

# Vasoconstrictor prostanoids

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**Abstract** In cardiovascular diseases and during aging, endothelial dysfunction is due in part to the release of endothelium-derived contracting factors that counteract the vasodilator effect of the nitric oxide. Endothelium-dependent contractions involve the activation of endothelial cyclooxygenases and the release of various prostanoids, which activate thromboxane prostanoid (TP) receptors of the underlying vascular smooth muscle. The stimulation of TP receptors elicits not only the contraction and the proliferation of vascular smooth muscle cells but also diverse physiological/pathophysiological reactions, including platelet aggregation and activation of endothelial inflammatory responses. TP receptor antagonists curtail endothelial dysfunction in diseases such as hypertension and diabetes, are potent antithrombotic agents, and prevent vascular inflammation.

**Keywords** Hypertension · Diabetes · Aging · Endothelium-dependent contraction · TP receptors · EDCF

## Introduction: the history

At the very beginning of the endothelial saga, the comparison of the endothelium-dependent responses of canine arteries and veins yielded the surprising finding that in the latter endothelial cells not only release relaxing factors, but also can initiate endothelium-dependent contractions of the underlying vascular smooth muscle cells [13]. A pivotal finding was that those endothelium-dependent contractions can be prevented by various inhibitors of cyclooxygenases [54]. The initial observations made in canine veins were soon extended to the basilar artery of the same species and the aorta of the rat [37, 38, 46]. Later bioassay studies demonstrated that the endothelium-dependent contractions were indeed caused by vasoconstrictor prostanoids [endothelium-derived contracting factor (EDCF)] produced by the endothelial cells and diffusing to the underlying vascular smooth muscle [101]. The EDCF-mediated responses evoked by stretching and agonists that elevate the endothelial intracellular calcium concentration shared the characteristic to be abrogated by inhibitors of cyclooxygenase. Thus, the increase in endothelial intracellular calcium must stimulate phospholipase A<sub>2</sub>, which frees arachidonic acid for further metabolism by cyclooxygenase. The breakdown of the fatty acid by this enzyme generates endothelium-derived constrictor prostanoids. They ultimately activate thromboxane prostanoid (TP) receptors of the smooth muscle to evoke contractions [5, 88]. Over the years, oxygen-derived free radicals [39, 100], thromboxane A<sub>2</sub> [26, 75], endoperoxides [23], prostacyclin [25], and prostaglandin

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$F_{2\alpha}$  [97] have been identified as cyclooxygenase-derived mediators of endothelium-dependent contractions.

To exemplify this phenomenon, this brief review will highlight the pathological role of endothelium-dependent contractions, especially in aging, hypertension, and diabetes, and in view of the central role of cyclooxygenases in these EDCF-mediated responses, will focus in particular on the two endothelial isoforms of the enzyme, cyclooxygenase-1 (COX-1), and cyclooxygenase-2 (COX-2).

### Arachidonic acid metabolism

Arachidonic acid, the most common precursor of prostaglandins, is generally released from the cell membrane phospholipids by phospholipases and can be metabolized by several enzymatic systems including cyclooxygenases, lipoxygenases, and cytochrome P450 monooxygenases [73].

The first cyclooxygenase (COX-1) was purified in 1976 and subsequently cloned in 1988 [16, 53, 55, 104]. In 1991, the product of a second gene (COX-2), possessing also both cyclooxygenase and peroxidase activities, was identified [33, 63]. It is generally assumed that COX-1, in most tissues, is expressed constitutively while COX-2 is induced mainly at sites of inflammation [12, 87]. However, COX-2 is also expressed constitutively in several organs and cell types, including the endothelial cells where its expression is regulated by shear stress [83]. In the vascular wall, both endothelial and vascular smooth muscle cells contain COXs; however, in healthy blood vessels, endothelial cells contain much more of the enzyme than the surrounding smooth muscle cells [15]. In most blood vessels, prostacyclin, first described as a potent anti-aggregating agent and as a vasodilator, is the principal metabolite of arachidonic acid, the endothelium being the major site of its synthesis [55, 56]. In the rat aorta, where both COXs isoforms are detected, the amount of COX-2 transcripts in endothelial or smooth muscle cells is markedly less than that of COX-1 [81]. However, the COX isoform expressed by the human vasculature has been a matter of controversy. Nevertheless, several lines of evidence indicate that in humans, although COX-2 is the predominant contributor of the systemic generation of prostacyclin, endothelial COX-1, in both healthy and diseased blood vessels, appears to be also a major source of vascular prostaglandins [19, 21, 51, 68].

