

## Endothelial dysfunction: a strategic target in the treatment of hypertension?

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**Abstract** Endothelial dysfunction is a common feature of hypertension, and it results from the imbalanced release of endothelium-derived relaxing factors (EDRFs; in particular, nitric oxide) and endothelium-derived contracting factors (EDCFs; angiotensin II, endothelins, uridine adenosine tetraphosphate, and cyclooxygenase-derived EDCFs). Thus, drugs that increase EDRFs (using direct nitric oxide releasing compounds, tetrahydrobiopterin, or L-arginine supplementation) or decrease EDCF release or actions (using cyclooxygenase inhibitor or thromboxane A<sub>2</sub>/prostanoid receptor antagonists) would prevent the dysfunction. Many conventional antihypertensive drugs, including angiotensin-converting enzyme inhibitors, calcium channel blockers, and third-generation  $\beta$ -blockers, possess the ability to reverse endothelial dysfunction. Their use is attractive, as they can address arterial blood pressure and vascular tone simultaneously. The severity of endothelial dysfunction correlates with the development of coronary artery disease and predicts future cardiovascular events. Thus, endothelial dysfunction needs to be considered as a strategic target in the treatment of hypertension.

**Keywords** Endothelium · Prostaglandin · Contraction · Free radical · Hypertensive rats

**Introduction** 33

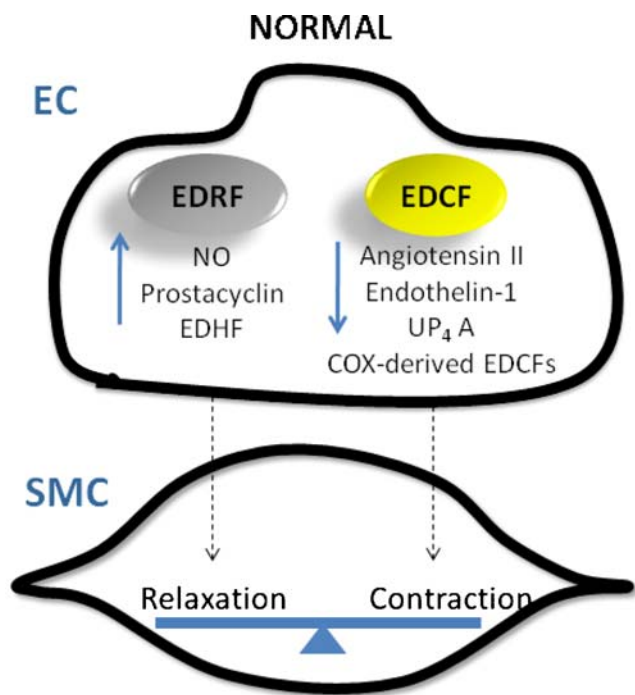
The endothelium, the thin layer of cells that lines the interior surface of blood vessels, can be activated by various chemical and physical stimuli to simultaneously release endothelium-derived relaxing (EDRFs) and contracting (EDCFs) factors. EDRFs and EDCFs act as acute functional antagonists and exert opposing effects on the underlying vascular smooth muscles to control their tone (Fig. 1). When endothelial cells are exposed to a chronic elevation in arterial blood pressure, they age prematurely, their turnover is accelerated, and they are replaced by regenerated endothelial cells [1, 2]. However, the regenerated endothelium has an impaired ability to release EDRFs (endothelial dysfunction)—in particular, nitric oxide (NO) [3, 4]—which results in the weakening of the inhibitory brake to oppose the action of EDCFs, with ensuing prominence of endothelium-dependent contractions (constrictions) [5]. Endothelial dysfunction can trigger a chain of undesired responses, including increases in platelet aggregation, expression of adhesion molecules, and vascular smooth muscle growth [1, 6]. Thus, a vicious cycle is established, ultimately contributing to thrombosis, inflammation, vascular remodeling, and atherosclerosis.

Endothelial dysfunction has been demonstrated both in resistance arteries and conduit arteries of several hypertensive animals, including the spontaneously hypertensive rat (SHR) [7–9], the two-kidney one-clip model [10, 11], deoxycorticosterone acetate salt-treated animals [12], and the Dahl salt-sensitive rat [13, 14]. Evidence of endothelial dysfunction in human hypertension has been characterized

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**Fig. 1** In healthy arteries, a normal vascular tone is maintained by the balanced release of EDRF and EDCF. This balance is tipped in hypertensive arteries with an increase in the release of EDCF and a decrease in the release of EDRF, favoring contractions. *EC* endothelial cell, *SMC* smooth muscle cell

by decreased forearm blood flow responses to endothelium-dependent vasodilator agonists, such as acetylcholine and bradykinin [15, 16], or by an increase in vasoconstrictor response to locally administered nitric oxide synthase inhibitors [17].

