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REVIEW

Endothelium-mediated control of vascular tone: COX-1 and COX-2 products

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Endothelium-dependent contractions contribute to endothelial dysfunction in various animal models of aging, diabetes and cardiovascular diseases. In the spontaneously hypertensive rat, the archetypal model for endothelium-dependent contractions, the production of the endothelium-derived contractile factors (EDCF) involves an increase in endothelial intracellular calcium concentration, the production of reactive oxygen species, the predominant activation of cyclooxygenase-1 (COX-1) and to a lesser extent that of COX-2, the diffusion of EDCF towards the smooth muscle cells and the subsequent stimulation of their thromboxane A2-endoperoxide TP receptors. Endothelium-dependent contractions are also observed in various models of hypertension, aging and diabetes. They generally also involve the generation of COX-1- and/or COX-2-derived products and the activation of smooth muscle TP receptors. Depending on the model, thromboxane A_2, PGH_2, $PGF_{2\alpha}$, PGE_2 and paradoxically PGI_2 can all act as EDCFs. In human, the production of COX-derived EDCF is a characteristic of the aging and diseased blood vessels, with essential hypertension causing an earlier onset and an acceleration of this endothelial dysfunction. As it has been observed in animal models, COX-1, COX-2 or both isoforms can contribute to these endothelial dysfunctions. Since in most cases, the activation of TP receptors is the common downstream effector, selective antagonists of this receptor should curtail endothelial dysfunction and be of therapeutic interest in the treatment of cardiovascular disorders.

LINKED ARTICLES

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Abbreviations

ATP, adenosine triphosphate; BMP4, bone morphogenic protein-4; $iPLA_2$, calcium-independent phospholipase A_2; cAMP, cyclic adenosine monophosphate; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; $cPLA_2$, cytosolic phospholipase A_2; DiHET, dihydroxyeicosatrienoic acids; EDCF, endothelium-derived contracting factor; EDHF, endothelium-derived hyperpolarizing factor; EETs, epoxyeicosatrienoic acids; EP, prostaglandin E_2 receptor; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; FP, prostaglandin $F_{2\alpha}$ receptor; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharides; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor κB; NO, nitric oxide; OLEFT, Otsuka Long-Evans Tokushima fatty rats; PPAR, peroxisome proliferator-activated receptor; PLA_2, phospholipase A_2; IP, prostacyclin receptor; PG, prostaglandin; PGD_2, prostaglandin D_2; DP, prostaglandin D_2 receptor; PGE_2, prostaglandin E_2; PGES, prostaglandin E synthase; cPGES, cytosolic PGE synthase; mPGES, membrane-bound; PGE-synthase; PGH_2, prostaglandin H_2, endoperoxide; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGG_2, prostaglandin G_2; PGI_2, prostaglandin I_2 or prostacyclin; PGIS, prostacyclin synthase; PGJ_2, prostaglandin J_2; $sPLA_2$, secreted phospholipase A_2; SHR, spontaneously hypertensive rat; SOC, store-operated calcium channels; TP, thromboxane/endoperoxide receptor; WKY, Wistar-Kyoto rats

Introduction

In 1980, Furchgott and Zawadzki (1980) unequivocally demonstrated that the presence of the endothelium was required in order to observe relaxations of isolated arteries to acetylcholine. This seminal discovery not only led to the identification of the L-arginine nitric oxide (NO) synthase pathway and the overwhelming role of NO as an intercellular messenger but also led to the quest for other endothelium-derived vasoactive factors, in particular endothelium-derived hyperpolarizing factor (EDHF) and endothelium-derived contracting factors (EDCF) (for review, Félétou and Vanhoutte, 2006a,b; Félétou *et al.*, 2009).

However, although the era of endothelium-derived relaxing factors truly began with the scientific breakthrough of Furchgott and Zawadzki (1980), prostaglandins (PG) were in fact the first endothelium-derived vasoactive paracrine substances to be identified (Moncada *et al.*, 1976; 1977). PGs and thromboxane A_2 are critical modulators of vascular tone and platelet activity under both physiological and pathophysiological conditions (Moncada and Vane, 1979; Félétou *et al.*, 2010a). The fatty acid arachidonic acid, the most common precursor of PGs, is released from the cell membrane phospholipids primarily by phospholipase A_2 and can be metabolized by several enzymatic systems including prostaglandin H (PGH) synthases, lipoxygenases and cytochrome P450 monooxygenases or be transformed in a radical catalyzed non-enzymatic manner into isoprostanes (Morrow *et al.*, 1980; Smith and Marnett, 1991). PGH synthase, the first and rate-limiting enzyme involved in the biosynthetic pathway of PGs, possesses both a cyclooxygenase (COX) catalytic activity leading to the formation of prostaglandin G_2 (PGG_2) and a peroxidase activity catalyzing the reduction of PGG_2 to prostaglandin H_2 (endoperoxide, PGH_2). Although this single protein is associated with both COX and peroxidase activities, PGH-synthases are usually termed COX (Vane *et al.*, 1998).

COX- and endothelium-dependent contractions have been reported in arteries and veins of different species in response to various agonists and substances that increase the endothelial intracellular calcium concentration ($[Ca^{2+}]_i$) in a receptor-independent manner as well as in response to physical stimuli such as stretch (Miller and Vanhoutte, 1985; Katusic *et al.*, 1987; 1988; Ihara *et al.*, 1999; Okon *et al.*, 2002; Yang *et al.*, 2004b; Tang *et al.*, 2007). Endothelium-dependent contractions have been observed in healthy blood vessels, suggesting that they play a physiological role in the endothelium-dependent regulation of vascular tone. For instance, the endothelium may contribute to the autoregulation of cerebral blood flow during increases in transmural pressure by the increased production and release of PGs, which causes activation of the underlying vascular smooth muscle (Katusic *et al.*, 1987). However, endothelium-dependent contractions are also frequently associated with cardiovascular disease in both animals and humans. These responses counterbalance the endothelium-dependent vasodilatations produced by NO and/or EDHF and contribute to endothelial dysfunction (Vanhoutte *et al.*, 2005; Félétou *et al.*, 2009; 2010b).

This brief review will highlight the physiological and pathological role of endothelial COX-derived vasoactive factors, especially in aging, hypertension and diabetes.

Mobilization of arachidonic acid

The fatty acid arachidonic acid, the most common precursor of PGs, is released from the cell membrane phospholipids. Two major phospholipases are implicated in prostanoid formation, phospholipase A2 (PLA_2) acting on phosphatidylethanolamine, phosphtidyl-choline or plasmalogens, as well as phospholipase C, which together with the diacylglycerol lipase acts sequentially on phosphatidyl-inositols derivatives (Smith and Marnett, 1991).

The phospholipase A_2 super family includes in mammals at least 25 enzymes identified with PLA_2 activity and is subdivided in five main groups: secreted PLA_2 ($sPLA_2$), cytosolic PLA_2 ($cPLA_2$), calcium-independent PLA_2 ($iPLA_2$), the platelet-activating factor acetylhydrolases and the lysosomal PLA_2. Although $sPLA_2$, $cPLA_2$ and $iPLA_2$ can generate arachidonic acid, $cPLA_2\alpha$ (or group IV-A) is the only PLA_2 enzyme that shows significant selectivity towards phospholipids containing arachidonic acid (Burke and Dennis, 2009). $cPLA_2\alpha$ is expressed ubiquitously and constitutively in most cells and tissues and is an essential component of the initiation of the metabolism of arachidonic acid. The translocation and activation of this intracellular enzyme is initiated by submicromolar calcium concentrations and by phosphorylations, both of which are critical events in the post-receptor signalling transduction (Kudo and Murakami, 2002). $iPLA_2$s, as their name indicates, do not require calcium for membrane association or enzymatic activity and have been implicated in a number of physiological and pathophysiological processes. In both endothelial and smooth muscle cells, $iPLA_2\beta$ is an important effector of calcium signalling and contributes with STIM and ORAI to capacitative Ca^{2+} entry (Bolotina, 2008).