Various biologically active eicosanoids are formed from the short-lived, but biologically active endoperoxides [prostaglandin  $H_2$  (PGH<sub>2</sub>)], through the action of a set of synthases namely PGD, PGE, PGF, PGI, and thromboxane synthases. Prostaglandins interact with specific seven transmembrane, G-protein-coupled receptors, which are

classified in five subtypes DP, EP, FP, IP, and TP receptors in function of their sensitivity to the five primary prostanoids, prostanglandins D<sub>2</sub>, E<sub>2</sub>, F<sub>2 $\alpha$</sub> , I<sub>2</sub> (prostacyclin), and thromboxane A<sub>2</sub>, respectively [84].

The stimulation of TP receptors elicits diverse physiological/pathophysiological responses, including platelet aggregation and smooth muscle contraction. Furthermore, the activation of endothelial TP receptors promotes the expression of adhesion molecules and favors adhesion and infiltration of monocytes/macrophages [61]. Although thromboxane A<sub>2</sub> is the preferential physiological ligand of the TP receptor [42], PGH<sub>2</sub> and the other prostaglandins, with a various range of potency, also can activate this receptor [25] (Fig. 1). Additionally, isoprostanes (prostaglandin isomers that are generally produced non-enzymatically from the oxidative modification of polyunsaturated fatty acids [59] but also in endothelial cells, in a COX-dependent manner [91]) as well as hydroxyeicosatetraenoic acids (HETEs, generated by lipoxygenases and cytochrome P450 monooxygenases or formed by nonenzymatic lipid peroxidation in endothelial cells and leukocytes) are also potent endogenous agonists at TP receptors [9, 20, 82, 86, 103].

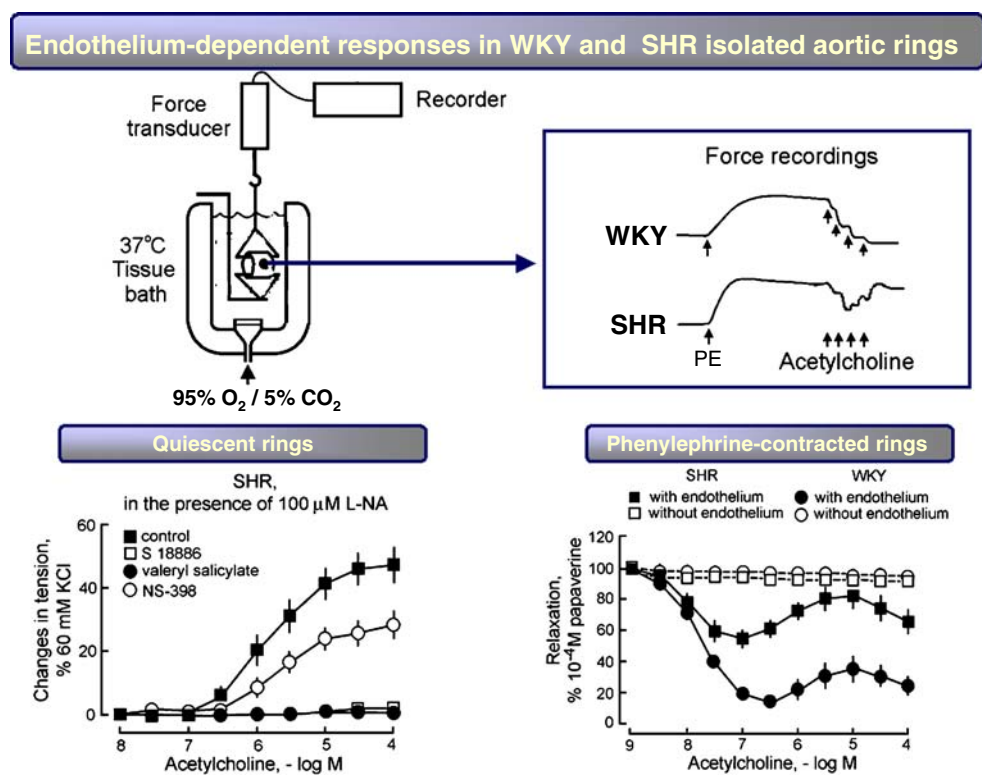
Prostacyclin is a potent inhibitor of platelet adhesion to the endothelial cell surface and of platelet aggregation, and is generally described as an endothelium-derived vasodilator [55–57, 65, 66]. Prostacyclin is the preferential ligand of IP receptors and most of its effects involve the activation of adenylyl cyclase and the subsequent elevation of intracellular cyclic-AMP [90, 94].