### Endothelium-derived relaxing factors

The endothelium produces a range of EDRFs, the most significant and well-characterized of which is NO. But prostacyclin and endothelium-derived hyperpolarizing factors are also important endothelium-derived vasodilator signals, with the latter prominently contributing to endothelium-dependent relaxations in resistance arteries [18]. The majority of studies on endothelial dysfunction have concentrated on the mechanisms underlying the decreased bioavailability of NO. This decrease may result from a decrease in NO production, from a decrease in activation of guanylyl cyclase, and/or an increase in NO degradation (Fig. 2). A decrease in NO production may result from a deficiency in substrates and cofactors for NO synthases (NOS), such as L-arginine or tetrahydrobiopterin (BH<sub>4</sub>) [13, 19]; from a decreased expression and presence of endothelial NOS (eNOS) [20]; from a decreased activation of NOS, such as phosphorylation of the enzyme

or interactions with proteins (e.g., heat shock protein 90 or calmodulin) [20]; or from an increased presence of endogenous inhibitors of NOS, asymmetric dimethyl arginine in particular [21] (Fig. 2). An increase in NO degradation can result from the binding of NO to molecules such as hemoglobin and albumin, or from increased inactivation of NO by its interaction with superoxide anions [22]—a reaction which leads to the production of peroxynitrite, a toxic vascular oxidant that further contributes to vasoconstriction and vascular injury (Fig. 2). Animal and clinical studies indicate that hypertension is associated with an increase in the production of reactive oxygen species (ROS), together with a decreased level of endogenous antioxidants [23–25]. The ability of vitamin C to restore NO production and improve endothelial function in essential hypertensive patients suggests a role of oxidative stress in endothelial dysfunction in humans [25].

### Endothelium-derived contracting factors

The endothelial cells can produce several EDCFs, including angiotensin II, endothelin-1, dinucleotide uridine adenosine tetraphosphate (UP<sub>4</sub>A), cyclooxygenase (COX)-derived prostanoids, and ROS [5, 26]. When these endothelium-derived vasoconstrictors are overproduced, such as in hypertension or diabetes, they oppose the vasodilator effects of the EDRFs, exacerbating endothelial dysfunction.

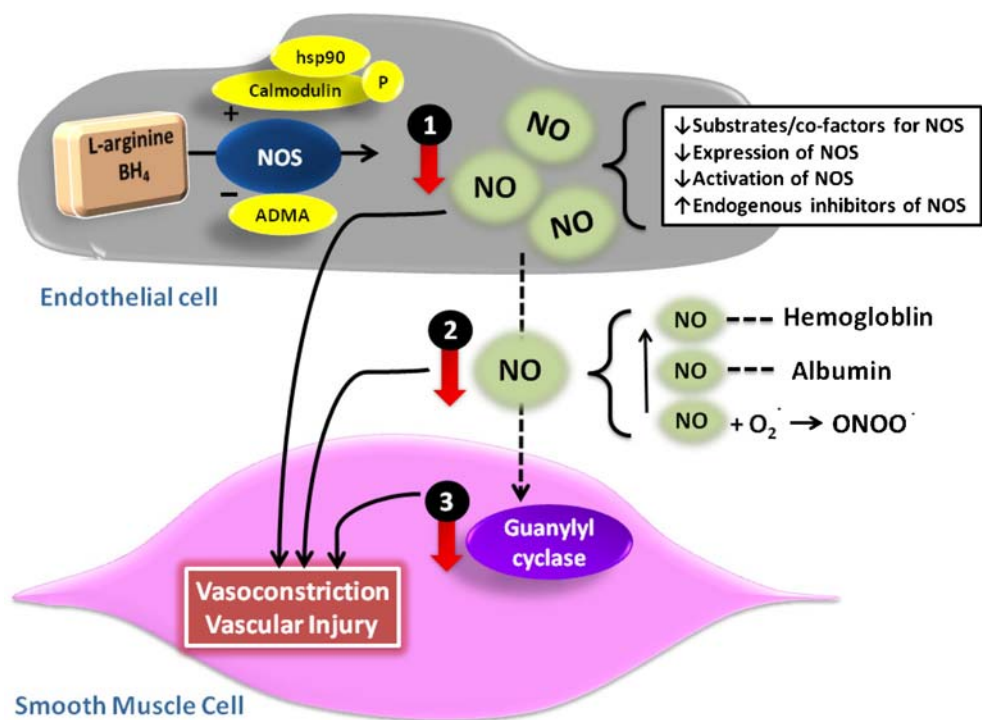
### Angiotensin II

Angiotensin I is metabolized into angiotensin II by endothelial angiotensin-converting enzyme (ACE). Angiotensin II can activate angiotensin receptors and trigger an increase in cytosolic calcium to mediate contractions [27]. In addition to causing vasoconstriction, angiotensin II can enhance the production of ROS—predominately through the activation of membrane-bound nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate oxidases—and thus, impairs NO bioavailability [28]. Furthermore, angiotensin II can directly stimulate the production and release of endothelin-1 and thus aggravate endothelial dysfunction [29].