COXs

Two COXs (COX-1 and COX-2) encoded by two different genes have been cloned and characterized. Although COX-1 and COX-2 share a high level of homology (65%), the activity and expression of these enzymes are regulated differentially, and they can function independently within the same cell type (Davidge, 2001). An enzymatically active splice variant of COX-1, termed COX-3, is expressed in the heart and cerebral cortex, but the regulation of its transcription appears similar to that of COX-1 (Park *et al.*, 2006). Fatty acids such as arachidonic acid are the preferential substrate of COX-1, while COX-2 used as substrates both and equally well fatty acids and 2-arachidonyl glycerol. Therefore, COX-2 can generate group of products that COX-1 cannot synthesize (Smith and Song, 2002). Both COXs depend on the presence of lipid peroxides for their activation, but the activation of COX-2 requires 10-fold lower concentrations of hydroperoxide than COX-1, suggesting that COX-2 can function in the presence of COX-1, without the latter being activated (Morita, 2002; Smith and Song, 2002). In most tissues, COX-1

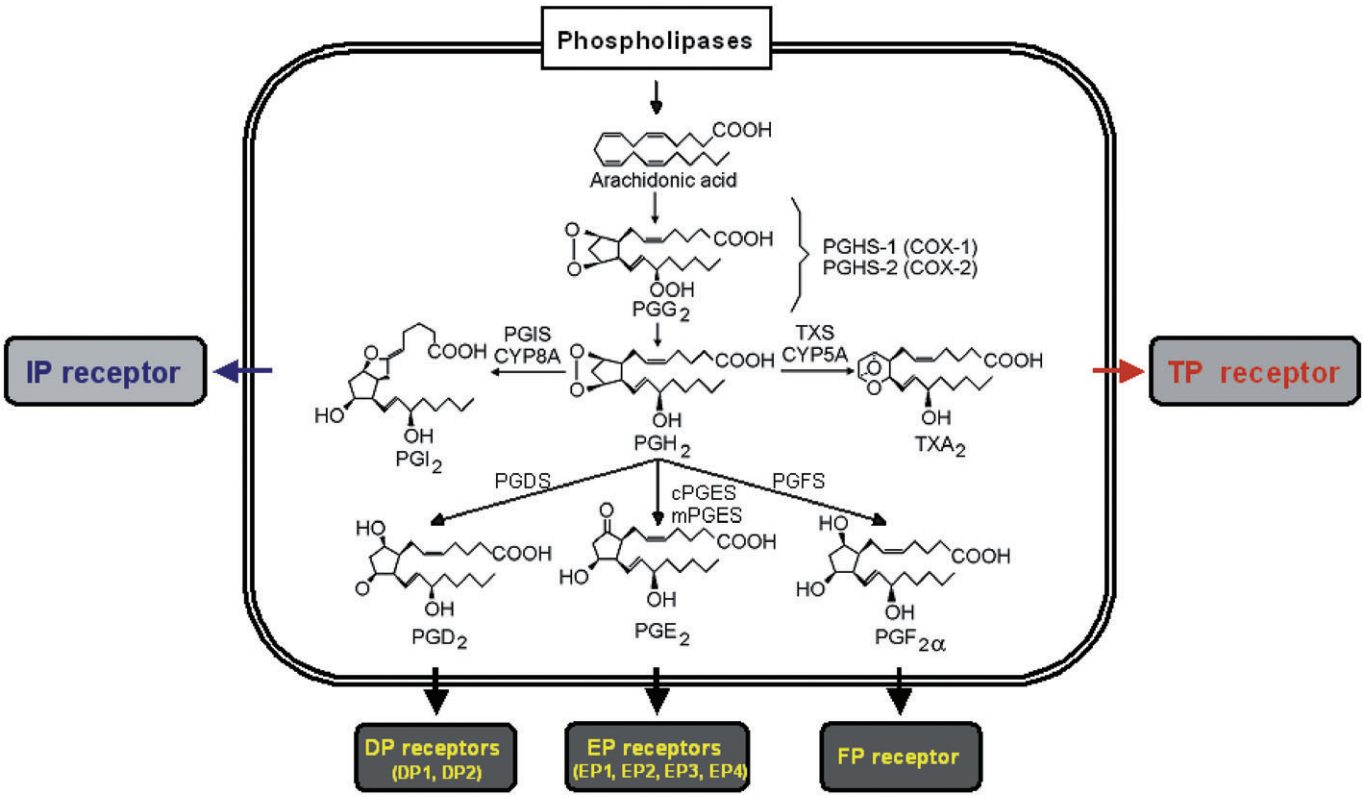

Figure 1

Cyclooxygenases and arachidonic acid metabolism. Prostacyclin and thromboxane synthases belong to the cytochrome P-450 superfamily (in the human, CYP8A1 and CYP5 respectively). The preferential receptors for the five primary prostaglandins and their subtypes are indicated: IP, DPs, EPs, FP and TP for prostacyclin, prostaglandin D_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$ and thromboxane A_2 respectively. PGG_2, prostaglandin G_2; PGH_2, prostaglandin H_2; PGI_2, prostacyclin; TXA_2, thromboxane A_2; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; COX, cyclooxygenase; PGHS, prostaglandin H synthase; PGIS, prostacyclin synthase; TXS, thromboxane synthase; PGDS, prostaglandin D synthases; cPGES, cytosolic prostaglandin E_2 synthase; mPGES, membrane prostaglandin E_2 synthase; PGFS, prostaglandin F_2 synthase.

is expressed constitutively but can also be overexpressed, for instance by shear stress (Doroudi *et al.*, 2000), while COX-2 is often induced at sites of inflammation. However, COX-2 is also expressed constitutively in several organs and cell types, including endothelial cells where its expression is also up-regulated by shear stress (Funk and Fitzgerald, 2007).

Various biologically active eicosanoids are formed from the short-lived but biologically active PGH_2, through the action of a set of PG synthases, PGD, PGE, PGF, PGI and thromboxane synthases. The five primary PGs formed, PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and thromboxane A_2, interact with prostanoid (P) receptors, which belong to the G-protein-coupled seven transmembrane domains family, and are classified in five subtypes (DP, EP, FP, IP and TP receptors; Figure 1) in function of their preferential affinity towards those PGs (Tsuboi *et al.*, 2002). Additionally, activation of COXs is a source of superoxide anions because of their ability to co-oxidize substances such as NADPH (Wolin, 2000; Tang *et al.*, 2007). The oxidative modification of polyunsaturated fatty acids via a non-enzymatic, free radical–catalyzed mechanism can generate isoprostanes (Morrow *et al.*, 1980). Isoprostanes are often used as markers of oxidative stress (Pratico *et al.*, 2001) and have been associated with various cardiovascular diseases, including coronary stenosis (Wang *et al.*, 2006a). However, these prostanoids are also biologically active compounds that stimulate PG receptors (Janssen, 2002; Yang *et al.*, 2004a; Figure 2). Furthermore, a COX-dependent production of 8-isoprostanes can take place in endothelial cells (Watkins *et al.*, 1999). For instance, the COX-2-dependent production of 8-iso-$PGF_{2\alpha}$, which activates TP receptors, may contribute to the hypoxia-induced hyperresponsiveness of pulmonary arteries (Delannoy *et al.*, 2010).

In healthy blood vessels, both endothelial and, to a lesser extent, vascular smooth muscle cells express the two COXs, COX-1 being the predominant isoform (De Witt *et al.*, 1983; Tang and Vanhoutte, 2008). Similarly, in humans, although COX-2 appears to be the major contributor of the overall systemic generation of prostacyclin, in endothelial cells of both healthy and diseased blood vessels, COX-1 is also an important source of PGs (McAdam *et al.*, 1999; Flavahan, 2007; Funk and FitzGerald, 2007; Rovati *et al.*, 2010).

