the SHR, but not in that of the WKY rat (Figure 11).<sup>22</sup> 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<sub>1</sub> expression in the SHR aorta yielded no difference between preparations with and without endothelium.<sup>22</sup> This lack of difference has fueled the suggestion that an undefined EDCF is released from the endothelium



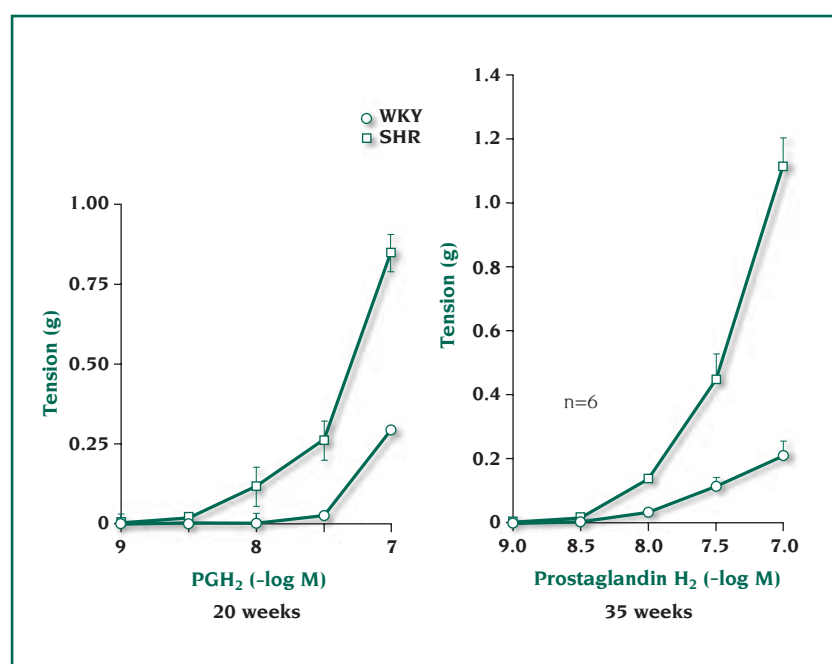
**Figure 10.** In the aorta of the spontaneously hypertensive rat, studied in the presence of nitro-L-arginine, acetylcholine causes endothelium-dependent contractions, which are abolished by S18886, a selective antagonist at thromboxane-prostanoid (TP)-receptors. These experiments demonstrate that activation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

**Data from reference 19:** Yang D, Féletou 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.

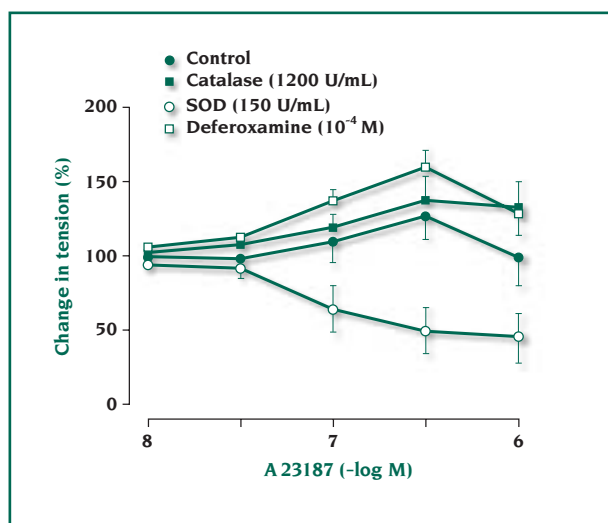


**Figure 12.** Contractions evoked by increasing concentrations of exogenous endoperoxides (prostaglandin  $H_2$ ,  $PGH_2$ ) in rings without endothelium of aortas from 20-week (left) and 35-week-old (right) normotensive (Wistar-Kyoto rats [WKY]) and spontaneously hypertensive rats (SHR). In both age 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.<sup>29</sup> 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).<sup>22</sup> 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.<sup>26</sup> 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.<sup>26</sup> 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).<sup>22</sup>



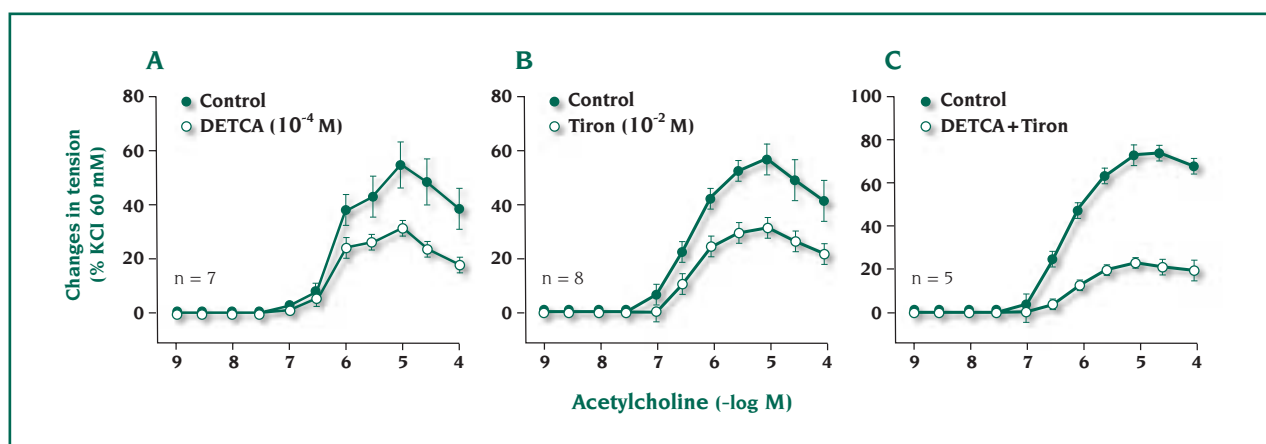
**Figure 13.** In rings with endothelium of canine basilar arteries, increasing concentrations of the calcium ionophore A23187 cause contractions [not observed in preparations without endothelium [Katusic et al, 1988]], which are not affected by catalase (which removes hydrogen peroxide) or deferoxamine (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.