### Endothelin-1

There are three isoforms of endothelin (identified as ET-1, ET-2, and ET-3) that activate two subtypes of receptors (ET<sub>A</sub> and ET<sub>B</sub>) [30]. ET<sub>A</sub> and ET<sub>B</sub> receptors are found in the vascular smooth muscle and are coupled to a G<sub>q</sub>-protein that leads to IP<sub>3</sub> formation [30]. IP<sub>3</sub> stimulates calcium release

**Fig. 2** Decreased bioavailability of nitric oxide may result from a decrease in NO production, an increase in NO degradation, or a decrease in the activation of guanylyl cyclase. Decreased NO production may result from deficiency in substrates and cofactors for nitric oxide synthase (NOS), decreased expression of NOS, decreased activation of NOS, or an increase in endogenous inhibitors of NOS. An increase in NO degradation can result from the binding of NO to molecules such as superoxide anions, hemoglobin, and albumin. *ADMA* asymmetric dimethyl arginine, *BH<sub>4</sub>* tetrahydrobiopterin, *EC* endothelial cell, *hsp90* heat shock protein 90, *NOS* nitric oxide synthase, *O<sub>2</sub>*, *ONOO<sup>-</sup>* peroxynitrite, *P* phosphorylation, *SMC* smooth muscle cell



130 from the sarcoplasmic reticulum, which contributes to the  
 131 contraction of the vascular smooth muscle [30]. Because of  
 132 its powerful vasoconstrictor properties, and the retention of  
 133 sodium that it causes, endothelin-1 (the main isoform  
 134 produced by endothelial cells) increases arterial blood  
 135 pressure. ET<sub>B</sub> receptors are primarily located on endothelial  
 136 cells, and when stimulated, they increase the release of NO  
 137 and augment natriuresis and diuresis, thus lowering blood  
 138 pressure [31]. The distribution of endothelin receptors on  
 139 endothelial and smooth muscle cells helps to explain the  
 140 phenomenon that systemic administration of endothelin-1  
 141 causes an initial transient vasodilatation (endothelial ET<sub>B</sub>  
 142 activation) and hypotension, followed by prolonged vaso-  
 143 constriction and hypertension (ET<sub>A</sub> and ET<sub>B</sub> activation of  
 144 vascular smooth muscle). Endothelin-1 can also induce the  
 145 secondary release of cyclooxygenase-dependent EDCFs  
 146 (presumably endoperoxides and thromboxane A<sub>2</sub>) that cause  
 147 the activation of thromboxane A<sub>2</sub>/prostanoid (TP) receptors  
 148 of vascular smooth muscle [32–34].

149 **Uridine adenosine tetraphosphate**

150 UP<sub>4</sub>A is a non-peptidic dinucleotide endothelium-derived  
 151 vasoconstrictor that is assumed to play a role in the  
 152 regulation of vascular tone [35]. UP<sub>4</sub>A possesses both purine  
 153 and pyrimidine moieties, and the contraction that it causes is  
 154 mediated predominately through P2X1, and probably also  
 155 through P2Y2 and P2Y4 purinoceptors. UP<sub>4</sub>A is released  
 156 from the endothelium in response to acetylcholine,

endothelin-1, the calcium ionophore A23187, adenosine, 157  
 and uridine triphosphate [35]. The role of UP<sub>4</sub>A in the 158  
 pathogenesis of hypertension is yet to be determined. 159

160 **COX-derived EDCFs**

161 The importance of COX-derived vasoconstrictor prostanoids 162  
 has gained significant recognition in the past decade. The 163  
 production of endothelium-derived prostanoids is augmented 164  
 in arteries with regenerated endothelium [36, 37], and in 165  
 normotensive aging and hypertensive arteries [5, 7, 9, 38]. 166  
 The endothelium of the renal arteries of healthy rats also 167  
 releases EDCF, suggesting that it may play a role in the 168  
 regulation of basal tone in this artery, and not only during 169  
 agonist-induced stimulated release [39, 40]. Studies in 170  
 humans show that the acetylcholine-induced vasodilatation 171  
 is diminished in conductance and resistance vessels of patients 172  
 with hypertension. In these hypertensive patients, intra-arterial 173  
 administration of the COX inhibitor indomethacin improved 174  
 the vasodilator response to acetylcholine [41, 42], suggesting 175  
 that the production of COX-derived EDCF contributes to the 176  
 onset of endothelial dysfunction in human hypertension.