In the cardiovascular system, thromboxane A_2, a powerful aggregating factor and a vasoconstrictor, is derived predominantly from platelet COX-1 but can also be generated by endothelial cells. 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 (Moncada

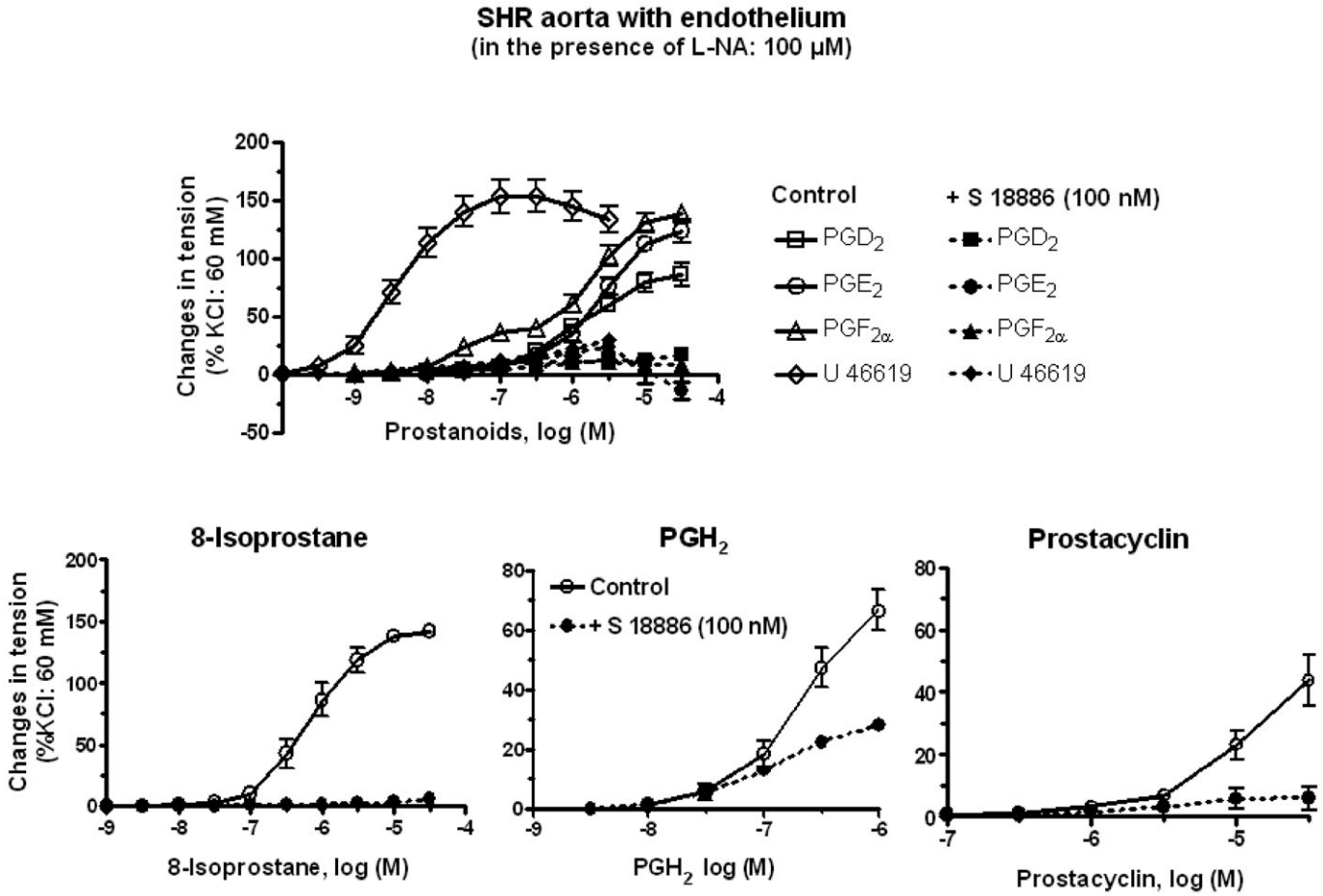

Figure 2

Prostanoids-induced contractions in SHR aorta and TP activation. In isolated aortic rings of SHR (in the presence of the NO synthase inhibitor L-nitro-arginine), PGD_2, PGE_2, $PGF_{2\alpha}$, the analogue of thromboxane A_2, U 46619, 8-isoprostane, PGH_2 and prostacyclin, all produce concentration-dependent contractions, which are inhibited by the selective TP antagonist, S 18886 (100 nM). SHR, spontaneously hypertensive rat; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

et al., 1976; 1977; Figure 3). However, in many cardiovascular diseases, the widespread vascular and organ inflammation and the associated oxidative stress enhance the production of eicosanoids and shift their production/effects from vasodilatation and anti-thrombosis to vasoconstriction, pro-thrombosis and further inflammation (Félétou *et al.*, 2010a).

In mice, disruption of COX-1 is associated with a decrease in constitutive PG synthesis, but the inducible synthesis remains unaffected. The knockout mice show normal survival but with an impaired platelet aggregation and inflammatory responses and with an increased tolerance to pain and some degree of airway hyper-responsiveness (Loftin *et al.*, 2002; Morita, 2002). They show a reduced hypertensive response to angiotensin II and, in isolated aortic rings, a complete inhibition of endothelium-dependent contractions (Tang *et al.*, 2005; Smyth *et al.*, 2009). COX-1 deletion prevents atherosclerotic lesion formation in ApoE null mice (McClelland *et al.*, 2009).

The phenotype associated with COX-2 deletion is more severe. Thirty to 40% of the pups from COX-2 knockout mice die within 48 h from a patent ductus arteriosus (Loftin *et al.*, 2001). Furthermore, the surviving animals have a shorter life span than the wild-type mice. The disruption of COX-2 is associated with a normal constitutive PG synthesis, but the inducible synthesis is impaired (Morham *et al.*, 1995). Females have a deficient reproductive function (Lim *et al.*, 1997), and in both sexes, the inflammatory responses are altered (Morita, 2002). COX-2 knockout mice have a compromised postnatal kidney development associated with a reduced renal blood flow, a decreased urine production and the development of nephropathy (Loftin *et al.*, 2001). COX-2 disruption decreases plasma renin in mice subjected to a low-salt diet. The knockout mice are hypertensive. and although platelet aggregation is normal, since mature platelets only express COX-1, they are more susceptible to thrombosis (Loftin *et al.*, 2002; Morita, 2002; Yu and Funk, 2007). They show an enhanced pressor effect in response to angiotensin II and no or minor inhibition of endothelium-dependent contractions. In mice with a specific deletion of COX-2 in the cardiomyocytes, heart failure and fibrosis are observed. However, in mouse models of abdominal aortic aneurysms, the disruption of COX-2 decreases their forma-