### Spontaneously hypertensive rats: the archetypal model

The first endothelium-dependent contractions associated with an endothelial dysfunction were observed in the isolated aorta of the spontaneously hypertensive rats (SHR) [17, 46]. In this artery, the endothelium-dependent relaxations are impaired because the generation of a diffusible EDCF opposes the relaxing effect of nitric oxide [46, 101]. These endothelium-dependent contractions are correlated with the severity of hypertension. They increase during the aging process and also occur in aging normotensive WKY [34, 43]. Endothelium-dependent contractions and the associated endothelial dysfunction are less pronounced in female SHR [29, 40].

Endothelium-dependent responses are associated with an increase in endothelial intracellular calcium concentration ( $[Ca^{2+}]_i$ ). Indeed, acetylcholine causes a rapid increase in  $[Ca^{2+}]_i$  in endothelial cells of SHR and to a much lesser extent in that of WKY. By contrast the calcium ionophore A 23187, which allows the free entry of extracellular calcium into endothelial cells, produces a similar increase in  $[Ca^{2+}]_i$  in the endothelial cells of both WKY and SHR.

**Fig. 1** Endothelium-dependent responses in WKY and SHR isolated aortic rings. The measurement of the changes in isometric tension in isolated aortic rings contracted with phenylephrine (PE) shows that the acetylcholine-induced endothelium-dependent relaxation is blunted in SHR rings when compared to that of normotensive WKY. In SHR quiescent rings treated with an inhibitor of NO synthase (L-nitroarginine, L-NA), acetylcholine produces endothelium- and concentration-dependent contractions, which are blocked by valeryl salicylate, a preferential COX-1 inhibitor or S 18886, a specific TP receptor antagonist, but are only partially inhibited by NS-398, a preferential COX-2 inhibitor (modified from Yang et al. [100])



Acetylcholine- and more generally receptor-mediated endothelium-dependent contractions are larger in SHR than in WKY while the maximal amplitude of these responses, when elicited by A 23187, are similar in the aortae of the two strains [27, 80, 103]. These results illustrate the first endothelial dysfunction associated with endothelium-dependent contractions, i.e., an abnormal calcium signaling in the endothelial cells of SHR in response to neuro-humoral agents.

Phospholipase A<sub>2</sub> catalyzes the breakdown of membrane phospholipids to arachidonic acid. There are two major cytosolic types of the enzyme, calcium-dependent (cPLA<sub>2</sub>) and calcium-independent (iPLA<sub>2</sub>) phospholipase A<sub>2</sub>. The increase in endothelial [Ca<sup>2+</sup>]<sub>i</sub>, irrespective of the stimulus, activates cPLA and provokes the mobilization of arachidonic acid. However, in response to acetylcholine, iPLA<sub>2</sub> is involved by producing lysophospholipids, which open store-operated calcium channels, permitting the influx of extracellular calcium and the subsequent activation of cPLA<sub>2</sub>. By contrast, the calcium ionophore bypasses the cell membrane receptors and causes increase in endothelial calcium and a direct activation of cPLA<sub>2</sub> [95].

The subsequent steps involve the activation of cyclooxygenase and the production of reactive oxygen species along with that of EDCFs and finally the activation of TP receptors [23, 25, 26, 46, 80, 100, 102]. In the SHR aorta, COX-1 is the preponderant enzyme involved in the generation of EDCF since endothelium-dependent contractions are blocked by specific inhibitors of COX-1 and minimally

affected by specific inhibitors of COX-2 [23, 25, 26, 80, 100–102]. Indeed, aortic endothelial cells of various species express preferentially COX-1 versus COX-2 [41, 64] and, in SHR endothelial cells, the mRNA and protein expression of COX-1 is enhanced when compared to that of WKY [23, 81]. In agreement with a preponderant role for COX-1 in endothelium-dependent contractions, these responses are abolished in aortae taken from COX-1 knockout mice while they are maintained in aortic rings of COX-2 knockout animals [79].