177 **Mechanisms underlying the production of COX-derived**  
 178 **EDCFs**

179 In brief, the chain of events leading to endothelium-  
 180 dependent contractions requires an abnormal increase in

181 intracellular calcium in the endothelial cells [5, 26]. The  
 182 rise in calcium activates phospholipase A<sub>2</sub> to release  
 183 arachidonic acid from the cell membrane phospholipids.  
 184 Then COX breaks down arachidonic acid to form  
 185 prostanoids that activate TP receptors located in the  
 186 vascular smooth muscle, resulting in contraction [5, 26].  
 187 During the production of prostanoids, COX simultaneously  
 188 produces ROS, which can subsequently stimulate COX  
 189 within the smooth muscle and produce more prostanoids  
 190 [5, 26], thus amplifying the TP receptor-mediated re-  
 191 sponse (Fig. 3).

192 Calcium overload

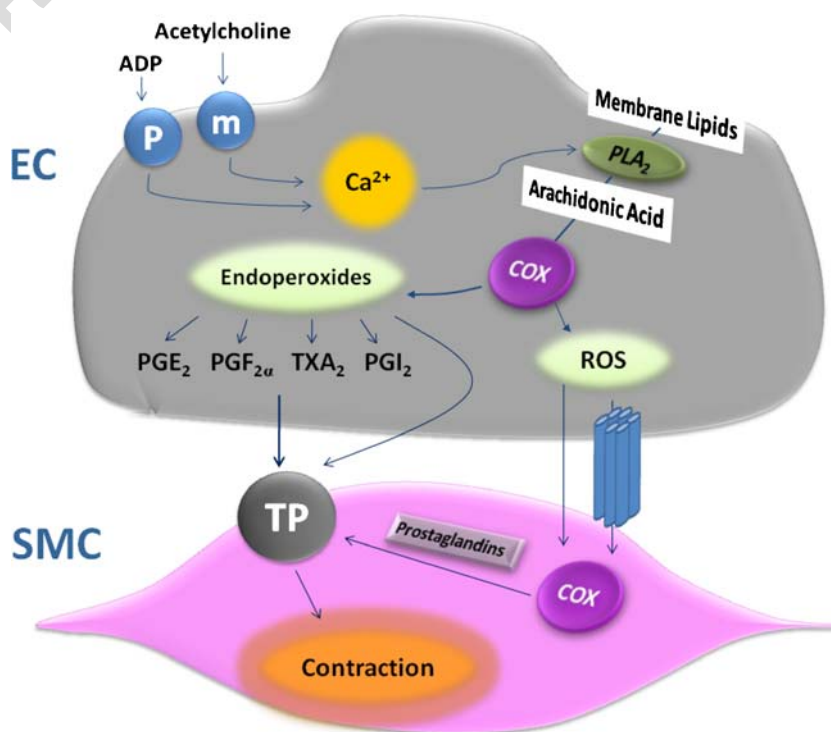
193 An abnormal, high accumulation of intracellular calcium in  
 194 endothelial cells is critical and triggers the production of  
 195 COX-derived EDCFs [43] (Fig. 3). Stimulation with acetyl-  
 196 choline results in calcium overload in the aortic endothelial  
 197 cells of SHR, but not in normotensive Wistar Kyoto rats  
 198 (WKY), signifying dysfunction of calcium handling in the  
 199 hypertensive strain [43]. When calcium overload is mimicked  
 200 in WKY arteries using calcium-increasing agents (such as the  
 201 calcium ionophore A23187 or cyclopiazonic acid),  
 202 endothelium-dependent contractions are evoked despite the  
 203 normal arterial blood pressure of the animals. Nonetheless,  
 204 the amplitude of the contraction remains larger in SHR than  
 205 in WKY [43]. This is explained best by the increased  
 206 expression of COX and prostanoid synthases, a greater

207 release of prostanoids, as well as a hyper-responsiveness of  
 208 the TP receptors in the aortas of SHR than in that of WKY  
 209 [5, 44–46]. Hence, all these downstream modifications are  
 210 not a prerequisite for the development of endothelium-  
 211 dependent contractions, but their presence amplifies the  
 212 response.