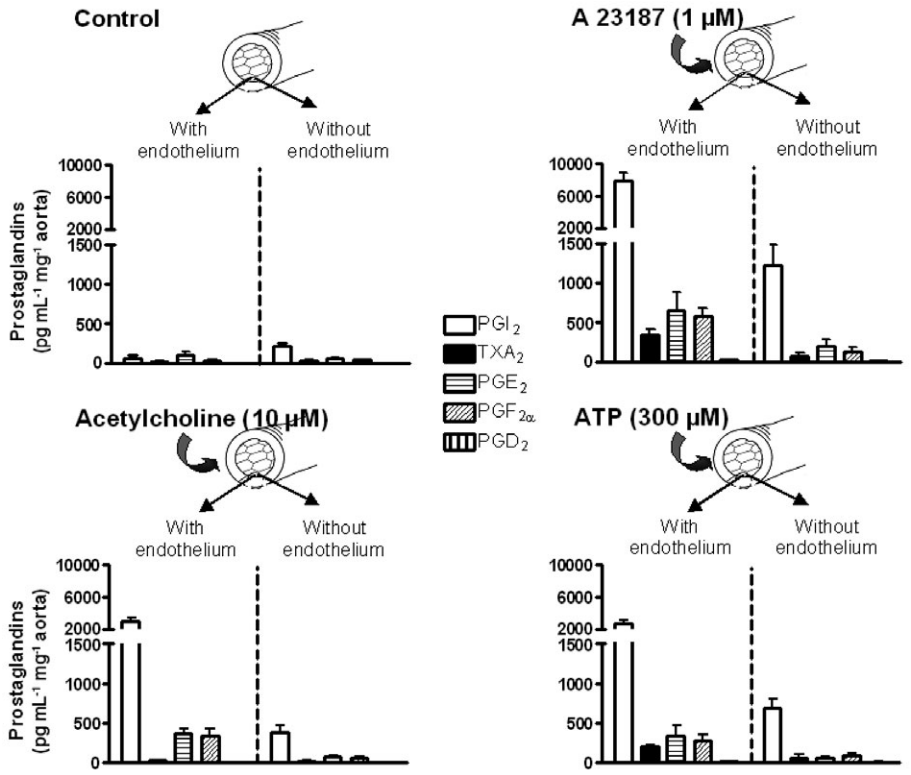

Figure 3

Release of prostaglandins by WKY isolated aortic rings with and without endothelium under resting conditions and upon stimulation by the calcium ionophore A 23187 (1 μM), acetylcholine (10 μM) and ATP (300 μM). In WKY aorta, as in most blood vessels, endothelium-derived prostacyclin is the predominant metabolite of arachidonic acid. WKY, Wistar-Kyoto rats.

tion, and in various experimental models of cancer, it reduces tumour formation (Tang *et al.*, 2005; Smyth *et al.*, 2009).

The double deletion of COX-1 and COX-2 is lethal within hours after birth because of patent ductus arteriosus (Davidge, 2001).

PGD_2 and DP receptors

PGD_2 is a major PG in the central nervous system and in immune cells. It can also be synthesized in the vascular wall as both types of PGD synthases are expressed in endothelial cells, where they are up-regulated in response to an increase in fluid shear stress (Taba *et al.*, 2000). However, PGD_2 is mainly involved in the regulation of sleep and in allergic responses (Smyth *et al.*, 2009).

Two distinct types of PGD synthases have been identified, the lipophilic ligand carrier protein (lipocalin) type enzyme and the haematopoietic enzyme. PGD_2 is dehydrated to produce the J series of PG (PGJ_2 analogues). PGJ_2 acts as a ligand for the PPARγ nuclear receptor, which is involved in the differentiation of adipocytes (Urade and Eguchi, 2002). PPARγ is also expressed in endothelial and smooth muscle cells (Jackson *et al.*, 1999; Law *et al.*, 2000). Two PGD_2 receptors, DP1 and DP2, have been identified, the latter being also known as CRTH2, a member of the chemokine receptor family (Hirai *et al.*, 2001; Figure 1). Activation of DP1 increases blood flow and vascular permeability (Smyth *et al.*, 2009). PGD_2 can produce not only endothelium- and NO-dependent relaxations (Abran *et al.*, 1997) but also endothelium-independent contractions, the latter effect involving activation of TP receptors (Gluais *et al.*, 2005; Figure 2).

PGE_2 and EP receptors

PGE_2, the most abundant PG in the human body, is involved in multiple physiological effects including gastric secretion and motility, bone formation, inflammation, female reproductive function, body temperature, sleep–wake activity and the regulation of kidney functions and blood pressure (glomerular filtration rate, tubular salt and water transports, renin release, renal blood flow and vascular tone) (Park *et al.*, 2006; Sugimoto and Narumiya, 2007; Legler *et al.*, 2010). The diverse effects of PGE_2 (for instance, this PG can produce both relaxation and contraction of vascular smooth muscle) can be attributed to the existence of four receptor subtypes, namely EP1, EP2, EP3 (of which three splice variants exist in the mouse and at least eight in the human) and EP4, which are coupled to different signalling pathways (Kobayashi and Narumiya, 2002; Hata and Breyer, 2004; Sugimoto and Narumiya, 2007; Figure 1).

Three distinct PGE synthases, responsible for the synthesis of PGE_2, have been identified and characterized, one cytosolic, cPGES, and two membrane bound, mPGES-1 and mPGES-2 (Kudo and Murakami, 2005). cPGES is a constitutive enzyme expressed ubiquitously and in abundance in many tissues and cell types. This cytosolic form is preferentially associated with COX-1, suggesting that this isoform contributes physiologically to the production of PGE_2 (Murakami *et al.*, 2002; Park *et al.*, 2006), and its function is thought to overlap that of COX-1. Knockout mice studies demonstrate that the deletion of cPGES is lethal (Hara *et al.*, 2010). The mPGES-1 is localized in perinuclear membranes, is up-regulated in response to stimuli that induce COX-2 expression and is associated preferentially with COX-2. In the vascular wall, mPGES-1 is also expressed by both endothelial and vascular smooth muscle cells (Murakami *et al.*, 2002). Cells from mice deficient in mPGES-1 display impaired LPS-stimulated PGE_2 generation, but the basal formation, which is cPGES-dependent, is preserved. mPGES-1 plays a critical role in fever, inflammation, pain, gastrointestinal and renal homeostasis as well as in tumorigenesis (Hara *et al.*, 2010). Additionally, the deletion of the mPGES-1 gene does not affect arterial blood pressure or thrombogenesis but delays atherogenesis, possibly by diverting the metabolism of PGH_2 towards prostacyclin (Cheng *et al.*, 2006; Wang *et al.*, 2006b) and prevents angiotensin-II-induced oxidative stress and aortic aneurysm formation (Wang *et al.*, 2008). Conversely, mPGES-1 deficient mice show adverse left ventricular remodelling after myocardial infarction (Degousee *et al.*, 2008) and can, depending on the model, develop severe hypertension when subjected to a high-salt diet (Jia *et al.*, 2006; Francois *et al.*, 2007). mPGES-2 is a constitutive, Golgi membrane–associated protein that can be coupled to both COX-1 and COX-2 (Kudo and Murakami, 2005). The real significance of its contribution in PGE_2 production under physiological or pathophysiological conditions is questionable since knockout of this enzyme does not result in a specific phenotype or a reduction in PGE_2 generation (Hara *et al.*, 2010).

Although PGE_2 is the common ligand of the four receptor subtypes, the amino acid homology among these receptors is limited. The stimulation of the EP1 receptor is predominantly coupled with an increase in intracellular calcium concentration (calcium channel gating and phospholipase C–dependent IP_3 formation), that of EP2 and EP4 with an increase in cAMP (activation of adenylyl cyclase) and that to EP3 with a decrease in cAMP (inhibition of adenylate cyclase) (Sugimoto and Narumiya, 2007; Legler *et al.*, 2010). In the rat aorta, the mRNAs of the four receptor subtypes are expressed, and in various rat arteries, EP1 activation is associated with contraction (Bolla *et al.*, 2004; Tang *et al.*, 2008). The studies performed on mice knockout for these various receptor subtypes as well as experiments with subtype selective agonists and antagonists indicate that the EP1 receptor is associated with stress responses and hyperalgesia; the EP2 receptor with ovulation and fertilization as well as pain and inflammation; the EP3 with fever, angiogenesis, duodenal secretion and pain; and the EP4 receptor with the closure of the ductus arteriosus, bone formation and possibly vascular headache in migraine as well as protection against inflammation (bowels) and generation of inflammation (arthritis) (Sugimoto and Narumiya, 2007; Jones *et al.*, 2009).