However, in both WKY and SHR endothelial cells, the induction of COX-2, especially in resistance arteries and during aging, is also associated with the generation of endothelium-derived contractile prostanoids. In these arteries, COX-2 contributes to the endothelial dysfunction [2, 7, 8, 22, 32, 71, 90, 99, 105]. Therefore, the enhanced endothelial expression of COX-1 and/or COX-2 is the second endothelial dysfunction associated with endothelium-dependent contractions.

Additionally, COX is also involved in the endothelial generation of reactive oxygen species. The enhanced COX-dependent generation of reactive oxygen species is the third endothelial abnormality associated with endothelium-dependent contractions observed in the SHR aorta [80]. Reactive oxygen species decrease NO bioavailability [30, 69] and activate COX [31]. This may involve a positive feedback loop on the endothelial cells by further activating COX and, since reactive oxygen species diffuse toward the vascular smooth muscle cells, they

can stimulate COX in smooth muscle cells and produce more contractile prostanoids [4, 39, 100]. Reactive oxygen species can also favor contraction of the vascular smooth muscle cells. Superoxide anions stimulate  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum of these cells [74]. In addition, exogenous hydrogen peroxide and/or the reactive oxygen species generated by the activation of TP receptors itself, enhances the stability and increases the density of functional TP receptors at the cell membrane [85, 93]. Thus, while the activated TP receptors are being internalized and degraded (a key component in limiting the action of agonists at these receptors), a reactive oxygen species-dependent pathway induces the enhanced biogenesis of new TP receptors. Activation of this positive feedback mechanism may underlie the augmented TP expression observed in cardiovascular diseases [36]. Finally, TP receptors are also expressed in endothelial cells and their stimulation can induce the inhibition of NO production [45]. This feed-forward loop involving a reactive oxygen species-dependent post-transcriptional stabilization of TP receptors associated with a decrease production of NO, further altering the unbalance between relaxing and contracting factors and exacerbating the endothelial dysfunction, suggest that TP receptors are very likely to play a pivotal role in cardiovascular diseases [93].

EDCFs diffuse toward the vascular smooth muscle cells and directly activate the TP receptors [101]. Inhibition of thromboxane  $\text{A}_2$  synthesis does not affect the endothelium-dependent contractions to acetylcholine but partially inhibits those in response to A23187, ADP, or endothelin, indicating that thromboxane  $\text{A}_2$  is only one of the EDCFs that can be released from SHR aortic endothelial cells [5, 23, 25–27, 35, 44, 46, 75, 100, 103].  $\text{PGH}_2$  is the second most potent agonist at TP receptors and is more effective in activating TP receptor in vascular smooth muscle from SHR than in that of WKY. Therefore,  $\text{PGH}_2$  is also a suitable candidate as EDCF [5, 23, 25–27, 35]. However, in SHR aortic endothelial cells, the massive expression of prostacyclin synthase [81] and its close association with COX-1 [41] are not in favor of a large  $\text{PGH}_2$  spill over. Paradoxically, prostacyclin is likely to be a major EDCF in SHR aorta. Because of an early and specific dysfunction of the IP receptors of the vascular smooth muscle [28], prostacyclin does not produce relaxations but evokes TP-receptor-dependent contractions [25, 67]. Furthermore, prostacyclin, as  $\text{PGH}_2$ , is also more potent in producing contraction in SHR than in WKY [25]. Therefore, the fourth dysfunction associated with endothelium-dependent contractions involves changes in the responses of the IP and TP receptors of the vascular smooth muscle without major changes in their respective expression [62, 74]. Prostacyclin is also a major contributing factor accounting for the endothelial dysfunction in

the aorta and mesenteric artery of WKY and SHR treated with aldosterone [7, 99]. Finally,  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  can also act as EDCF when prostacyclin synthase is inhibited and the metabolism of  $\text{PGH}_2$  diverted [25], a phenomenon that may occur when severe oxidative stress leads to the tyrosine nitration of prostacyclin synthase [107]. Thus, in the SHR aorta, thromboxane  $\text{A}_2$ ,  $\text{PGH}_2$ ,  $\text{PGI}_2$ , and, depending on the circumstances,  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  can all act as EDCF (Fig. 2).