213 COX activity

214 The activity of COX is required for the generation of  
 215 vasoconstrictor prostanoids. Two isoforms of COX, a  
 216 constitutive form (COX1) and an inducible form (COX2),  
 217 have been cloned and characterized [47]. Yet COX1—  
 218 termed as the constitutive isoform—can be over-expressed  
 219 under certain conditions, such as increases in shear stress  
 220 [47]. Inflammation is the most common cause for the up-  
 221 regulation of COX2 [47]. Multiple studies using arteries  
 222 from mice and rats have confirmed that COX1 is the  
 223 primary isoform involved in endothelium-dependent con-  
 224 tractions. For example, endothelium-dependent contractions  
 225 are abolished by selective COX1 inhibitors, but are  
 226 relatively insensitive to selective COX2 inhibitors [9, 48].  
 227 Furthermore, endothelium-dependent contractions occur in  
 228 the aortas of wild-type and COX2<sup>-/-</sup> knockout mice, but  
 229 not in those of COX1<sup>-/-</sup> knockout mice [49]. Later studies  
 230 using hamster aortas [50] and aging rats [51], however,  
 231 showed that COX2 can contribute equally to the contraction  
 232 when present or induced in the endothelial cells.

**Fig. 3** Endothelium-dependent contraction has two components: the generation of prostaglandins and ROS. A rise in calcium activates phospholipase A<sub>2</sub> (PLA<sub>2</sub>) to release arachidonic acid, which is subsequently metabolized by cyclooxygenase (COX) to form endoperoxides and various prostaglandins that activate TP receptors located at the vascular smooth muscle. COX also produces ROS, which diffuses or possibly transmigrates via gap junctions and stimulates COX within the smooth muscle, producing more prostanoids and amplifying TP receptor-mediated contractions. ADP adenosine diphosphate, *m* muscarinic receptors, *P* purinergic receptors, *PGE*<sub>2</sub> prostaglandin E<sub>2</sub>, *PGF*<sub>2α</sub> prostaglandin F<sub>2α</sub>; *PGI*<sub>2</sub> prostacyclin, *ROS* reactive oxygen species, *TXA*<sub>2</sub> thromboxane A<sub>2</sub>