As regards the cardiovascular role of EP receptor subtypes (Kobayashi and Narumiya, 2002), the deletion of the various EP receptor genes show that in the kidney, PGE_2 stimulates renin release via the stimulation of EP2 and EP4 receptors, but that the four subtypes are involved in the control of renal vascular tone, with EP1 and EP3 increasing and EP2 and EP4 decreasing it (Schweda *et al.*, 2004). EP1-deficient mice have a urine concentration defect due to decrease in vasopressin release resulting in hypotension. EP1 receptor deletion reduces the elevated blood pressure and cardiac hypertrophy induced by angiotensin-II (Guan *et al.*, 2007; Smyth *et al.*, 2009). Paradoxically in EP1 knockout males, but not in female, the depressor response to exogenous administration of PGE_2 is reduced. However, in both genders, EP2 receptors contribute to the decrease in blood pressure produced by PGE_2 (Audoly *et al.*, 1999), and EP2 knockout mice develop salt-sensitive hypertension (Ma *et al.*, 2001). EP3 receptor–deficient mice have a bleeding tendency and are resistant to thromboembolism (Yuhki *et al.*, 2010). In male mice, EP3 receptor activation opposes the vasodepressor response to PGE_2, while in female mice, EP4 receptors contribute also to the depressor action of PGE_2 (Audoly *et al.*, 1999). Endothelium- and NO-dependent vasodilatation to PGE_2 involves EP4 activation (Hristovska *et al.*, 2007). The germline disruption of the EP4 gene enhances infarct size in the heart following ischaemia–reperfusion (Xiao *et al.*, 2004), and specific deletion of EP4 in cardiomyocytes exacerbates the decline in cardiac function observed after myocardial infarction (Smyth *et al.*, 2009).

Prostaglandin $F_{2\alpha}$ and FP receptors

Prostaglandin F_2 isoforms are synthesized not only from PGH_2 by the membrane-associated 9,11-endoperoxide reductase but also from PGD_2 and PGE_2 by cytosolic PGD_2 11-ketoreductase and PGE_2 9-ketoreductase (or in the endometrium by the 20α-hydroxysteroid deshydrogenase) respectively (Watanabe, 2002; Helliwell *et al.*, 2004).

Prostaglandin $F_{2\alpha}$ interacts with its preferential receptor the FP receptor (Figure 1), which generally generates an increase in the intracellular concentration of calcium. Prostaglandin $F_{2\alpha}$ is required in the female reproductive system for normal parturition. In the kidney, this PG is involved in water absorption, causing natriuresis and diuresis and, in the eye, is involved in the regulation of intraocular pressure. Prostaglandin $F_{2\alpha}$ is produced in the vascular wall, is a potent vasoconstrictor and may be involved in cardiac hypertrophy (Hata and Breyer, 2004; Jones *et al.*, 2009; Smyth *et al.*, 2009). Additionally, FP receptor can be expressed in endothelial cells and induce endothelium- and NO-dependent relaxations when stimulated (Chen *et al.*, 1995). Deletion of FP receptors reduces arterial blood pressure and delays atherogenesis in hyperlipidemic mice (Yu *et al.*, 2009).

Prostacyclin and IP receptor

Prostacyclin is an unstable substance formed by prostacyclin synthase, which belongs to the cytochrome P450 superfamily

(in the human CYP8A1) of enzymes. In endothelial cells, prostacyclin synthase is highly expressed (Tang and Vanhoutte, 2008), and the enzyme is closely associated with COX-1 (Kawka *et al.*, 2007). Prostacyclin synthase is also expressed in vascular smooth muscle cells, neurons, oviducts, intestinal epithelial cells and embryonic cells (Wu and Liou, 2005).

The deletion of this enzyme generates hypertensive mice with thickening and sclerosis of the arterial wall and with kidney infarction associated with interstitial fibrosis and nephrosclerosis (Smyth and FitzGerald, 2002; Wu and Liou, 2005). Conversely, overexpression of prostacyclin synthase, alone or in association with COX-1, prevents injury-induced intimal hyperplasia, pulmonary hypertension and vascular remodelling and protects brain tissue from ischaemia–reperfusion injury (Wu and Liou, 2005). Prostacyclin synthase is amongst the most sensitive targets of peroxynitrite and is inactivated by concentrations as low as 50 nM (Zou *et al.*, 2002a,b; Schmidt *et al.*, 2003). When PGI_2 synthase is inactivated, the excess PGH_2 is shunted towards other metabolic pathways leading to a variety of products that are, in general, deleterious to vascular function (Gluais *et al.*, 2005).

Prostacyclin is not only a potent platelet inhibitor preventing their aggregation and their adhesion to the endothelial cell surface and an endothelium-derived vasodilator (Moncada *et al.*, 1976; 1977; Moncada and Vane, 1979; Radomski *et al.*, 1987a,b) but is also involved in renal function by regulating renal blood flow, glomerular filtration rate and renin release. In addition, prostacyclin is involved the development, transport and implantation of the embryo, in pain tolerance, in gastric acid secretion, and in intracelullar signalling by interacting with nuclear receptors and regulating gene transcription (Smyth and FitzGerald, 2002; Wise, 2003; Wu and Liou, 2005; Smyth *et al.*, 2009).

Prostacyclin is the preferential ligand of IP receptors (Figure 1), and its effects often, but not always, involve the activation of adenylyl cyclase and the subsequent elevation of intracellular cyclic AMP (Wise and Jones, 1996; Clapp *et al.*, 2002; Wise, 2003; Orie *et al.*, 2006). The genetic deletion of IP receptors is associated with increased injury-induced restenosis (Cheng *et al.*, 2002), thrombotic events (Murata *et al.*, 1997), atherosclerosis (Egan *et al.*, 2004; Kobayashi *et al.*, 2004) and reperfusion injury (Xiao *et al.*, 2001). Some of the negative effects observed with IP receptor deletion, including restenosis and enhanced platelet activation, can be abrogated by coincidental TP receptor deletion indicating that prostacyclin regulates the cardiovascular effects of thromboxane A2 (Fetalvero *et al.*, 2007). A cross-talk with the two prostanoid binding sites may involve the heterodimerization of these two receptors. TP receptor agonists stimulating these heterodimers would produce accumulation of cAMP. This and regulation of receptor endocytosis and trafficking would explain some of the braking effects of IP receptors on the cellular effects of TP receptor activation (Wilson *et al.*, 2004; 2007). In humans, a defect in prostacyclin receptor signalling, as observed in patients with a dysfunctional IP receptor mutation, leads to accelerated atherothrombosis (Arehart *et al.*, 2008). The accelerated cardiovascular disease associated with this IP^{R212C} mutation is observed in individuals carrying only one copy of the variant allele and thus can be attributed to a dominant-negative action not only when dimerized with the wild-type IP receptor but also through dimerization with the TP receptor; activation of such dimers no longer leads to cAMP production (Ibrahim *et al.*, 2010).

Selective and potent agonists and antagonists of the IP receptors have been synthesized. IP receptor antagonists could be of interest for the treatment of pain, inflammation and overactive bladder providing that cardiovascular side effects could be avoided (Jones *et al.*, 2009). IP receptor agonists are prescribed for pulmonary hypertension but are currently administered intravenously or by inhalation and are associated with serious side effects. The recent synthesis of orally active and long-acting IP receptor agonists may prove beneficial not only in pulmonary hypertension but possibly also in atherosclerosis obliterans (Fetalvero *et al.*, 2007; Kuwano *et al.*, 2007).

Thromboxane A_2 and TP receptors

Thromboxane A_2 is a highly unstable intermediate enzymatically produced from PGH_2 as a substrate by thromboxane synthase, which belongs also to the cytochrome P450 superfamily (in the human, CYP5; Yokoyama *et al.*, 1991). Thromboxane A_2 not only induces platelet aggregation and contraction of vascular smooth muscle but is also involved in allergies, modulation of acquired immunity, atherogenesis, neovascularization and metastasis of cancer cells (Nakahata, 2008).