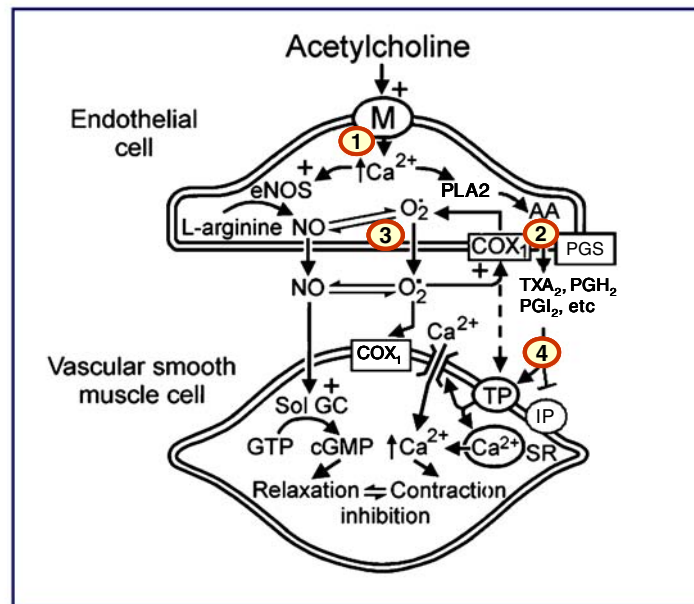
The contribution of EDCF- and TP-receptor-mediated responses in endothelial dysfunction, first observed in the SHR, has been reported in numerous other models of hypertension and is likely to occur in patients with essential hypertension [18]. Additionally, non-endothelium-derived contractile prostanoids can contribute to vascular dysfunction. For instance, in the aorta of the hypertensive eNOS knockout mice, smooth muscle- and COX-2-derived thromboxane  $\text{A}_2$  contributes to the enhanced contractions in response to endothelin-1 [106].

### Enhanced action of COX-derived EDCF in aging

Aging favors a shift of the fine balance between NO-mediated endothelium-dependent relaxations and COX-dependent contractions towards the latter. Impaired endothelium-dependent relaxations to acetylcholine have been demonstrated in the aorta and superior mesenteric arteries of aged rats. This is accompanied by an increased expression of COX isoforms. The relaxations are potentiated by a non-selective COX inhibitor indomethacin, suggesting a critical role of COX-derived vasoconstrictor prostanoids in impairing endothelium-dependent relaxations [49]. Similar findings are reported in small mesenteric arteries [1], in which indomethacin and a specific COX-2 inhibitor NS-398 restored the attenuated endothelium-dependent relaxations and eliminated contractions caused by high concentrations of acetylcholine.

Endothelium-dependent contractions are usually unveiled in pathological models, owing to a reduction of NO bioavailability that allows the emergence of endothelium-dependent contractions. However, there are exceptions, one being the occurrence of endothelium-dependent contractions in the aorta of young and healthy hamsters [96, 97]. In this preparation, COX-2 is expressed constitutively and incubation with an inhibitor of eNOS unmasks the ability of acetylcholine to elicit endothelium-dependent contractions which are sensitive to COX-2 inhibition and TP receptor antagonism, while these responses are unaffected by COX-1 inhibitors and reactive oxygen species scavengers. By contrast to the SHR aorta in which the proposed EDCFs are prostacyclin,  $\text{PGH}_2$ , and thromboxane  $\text{A}_2$ , the endothelium-derived vasoconstrictor prostanoid responsible

## Acetylcholine and endothelium-dependent contractions in SHR aorta



**Fig. 2** Mechanisms of endothelium-dependent contractions in WKY and SHR aortic rings. *M* muscarinic receptor, *AA* arachidonic acid, *eNOS* endothelial nitric oxide synthase, *NO* nitric oxide,  $O_2^-$  superoxide anion, *PGS* prostaglandin synthases, *COX-1* cyclooxygenase-1, *PGI<sub>2</sub>* prostacyclin, *TXA<sub>2</sub>* thromboxane A<sub>2</sub>, *TP* TP receptor, *IP* IP receptor, *SR* sarcoplasmic reticulum, *Sol GC* soluble guanylyl cyclase, *GTP* guanosine triphosphate, *cGMP* cyclic guanosine monophosphate. The

number ①, ②, ③, and ④ indicates identified abnormalities which contribute to the exacerbated endothelium-dependent contractions in SHR aorta, i.e., endothelial calcium handling, enhanced endothelial COX-1 expression and activity, increased generation of endothelial reactive oxygen species, and dysfunctional smooth muscle TP and IP receptors, respectively (modified from Vanhoutte et al. [88])

in the hamster aorta appears to be  $PGF_{2\alpha}$  [96, 97]. In the hamster, aging not only exaggerates endothelium-dependent contractions, which are again attenuated by COX-2 inhibitors, but also increases COX-2 expression and augments the release of and the vascular sensitivity to  $PGF_{2\alpha}$  [97]. One distinctive feature of the endothelium-dependent contraction in the aorta of aged hamsters compared with their younger counterparts is that the response can be observed in the absence of inhibitors of eNOS. On the other hand, the endothelium-dependent relaxations are diminished in preparations from aging hamsters, reinforcing the interpretation that endothelium-dependent contractions are unmasked by a reduction in NO bioavailability in aged animals.