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.<sup>33</sup> 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),<sup>20</sup> and that chronic in vivo depletion of superoxide anions also unmasks

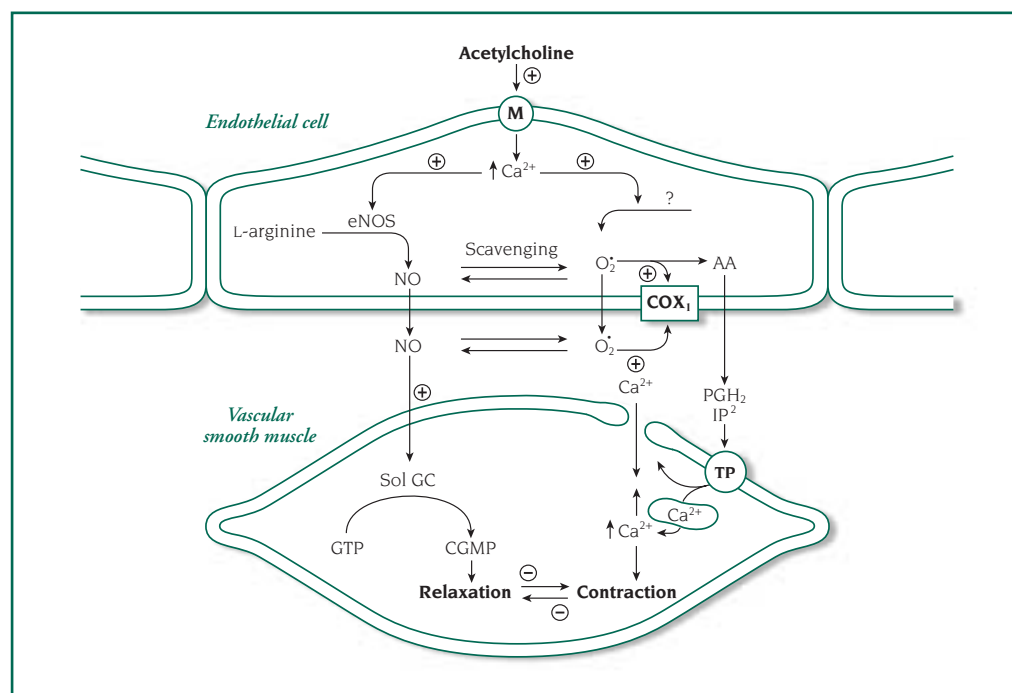
### Contribution of oxygen-derived free radicals

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).<sup>30</sup> 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.<sup>30-32</sup> 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.



**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<sub>1</sub>, cyclooxygenase 1; eNOS, endothelial nitric oxide synthase; IP, isoprostanes; M, muscarinic receptor; NO, nitric oxide; O<sub>2</sub><sup>-</sup>, superoxide anions; PGH<sub>2</sub>, endoperoxides (prostaglandin H<sub>2</sub>); 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.<sup>20</sup> Conversely, the exogenous generation of free radicals in the extracellular space exacerbates the endothelium-dependent contractions to acetylcholine in the SHR aorta.<sup>34</sup> 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.<sup>35</sup> 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,<sup>36</sup> 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<sub>1</sub> 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<sub>1</sub> and the hyperresponsiveness of the TP receptors are required.<sup>22,23</sup> 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.<sup>20,26,37</sup>

### 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.<sup>38</sup> 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<sub>1</sub>, is already present in the aorta of younger SHR, at a time when they are not yet overtly hypertensive

### 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.

(*Figures 9 and 12, left*).<sup>22,23,39,40</sup> 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<sub>1</sub>. This interpretation is strengthened by the finding that endothelium-dependent contractions indeed appear progressively in the arteries of aging normotensive animals.<sup>41-43</sup> Likewise, in porcine coronary arteries covered with regenerated, senescent endothelial cells, an EDCF-mediated response can be observed.<sup>28,44</sup> 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,<sup>45,46</sup> which eventually initiates the atherosclerotic process, the most feared complication of both hypertension and diabetes.<sup>47</sup> This conclusion is considerably reinforced by the fact that the TP-receptor antagonist S18886 is able to inhibit the atherosclerotic process.<sup>48-50</sup>

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