Thromboxane A_2 is the preferential ligand of TP receptors (Figure 1), but PGH_2 and higher concentrations of other PGs, isoprostanes and hydroxyeicosatetraenoic acids (HETEs) can activate them with a various range of potency (Félétou *et al.*, 2010b; Figure 2). By contrast, epoxyeicosatrienoic acids (EETs), which act as endothelium-derived hyperpolarizing factors in some vascular beds (Félétou and Vanhoutte, 2006b), and their dihydro-derivatives (DiHETs) are endogenous antagonists of TP receptors (Behm *et al.*, 2009). The transduction signal associated with TP receptor activation involves mainly two types of G-proteins, the Gq and the G13 families, resulting in the stimulation of phospholipase C and RhoGEF respectively. In humans, but not in rodents, two alternatively spliced isoforms of TP, TPα and TPβ, have been described, which differ only in their C-terminal portions. Both isoforms are coupled to phospholipase C activation, but TPα stimulates adenylyl cyclise, whereas TPβ inhibits it, at least in transfected cells (Hirata *et al.*, 1996). Furthermore, TP receptors can be engaged in cross-talks with receptor tyrosine kinases, such as the EGF receptor, to induce cell proliferation and differentiation (Nakahata, 2008). Reactive oxygen species enhance the stability and increase the density of functional TP receptors at the cell membrane (Valentin *et al.*, 2004; Wilson *et al.*, 2009), and, in endothelial cells, the activation of TP receptors inhibits NO production (Liu *et al.*, 2009). Metabolically stable agonists of TP receptors with high affinity and potency have been synthesized and numerous synthetic TP antagonists have been designed including S 18886 (terutroban), which is an orally active, potent and selective antagonist of TP receptors (Simonet *et al.*, 1997).

Mice genetically deficient in TP receptors are normotensive but have abnormal vascular responses to thromboxane A_2 and show a tendency to bleeding (Thomas *et al.*, 1998).

The deletion of TP receptors decreases vascular proliferation and platelet activation in response to intimal lesions (Cheng *et al.*, 2002), delays atherogenesis in apoE$^{-/-}$ mice (Kobayashi *et al.*, 2004), prevents angiotensin-II- and L-NAME-induced hypertension and the associated cardiac hypertrophy but reduces the extent of kidney injury only in the former hypertensive model (Francois *et al.*, 2004; 2008). TP receptor knockout mice are also protected against various lipopolysaccharide-induced responses such as the increase in iNOS expression (Yamada *et al.*, 2003), acute renal failure (Boffa *et al.*, 2004) and inflammatory tachycardia (Takayama *et al.*, 2005).

The phenotype of thromboxane A_2 synthase knockout mice is much less pronounced (Yu *et al.*, 2004) most likely because thromboxane A_2 is only one of the endogenous agonists of TP receptor and also because the deletion of this enzyme may redirect the arachidonic cascade towards less pathogenic synthases.

COX products and endothelium-dependent relaxations

Since inhibitors of COX abolish the basal and stimulated generation of PGI_2, and potent and selective antagonists of the IP receptor block vasodilator responses that it causes (Gluais *et al.*, 2005; Gomez *et al.*, 2008), the contribution of PGI_2 in endothelium-dependent responses can be assessed. This PG plays a role in flow-mediated vasodilatation (Koller *et al.*, 1993; Duffy *et al.*, 1998), but its contribution to acute endothelium-dependent relaxations in response to neurohumoral mediators is often considered as minimal, because COX inhibitors, in particular indomethacin, do not affect these responses. However, the role of prostacyclin as an endogenous mediator of endothelium-derived relaxation has been generally overlooked since in some vascular beds, a major vasodilator effect of COX derivatives can only be observed when the other pathways leading to endothelium-dependent relaxations have been inhibited (Corriu *et al.*, 1996; Zygmunt *et al.*, 1998). The contribution of PGI_2 to endothelium-dependent responses is increased in eNOS knockout mice (Chataigneau *et al.*, 1999; Sun *et al.*, 1999). Similarly, in human with cardiovascular diseases, COX-2-derived PGs can play a compensatory role for the decreased NO bioavailability (Bulut *et al.*, 2003; Szerafin *et al.*, 2006) possibly explaining some of the detrimental cardiovascular effects associated with COX-2 inhibitors (Andersohn *et al.*, 2006).

The vascular relaxation to PGI_2, or its synthetic analogues, is often associated with a concomitant hyperpolarization of the smooth muscle cells, which, depending on the blood vessels and the species, can involve the opening of various populations of potassium channels (Corriu *et al.*, 2001; Félétou and Vanhoutte, 2007). Therefore, in numerous vascular beds, PGI_2 can act also as an endothelium-derived hyperpolarizing substance (Parkington *et al.*, 2004).

Although PGE_2 can be a potent vasodilator and is acutely released by endothelial cells (Gluais *et al.*, 2005), there is little evidence for a role of this PG in endothelium-dependent relaxations, at the possible exception of some rabbit veins (Rouaud *et al.*, 1999). The concomitant activation of contractile EP1 and/or EP3 receptors could mask a potential relaxing effect of endothelium-derived PGE_2 in other blood vessels.

COX products and endothelium-dependent contractions

COX-1

Hypertension. Endothelium-dependent contractions, associated with endothelial dysfunctions, were observed first in the isolated aorta of spontaneously hypertensive rats (SHR; Luscher and Vanhoutte, 1986). They have been extensively characterized thereafter in that blood vessel. In the arteries of this hypertensive model, the generation of a diffusible EDCF opposes the relaxing effect of NO. Endothelium-dependent contractions are positively correlated with the severity of hypertension and the aging process, are delayed in female SHR and also occur in aging normotensive Wistar–Kyoto control rats (WKY; Félétou *et al.*, 2009).

In SHR aorta, endothelium-dependent contractions are associated with multiple dysfunctions in both the endothelial and the smooth muscle cells. In the endothelial cells, they include (i) abnormal calcium handling, (ii) an increased expression of COX-1, (iii) the associated enhanced production of reactive oxygen species, (iv) a major increase in prostacyclin synthase expression, (v) the enhanced release of prostacyclin, thromboxane A_2, and possibly PGH_2. In vascular smooth muscle cells, they include (i) an exacerbated response of the TP receptor to prostacyclin and PGH_2, (ii) a deficient IP receptor function and (iii) an early dysfunction in the adenylyl cyclase pathway (Félétou *et al.*, 2009; 2010a,b).

When compared with WKY aorta and in response to receptor-mediated stimuli (acetylcholine), the amplitude of the endothelium-dependent contractions and the increase in intracellular calcium ($[Ca^{2+}]_i$) in SHR endothelial cells are exacerbated while in response to receptor-independent stimuli (calcium ionophore, A 23187) the maximal amplitude of the endothelium-dependent contractions and the changes in $[Ca^{2+}]_i$ in both strains are similar (Gluais *et al.*, 2005; 2006; Tang *et al.*, 2007). Any event leading to an increase in endothelial $[Ca^{2+}]_i$, activates the calcium-dependent phospholipase A_2 ($cPLA_2$) and provokes the mobilization of arachidonic acid. However, in response to receptor-dependent stimuli, the activation of the calcium-independent phospholipase A_2 ($iPLA_2$) allows the store-operated calcium channels (SOC)–dependent influx of extracellular calcium and the subsequent activation of $cPLA_2$. It mediates the initial part of the signalling cascade leading to endothelium-dependent contractions of the SHR aorta in response to acetylcholine. Substances, such as calcium ionophores, that bypass the cell membrane receptors causes an increase in $[Ca^{2+}]_i$, and a direct activation of $cPLA_2$ (Wong *et al.*, 2010b). Therefore, the $iPLA_2$ pathway associated with calcium mobilization is defective in SHR endothelial cells (Figure 4).