The endothelial alterations observed in various animal models of aging suggest that the endothelial dysfunction observed in hypertension can be considered as the consequence of the premature aging of the blood vessel wall [17].

### Role of prostanoids in endothelial dysfunction in diabetes

Micro- and macrovascular diseases are currently the major causes of morbidity and mortality in patients with diabetes

mellitus and endothelial dysfunction plays also a key role in the pathogenesis of these diabetic vascular diseases. Impaired endothelium-dependent vasodilatation has been demonstrated in various vascular beds of different animal models of diabetes and in humans with types 1 and 2 diabetes. However, the mechanisms of endothelial dysfunction appear to differ according to the diabetic model and the vascular bed under study and include impaired signal transduction or substrate availability, impaired release of NO, increased destruction of NO, decreased sensitivity of the vascular smooth muscle to NO, and enhanced release of endothelium-derived constricting factors. These dysfunctions are again generally associated with the over-generation of reactive oxygen species, lipid peroxidation, and elevated production of adhesion molecules [14, 50].

Streptozotocin-induced diabetes leads to a diminished endothelium-dependent NO-mediated relaxation in rat conduit arteries. TP receptor antagonism, again, restores the impaired relaxation and prevents the endothelium-dependent contraction. Neither thromboxane A<sub>2</sub> nor prostacyclin plays a significant role as the relaxation is unaffected by thromboxane A<sub>2</sub> synthesis inhibition and since prostacyclin does not cause contractions in those arteries [72]. Likewise, indomethacin inhibits the occurrence of endothelium-dependent contraction in the femoral

artery of streptozotocin-treated rats whereby COX-1-derived products appear to play a dominant role [70]. Thromboxane  $A_2$  does not play a direct role in reducing endothelial function in type 1 diabetes, but endothelium-derived thromboxane  $A_2$  may be involved in the enhanced contractile response to endothelin-1 as thromboxane  $A_2$  synthesis inhibition attenuates the exaggerated contraction to endothelin-1 in the mesenteric arteries of streptozotocin-treated rats [3]. In the mesenteric artery of type 2 diabetic OLETF rats, the impaired endothelium-dependent relaxation to acetylcholine is normalized and the contraction to the muscarinic agonist inhibited by indomethacin [48]. The acetylcholine-stimulated release of thromboxane  $A_2$  and  $PGE_2$  are greater in OLETF than non-diabetic rats; but it is not clear which COX-derived prostanoid is most likely involved [48]. The impaired endothelium-dependent relaxations in the mesenteric vascular bed of streptozotocin-induced diabetic mice is opposed by a compensatory up-regulation of both expression and activity of COX-2 and selective inhibition of COX-2 unmasks endothelial dysfunction [60], suggesting a vascular benefit of COX-2-derived products.

Despite a clearly demonstrated role of COX-derived prostaglandins in the regulation of vascular reactivity in conduit arteries, their link to endothelium-dependent hyperpolarization of vascular smooth muscle (EDHF-mediated responses) in resistance blood vessels is unclear. Indomethacin augments the endothelium-dependent EDHF-mediated relaxation in mesenteric resistance arteries from streptozotocin-induced diabetic but not in those from control mice [58], suggesting that