233	Production of prostanoids	
234	The immediate products of COX are the endoperoxides,	
235	which themselves function as vasoconstrictors by binding	
236	to TP receptors [45]. Endoperoxides are further transformed	
237	into prostacyclin, thromboxane A <sub>2</sub> , prostaglandin E <sub>2</sub> ,	
238	prostaglandin F <sub>2α</sub> , and prostaglandin D <sub>2</sub> by their respective	
239	prostanoid synthases (Fig. 3). Prostacyclin synthase is by	
240	far the most abundant prostanoid synthase expressed in the	
241	endothelium [52]. Its expression is augmented in the aorta	
242	of SHR compared with that of WKY [52, 53], suggesting	
243	that chronic hypertension induces the protein. In line with	
244	this observation, there is an exaggerated release of	
245	prostacyclin in the aorta of the hypertensive rat [46, 54,	
246	55]. Since this classical vasodilator prostanoid does not	
247	mediate relaxation in this artery, it instead evokes contrac-	
248	tion through activation of TP receptors at high concen-	
249	trations [44]. In response to acetylcholine, prostacyclin and	
250	endoperoxides are the key mediators of endothelium-	
251	dependent contractions in the rat aorta [5, 44]. Whether or	
252	not prostacyclin plays a detrimental role as EDCF in other	
253	animal models or in humans remains to be demonstrated.	
254	Under certain pathological conditions involving en-	
255	hanced oxidative stress, ROS interacts with NO to form	
256	peroxynitrite [22], which can significantly inhibit the	
257	activity of prostacyclin synthase by tyrosine nitration of	
258	the enzyme [56, 57]. Under such circumstances, there is a	
259	marked compensatory production of prostaglandin E <sub>2</sub> and	
260	prostaglandin F <sub>2α</sub> , leading to greater importance of these	
261	two prostanoids [46, 56, 58]. In the hamster aorta and in	
262	human renal arteries, there is a high expression of COX2	
263	and a prominent release of prostaglandin F <sub>2α</sub> , indicating the	
264	importance of this prostanoid as the EDCF in these arteries	
265	[50]. Likewise, prostaglandin F <sub>2α</sub> is the major EDCF	
266	released from re-endothelized femoral rat arteries [36].	
267	When endothelium-dependent contractions are evoked	
268	by the calcium ionophore A23187 or adenosine diphos-	
269	phate (ADP) in the aorta of SHR, the response is partly	
270	sensitive to inhibitors of thromboxane synthase [54, 55,	
271	59], implying the involvement of thromboxane A <sub>2</sub> . The	
272	mRNA expression of thromboxane synthase is enhanced in	
273	the aorta of SHR compared to WKY [52]. Direct chemical	
274	detection with immunoassays has revealed that A23187 and	
275	ADP stimulate the release of thromboxane A <sub>2</sub> and	
276	endoperoxides [46, 54, 55], suggesting that these prosta-	
277	noids are the key mediators of endothelium-dependent	
278	contraction during exposure to these agonists.	
279	On the whole, there is a marked heterogeneity in the	
280	formation of EDCF. The precise chemical identity of EDCF	
281	varies depending on the stimulus, the vascular bed, the age,	
282	and the physiopathological condition of the donor animal.	
283	Thus, prostacyclin, thromboxane A <sub>2</sub> , prostaglandin E <sub>2</sub> ,	
284	prostaglandin F <sub>2α</sub> , and ROS all have been proposed as	
	COX-derived EDCF. It is important to keep in mind that	285
	endothelium-dependent contractions are unlikely to be due	286
	a single substance, but rather likely are evoked by a mixture	287
	of these endothelium-derived products (Fig. 3).	288
	<b>The involvement of TP receptors</b>	289
	Prostanoid receptors are classified into five discrete types	290
	based on their sensitivity to the five naturally occurring	291
	prostanoids: prostacyclin I <sub>2</sub> , thromboxane A <sub>2</sub> , prostaglandin	292
	D <sub>2</sub> , prostaglandin E <sub>2</sub> , and prostaglandin F <sub>2α</sub> . They are	293
	termed P receptors—IP, TP, DP, EP, and FP—with the	294
	preceding letter indicating the prostanoid to which they are	295
	the most sensitive. The effectiveness of TP receptor	296
	inhibitors in abolishing endothelium-dependent contrac-	297
	tions pinpoints the involvement of this prostanoid receptor	298
	subtype in the response [48, 60–62]. Although thrombox-	299
	ane A <sub>2</sub> is the most potent agonist towards TP receptors, it is	300
	not its exclusive ligand. All other prostanoids can bind to	301
	TP receptors and mediate contraction, but with varying	302
	potency. The mRNA and protein expression of TP receptors	303
	does not differ in the aortas of WKY and SHR, indicating	304
	that their expression level is not altered by the hypertensive	305
	process [52, 63]. However, the vascular smooth muscle of	306
	the SHR aorta exhibits a greater responsiveness than that of	307
	the WKY to the constrictor effect of endoperoxides acting	308
	at TP receptors [45]. An involvement of other prostanoid	309
	receptors in endothelium-dependent contractions has been	310
	suggested [63–65], but non-TP receptor endothelium-	311
	dependent component appears to constitute a small part of	312
	the full response.	313
	<b>A separate ROS component</b>	314
	During the production of prostanoids by endothelial COX,	315
	ROS are formed simultaneously. These COX-derived ROS	316
	can act as vasoconstrictors [43, 62]. Thus, COX-derived	317
	EDCF-mediated contractions can be attributed to two	318
	components—prostanoids or ROS [5] (Fig. 3). The possible	319
	existence of a separate ROS component in endothelium-	320
	dependent contractions is strengthened by the following	321
	observations: First, that the generation of ROS by xanthine	322
	plus xanthine oxidase in the extracellular bathing fluid	323
	evokes a contraction in the aorta without endothelium that	324
	requires the activity of COX and stimulation of TP	325
	receptors [62, 66], suggesting that endothelium-derived	326
	ROS could stimulate COX in the vascular smooth muscle	327
	with resulting prostanoid production, causing more TP	328
	receptor-mediated contraction. Second, the direct applica-	329
	tion of hydrogen peroxide, but not that of superoxide	330
	anions or hydroxyl radicals, triggers contractions in the rat	331

332 aorta that are sensitive to cyclooxygenase inhibitors and TP  
 333 receptor antagonists [66–69], suggesting that hydrogen  
 334 peroxide is the mediator responsible for the ROS compo-  
 335 nent of endothelium-dependent contraction. Myoendothe-  
 336 lial gap junctions may facilitate the transfer of ROS from  
 337 endothelial cells to smooth muscle cells [70]. In the aorta of  
 338 the SHR, both the prostanoid and ROS component appear  
 339 to contribute equally to the final endothelium-dependent  
 340 contractions, as antioxidants only partly reduce the re-  
 341 sponse [62]. By contrast, in the canine basilar artery,  
 342 endothelium-dependent contractions are fully prevented by  
 343 superoxide dismutase plus catalase [71], indicating that the  
 344 response is dominated by the endothelial ROS component.