The subsequent steps involve the activation of COX and the production of reactive oxygen species along with that of prostanoids. Aortic endothelial cells express preferentially COX-1 versus COX-2 (Kawka *et al.*, 2007; Tang and Vanhoutte, 2008). In SHR endothelial cells, the mRNA and

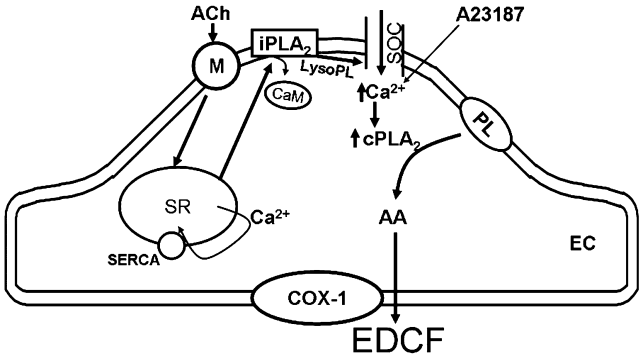

Figure 4

Calcium signalling and the COX-1 production of endothelium-derived contracting factors (EDCF). Acetylcholine (ACh) activates muscarinic receptors (M) on the endothelial cell membrane and triggers the release of calcium from intracellular stores. The resulting calcium depletion process displaces the inhibitory calmodulin (CaM) from iPLA2. Activated iPLA2 produces lysophospholipids (LysoPL), which in turn open store-operated calcium channels (SOCs) leading to the influx of extracellular calcium into the endothelial cells. This large influx of calcium ions then activates cPLA2, which catalyses the production of arachidonic acids (AA). The later is then metabolized by cyclooxygenase-1 (COX-1) to prostanoids. cPLA2, calcium dependent phospholipase A2; EC, endothelial cells; iPLA2, calcium independent phospholipase A2 (modified from Wong and Vanhoutte, 2010).

protein expression of COX-1 are enhanced when compared with that of WKY, and in the two strains, both are augmented by aging (Ge *et al.*, 1995; Tang and Vanhoutte, 2008). Conversely, in SHR, the decrease expression of COX-1 produced by a chronic treatment with vitamin D reduces the endothelium-dependent contractions (Wong *et al.*, 2010a). In response to acetylcholine, endothelium-dependent contractions and the associated generation of PGs are blocked consistently by selective inhibitors of COX-1 and partially inhibited, although to various extent depending on the experimental conditions, by selective inhibitors of COX-2 (Ge *et al.*, 1995; Yang *et al.*, 2003a; Gluais *et al.*, 2005; 2006). However, if the endothelium-dependent contractions and the release of PGs by A 23187 are also fully blocked by COX-1 inhibitors, these responses are less sensitive to COX-2 inhibition (Figure 5). This could possibly be explained by the fact that low concentrations of arachidonic acid are preferentially oxygenated by COX-2, while higher ones are preferentially metabolized by COX-1 (Morita, 2002). Alternatively, the effects observed with the COX-2 inhibitors could nevertheless be attributed to COX-1 inhibition. Indeed, the ability of COX-2 inhibitors to inhibit COX-1 depends obviously not only on the degree of selectivity of any given inhibitor but also on other factors such as substrate availability, endogenous lipid peroxide levels and plasma protein concentration, explaining why COX-2 inhibitors are systematically more potent in preventing the endothelial production of PGI_2 than the platelet production of thromboxane A_2 (Mitchell *et al.*, 2006; Warner *et al.*, 2006). 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 (Tang *et al.*, 2005).

Additionally, COX is also involved in the endothelial generation of reactive oxygen species, a key factor in the generation of endothelium-dependent contractions (Yang *et al.*, 2002; Tang *et al.*, 2007). Reactive oxygen species decrease NO bioavailability (Gryglewski *et al.*, 1986; Rubanyi and Vanhoutte, 1986) and, as a positive feedback loop, the formation of hydroperoxides further activates COX (Morita, 2002). In addition, since reactive oxygen species diffuse towards the vascular smooth muscle cells, they can stimulate COX in these cells and produce more contractile prostanoids.

The generated PGs diffuse towards the vascular smooth muscle cells and directly activate TP receptors (Luscher and Vanhoutte, 1986; Auch-Schwelk *et al.*, 1990; Yang *et al.*, 2003a). In the rat aorta, the five major PGs and 8-isoprostane produce contractions that predominantly involve TP receptor activation (Figure 2). However, the involvement of PGD_2 and 8-isoprostane in endothelium-dependent contractions can be ruled out since their generation is not affected by acetylcholine (Gluais *et al.*, 2005).

In SHR aortic endothelial cells, the expression of thromboxane synthase is enhanced when compared with that in WKY endothelium (Tang and Vanhoutte, 2008). In response to ATP or the calcium ionophore A 23187, this is associated with an increase generation of thromboxane A_2, and the endothelium-dependent contractions are partially inhibited by dazoxiben, a selective inhibitor of thromboxane synthase that abrogates the production of thromboxane A_2 (Gluais *et al.*, 2006; 2007). By contrast, acetylcholine produces only a minor dazoxiben-sensitive increase in thromboxane A_2 production, and the endothelium-dependent contractions that it evokes are not affected by the presence of the thromboxane synthase inhibitor, indicating that thromboxane A_2 is only one of the EDCFs that can be released from SHR aortic endothelial cells (Koga *et al.*, 1989; Kato *et al.*, 1990; Ge *et al.*, 1995; Gluais *et al.*, 2005; 2006; 2007).

Paradoxically, prostacyclin is likely to be a major EDCF in SHR aorta. In SHR endothelial cells, prostacyclin is by far the most abundant PG released in response not only to receptor-dependent stimuli but also to calcium ionophores (Gluais *et al.*, 2005; 2006; 2007). This may come as a surprise since prostacyclin synthase is rapidly nitrosylated and inactivated by peroxynitrite (Zou *et al.*, 2002a,b; Schmidt *et al.*, 2003). However, in the SHR aorta, the massive increase in the expression of prostacyclin synthase (Tang and Vanhoutte, 2008) may compensate the loss of activity due to peroxynitrite-dependent tyrosine nitration. Furthermore, in that preparation, prostacyclin does not produce relaxations but evokes TP receptor–dependent contractions (Rapoport and Williams, 1996; Gluais *et al.*, 2005; Figure 2). In fact, prostacyclin, like PGH_2, is also more potent in producing contraction in SHR than in WKY aortae (Ge *et al.*, 1995; Gluais *et al.*, 2005). The absence of relaxation in response to prostacyclin is attributed to an early (as young as 12 weeks old) dysfunction of the IP receptors of vascular smooth muscle. This dysfunction is tissue specific since the platelet response to prostacyclin (or its analogues) is unaffected or even enhanced (Anand-Srivastava, 1993; Gomez *et al.*, 2008). In order to explain this specific smooth muscle cell dysfunction, a decrease in the aortic expression of IP receptors (Numaguchi *et al.*, 1999) and