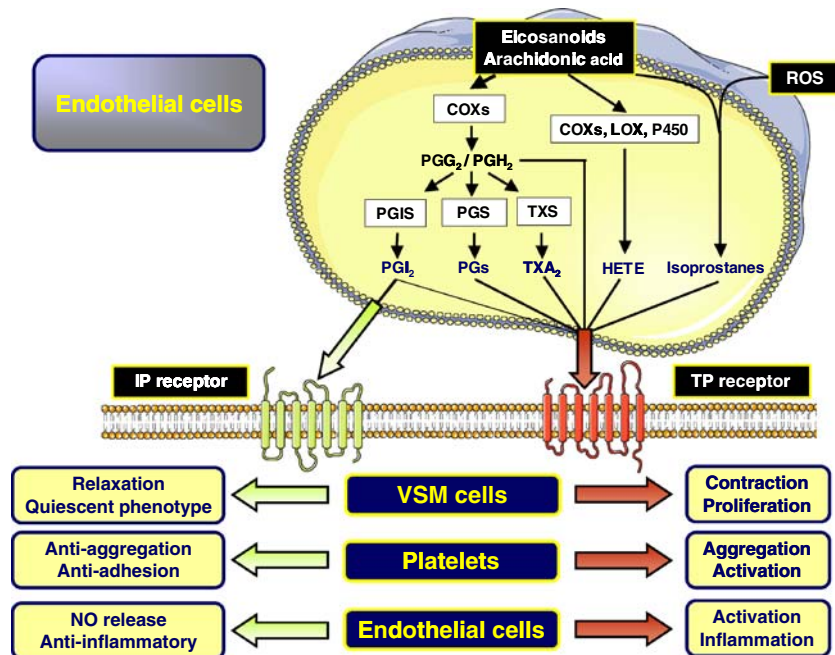
COX-derived prostanoids inhibit either the release of EDHF from the endothelium or its action on vascular smooth muscle [58].

### Clinical relevance

The information available in animal models demonstrates that in aging and in a number of diseases such as hypertension, diabetes, and atherosclerosis, as the endothelium becomes dysfunctional, the release of EDCF is favored and endothelium-dependent contractions become more prominent [11, 24, 44, 46, 48]. The indirect evidence available in people suggests that the same is true. Indeed, indomethacin potentiates the relaxations to acetylcholine in isolated renal arteries of aged patients [47] and the vasodilator response to the muscarinic agonist in the forearm of people with essential hypertension [76–78]. The comparison of the effect of the non-selective inhibitor of cyclooxygenases in different age groups further suggests that the contribution EDCF augments with advancing age [77, 78], as it does in the animal. In patients with endothelial dysfunction an improvement was observed with selective COX-2 inhibitors [10, 92], which may imply an important role for that isoform of the enzyme. The TP-receptor blocker S18886 improves endothelial function in patients with coronary disease [6], which further illustrates the role of vasoconstrictor prostanoids in human endothelial dysfunction.

The role of the specific COX isoforms and arachidonic acid metabolites in the regulation of vascular function in

**Fig. 3** Involvement of TP and IP receptors in vascular dysfunction. *COXs* cyclooxygenases, *LOX* lipoxygenase, *P450* cytochrome P450 monooxygenase, *ROS* reactive oxygen species, *PGS* prostaglandin synthases, *PGIS* prostacyclin synthase, *TXS* thromboxane synthase, *PGs* prostaglandins, *PGG<sub>2</sub>* prostaglandin G<sub>2</sub>, *PGH<sub>2</sub>* prostaglandin H<sub>2</sub>, *PGI<sub>2</sub>* prostaglandin I<sub>2</sub> (prostacyclin), *TXA<sub>2</sub>* thromboxane A<sub>2</sub>, *HETE* hydroxyeicosatetraenoic acid



human diabetes is less well defined. In young patients with type 1 diabetes, the impaired NO-dependent relaxation may be compensated by an increase in prostacyclin-mediated responses [52]. Indeed, indomethacin-sensitive blood flow is greater in patients with type 1 diabetes than in non-diabetic subjects [98], further suggesting a compensatory response for prostacyclin when the bioavailability of NO is declining. On the other hand, COX-derived prostanoids contribute to the appearance of endothelium-dependent contractions in arteries from older patients with diabetes and hypertension [97].

Since the stimulation of TP receptors elicits not only the contraction and the proliferation of vascular smooth muscle cells but also diverse physiological/pathophysiological reactions in platelets (adhesion and aggregation), and endothelial cells (expression of adhesion molecules associated with the subsequent adhesion and infiltration of monocytes/macrophages), TP receptor antagonists may have a unique potential for the treatment of cardiovascular disorders [61, 89] (Fig. 3). However, in contrast to many examples of animal models of cardiovascular diseases, in patients, no study is yet available demonstrating that reversal of endothelial dysfunction is independently associated with a better clinical outcome. The results of large-scale clinical trials are awaited in order to determine the proper therapeutic indication of TP antagonists and to confirm their potential beneficial effect.

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