345 **Therapeutic interventions to improve endothelial**  
 346 **function in hypertension**

347 Considering the marked endothelial dysfunction in hyper-  
 348 tension and since its severity correlates with the develop-  
 349 ment of coronary artery disease and predicts future  
 350 cardiovascular events [72], this dysfunction has to be  
 351 considered as a central target in the treatment of hyperten-  
 352 sion. Theoretically, drugs targeted to increase the release of  
 353 EDRF (and in particular, NO), and drugs that decrease the  
 354 production or action of EDCF, should reduce endothelial  
 355 dysfunction.

356 Improving NO production

357 Direct NO releasing compounds, such as nitroglycerin, are  
 358 effective vasodilators. However, continuous administration  
 359 comprises a clinical problem due to the desensitization of  
 360 the target enzyme guanylyl cyclase, leading to cross-  
 361 tolerance to other endothelium-dependent vasodilators  
 362 [73]. Other concerns involve the ability of nitroglycerin to  
 363 increase ROS indirectly [74].

364 Acute supplementation with BH<sub>4</sub>, an essential cofactor  
 365 of NOS, improves endothelial dysfunction by increasing  
 366 NO and reducing ROS in many experimental animal studies  
 367 [75]. But a clinical trial of the effects of BH<sub>4</sub> on arterial  
 368 blood pressure in subjects with poorly controlled systemic  
 369 hypertension has been terminated for lack of significant  
 370 beneficial effect [76]. By contrast, positive results have  
 371 been reported with the use of BH<sub>4</sub> to treat endothelial  
 372 dysfunction in patients with sickle-cell disease [76]. The  
 373 dissimilar results in these clinical trials highlight the  
 374 importance of fully addressing basic questions about  
 375 the mechanism of endothelial regulation that will be critical  
 376 in the design of BH<sub>4</sub>-based therapies.

377 Endogenous NO formation is largely dependent on the  
 378 extracellular concentrations of its substrate, L-arginine.  
 379 Supplementation of L-arginine leads to a measurable

decline in blood pressure and improved endothelial func- 380  
 tions in experimental animals and in hypertensive patients 381  
 [77, 78]. Most L-arginine studies to date have used high 382  
 daily doses, due to the pharmacokinetics of oral L-arginine, 383  
 which reaches its highest concentration in the blood within 384  
 an hour and then diminishes quickly [77]. The use of 385  
 sustained-release L-arginine products in hypertensive 386  
 patients shows promising signs of improving endothelial 387  
 function [79]. 388

When arteries are exposed to NO, whether released from 389  
 the endothelial cells or added exogenously, this causes a 390  
 long-term inhibition of endothelium-dependent contractions 391  
 [80–83]. This implies a suppressed occurrence of EDCF- 392  
 mediated contractions under conditions where there is an 393  
 adequate release of NO. Thus, NO-enhancing agents not 394  
 only will enhance vasodilatation, but also will hamper the 395  
 occurrence of endothelium-dependent contractions. 396

Reducing arterial blood pressure 397

Antihypertensive treatments—such as ACE inhibitors, 398  
 calcium channel blockers, and third generation β- 399  
 blockers—reverse endothelial dysfunction in experimental 400  
 animals and in hypertensive patients [84, 85]. Several 401  
 effects of ACE inhibitors enhance NO release and 402  
 bioactivity, including preventing the breakdown of endog- 403  
 enous bradykinin (a potent NO releaser) [85]. ACE 404  
 inhibitors also protect NO bioavailability [85]. The 405  
 beneficial effect of calcium channel blockers on endothe- 406  
 lial dysfunction can be attributed to their ability to reduce 407  
 calcium entry through voltage-dependent channels of the 408  
 vascular muscle cells, thereby dilating large conduit and 409  
 resistance arteries [86]. In addition, drugs such as amlodi- 410  
 pine activate eNOS to release more NO [87, 88]. Other 411  
 calcium channel blockers, such as lacidipine, possess 412  
 antioxidant properties [89], while third-generation β- 413  
 blockers such as carvedilol and nebivolol, in addition to 414  
 their adrenergic blocking characteristics, substantially im- 415  
 prove endothelial dysfunction through their strong stimula- 416  
 tory effect on the activity of endothelial NOS and their 417  
 antioxidative properties [90]. Blood pressure reduction per se 418  
 does not guarantee improvement in endothelial dysfunction. 419  
 Other antihypertensive drugs, such as conventional β- 420  
 adrenergic blockers, reduce arterial blood pressure but fail 421  
 to restore normal endothelial function [85]. 422

Preventing EDCF-mediated responses 423

Because prostacyclin is one of the main mediators of 424  
 endothelium-dependent contractions in the response of 425  
 acetylcholine, inhibition of its production may result in 426  
 the improvement of endothelial function. But prostacy- 427  
 clin also is beneficial to the vascular system because of 428

429 its ability to prevent aggregation of platelets and avoid  
 430 thrombosis [91]. In addition, inhibition of prostacyclin  
 431 synthase results in the build-up of endoperoxides (which  
 432 by themselves activate TP receptors) and the shunting  
 433 of the latter to other synthases, which produce more  
 434 potent vasoconstrictor prostanoids [46, 54, 55]. There-  
 435 fore, selective inhibition of prostacyclin synthase would  
 436 not reduce the occurrence of unwanted endothelium-  
 437 dependent contractions, but rather would result in ampli-  
 438 fied worsening of the vascular complications. In the SHR  
 439 aorta, thromboxane A<sub>2</sub>, and endoperoxides are the main  
 440 EDCF in response to A23187 and adenosine diphosphate  
 441 [44, 54, 55]. In the aorta of the hamster, in response to  
 442 acetylcholine, the main EDCF is prostaglandin F<sub>2α</sub> [50].  
 443 Thus, the contribution of various prostaglandins released  
 444 during endothelium-dependent contractions varies  
 445 depending on the stimulus, the artery, the species, and  
 446 the disease state of the donor. It therefore appears more  
 447 desirable to design drugs that target either upstream or  
 448 downstream of the EDCF cascade, rather than individual  
 449 prostanoid synthases, to alleviate EDCF-mediated endo-  
 450 thelial dysfunction.

451 Depending on the availability of the enzyme, both  
 452 COX1 and COX2 can contribute to endothelium-  
 453 dependent contractions. Thus, the use of selective drugs  
 454 targeting a specific isoform of COX is not the rationale of  
 455 choice to inhibit endothelium-dependent contractions in  
 456 hypertension. Moreover, the use of non-selective COX  
 457 inhibitors are linked with multiple adverse effects, includ-  
 458 ing peptic ulceration and dyspepsia, while selective COX-2  
 459 inhibition increases the risk of myocardial infarction,  
 460 thrombosis, and stroke [92].

461 EDCFs ultimately converge to activate TP receptors [48,  
 462 60–62]. Although other prostanoid receptors may contrib-  
 463 ute [63–65], it seems—at least from data obtained in animal  
 464 studies—that TP receptors are the dominant receptor  
 465 subtype involved. The TP receptor blocker terutroban  
 466 improves endothelial function in patients with coronary  
 467 disease [93], which illustrates the role of vasoconstrictor  
 468 prostanoids in human endothelial dysfunction. Thus, selec-  
 469 tive TP receptor antagonists may be the most logical  
 470 therapeutic tools to intervene with endothelium-dependent  
 471 contractions in hypertension. Epoxyeicosatrienoic and  
 472 dihydroxyeicosatrienoic acids function as endogenous TP-  
 473 receptor antagonists and induce vasodilatation [94], sug-  
 474 gesting their use as novel TP receptor inhibitors. Synthetic  
 475 TP receptor blockers (such as terutroban) effectively  
 476 prevent endothelium-dependent contraction in numerous  
 477 hypertensive experimental animal models [7, 48, 51, 60–  
 478 62]. The prospective use of TP-receptor antagonists in  
 479 correcting the consequences of the imbalanced release of  
 480 endothelium-derived vasoactive substances in hypertensive  
 481 patients deserves further exploration.

**Conclusion**

482  
 483 The endothelium is one of the major target organs that are  
 484 damaged by high blood pressure. Chronic elevation in blood  
 485 pressure accelerates the turnover of endothelial cells, causing  
 486 them to age prematurely. The regenerated endothelium has an  
 487 impaired ability to release EDRF and favors the occurrence of  
 488 endothelium-dependent contractions. Endothelial dysfunction  
 489 triggers a chain of undesired responses, including increased  
 490 platelet aggregation, expression of adhesion molecules, and  
 491 vascular muscle growth—ultimately leading to thrombosis,  
 492 inflammation, vascular remodeling, and atherosclerosis. En-  
 493 dothelial dysfunction therefore should be considered as a  
 494 central target in the treatment of hypertension. Mechanisms  
 495 that increase EDRF or decrease the release/bioavailability  
 496 action of EDCF are promising drug targets to mitigate the  
 497 damage caused by endothelial dysfunction.

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