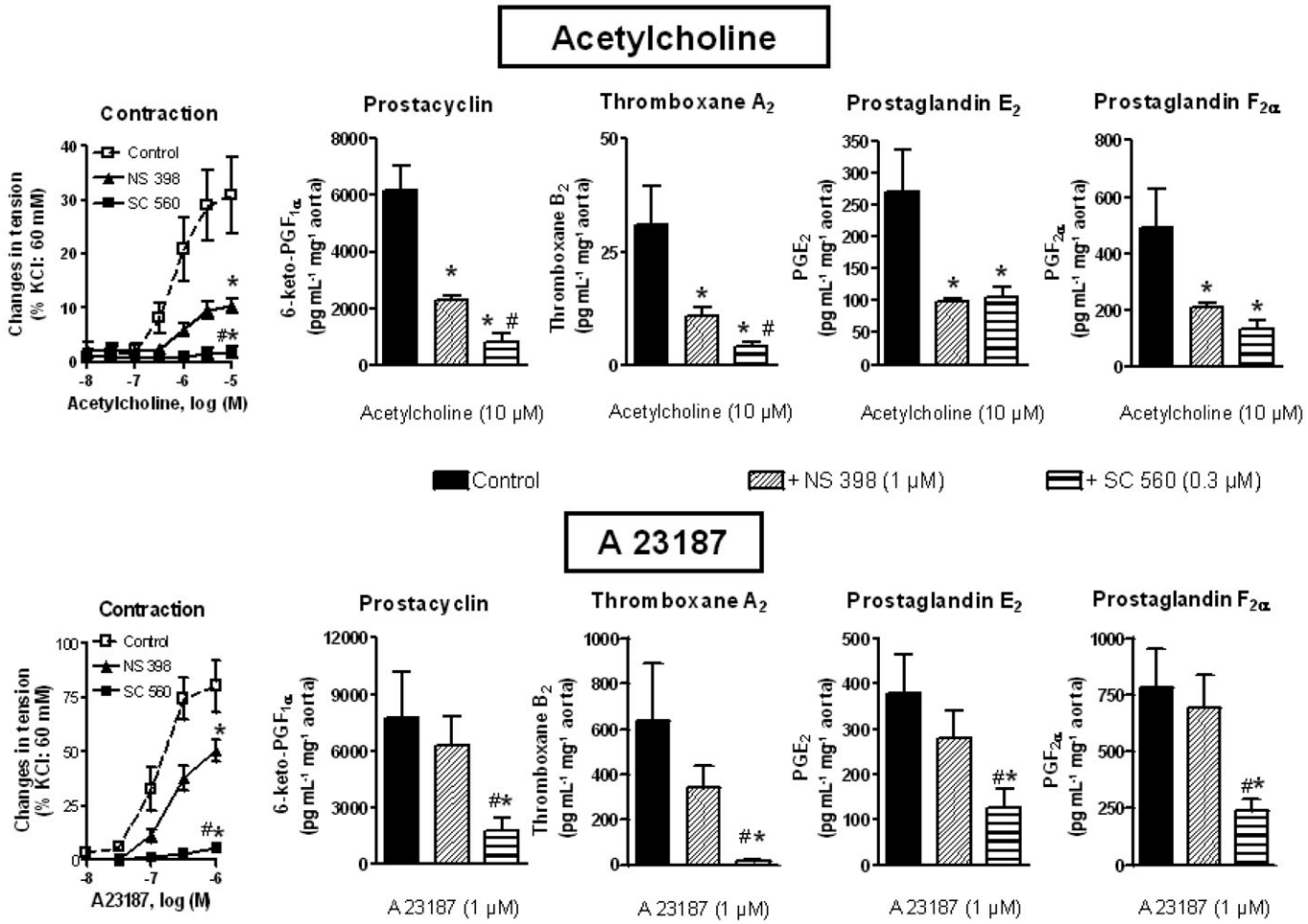

Figure 5

Effects of inhibitors of COX-1 and COX-2 on endothelium-dependent contractions and prostaglandins production in SRH aortic rings. Top panels: effects of acetylcholine. Lower panels: effects of A 23187. The effects of A23187 are less sensitive to the COX-2 inhibitor, NS 398, than those produced by acetylcholine. Data are shown as means ± SEM. The asterisk indicates a statistically significant effect of a COX inhibitor. The sharp sign indicates that the response in presence of the COX-1 inhibitor, SC 560, is significantly different from the response observed in the presence of NS 398. COX, cyclooxygenase; SHR, spontaneously hypertensive rat.

an early impairment of adenylyl cyclase signalling have been evoked (Anand-Srivastava, 1988; Masuzawa *et al.*, 1989). However, these two hypotheses can only, at best, partially explain the total disappearance of IP receptor–mediated relaxations in SHR aorta. Indeed, the decrease expression of the IP receptor has not been confirmed in latter experiments (Tang and Vanhoutte, 2008), and when compared with WKY, the relaxations to prostacyclin in SHR aorta are much more severely affected than those produced by other agents that stimulate adenylyl cyclase, such as isoproterenol and forskolin (Gomez *et al.*, 2008). A potential additional/alternative hypothesis, which requires proper validation, could be the oxidative damage of the IP receptor itself, which contain redox-sensitive cysteines that play an essential role in determining its structure, addressing and function (Stitham *et al.*, 2006).

Prostacyclin has also been identified as a major contributing factor accounting for the endothelial dysfunction in the aorta and mesenteric artery of WKY and SHR treated with aldosterone (Blanco-Rivero *et al.*, 2005; Xavier *et al.*, 2008). Thus, although as a rule prostacyclin is a vasodilator and an anti-aggregating agent, depending on the circumstances, the prostanoid can also act as an EDCF.

Any levels of prostacyclin synthase inactivation would theoretically lead to an excess of free PGH_2. Since PGH_2 is the second most potent agonist at TP receptors and is more effective in activating TP receptors in vascular smooth muscle from SHR than in that of WKY, the endoperoxide is also a suitable candidate as EDCF (Kato *et al.*, 1990; Ge *et al.*, 1995; Gluais *et al.*, 2005; Figure 2). Finally, the shunting of PGH_2 metabolism towards other metabolic pathways can lead to a variety of products, including PGE_2 and/or $PGF_{2\alpha}$, which also produce contractions by activating TP receptors (Figure 2). Therefore, thromboxane A_2, PGH_2, PGI_2, PGE_2 and $PGF_{2\alpha}$ can all act theoretically as EDCF (Gluais *et al.*, 2005; Félétou *et al.*, 2010a,b).

In addition, in the SHR aorta, PGE_2-mediated relaxations are impaired, which could contribute to the observed endot-

helial dysfunction (Tang *et al.*, 2008) and, in the femoral artery of diabetic rats, activation of the EP1 receptor contributes to the endothelium-dependent contractions (Shi *et al.*, 2007).

Furthermore, some alterations at the level of the TP receptors should also be considered. Hydrogen peroxide prevents the translocation and degradation of TP receptors, increasing their density at the cell membrane and TP activation enhances TP stability through a reactive oxygen species–dependent post-transcriptional mechanism (Valentin *et al.*, 2004; Wilson *et al.*, 2009). This may explain the enhanced TP receptor–dependent contractions in response to PGH_2, prostacyclin and exogenously generated reactive oxygen species observed in SHR aorta (Auch-Schwelk *et al.*, 1989; Ge *et al.*, 1995; Yang *et al.*, 2002; 2003b, Gluais *et al.*, 2005; García-Redondo *et al.*, 2009). In addition, TP receptors are also expressed in endothelial cells and their stimulation induces the Rho kinase–dependent inhibition of NO production (Liu *et al.*, 2009). Conversely, the isoform α of the human TP receptor is negatively and independently regulated by either NO or prostacyclin, following the phosphorylation of serine residues by protein kinase G and A respectively (Reid and Kinsella, 2003). Additionally, NO can inhibit the activity of thromboxane synthase (Wade and Fitzpatrick, 1997), indicating that a decrease in NO bioavailability may facilitate the TP receptor–dependent signalling pathway. Finally, EDCF- and TP-mediated responses, first observed in the aorta of the SHR, are not ubiquitous in SHR arteries but have been reported in other vascular territories such as the mesenteric, skeletal muscle and renal vascular beds (Félétou *et al.*, 2009). In these peripheral arteries, the endothelial dysfunction additionally includes a marked attenuation of the EDHF-mediated component of the endothelium-dependent relaxations (Félétou and Vanhoutte, 2006b). TP receptor stimulation induces a loss in the activity of endothelial small conductance calcium-activated potassium channels (Crane and Garland, 2004; McNeish and Garland, 2007), an essential component of EDHF-mediated responses (Félétou and Vanhoutte, 2006b). Conversely, the impairment of EDHF-mediated responses can favour the development of endothelium-dependent contractions (Michel *et al.*, 2008).

COX-2

COX-2 is traditionally believed to be the major generator of the vasodilator PGI_2, which functions especially when NO bioavailability is diminishing. Endothelial dysfunction is reversed by a complementary up-regulation of COX-2 expression and activity in the mesenteric vascular bed of mice with streptozotocin-induced diabetes (Nacci *et al.*, 2009), while attenuated NO-dependent vasodilatations in diabetic patients may be compensated for by the emerging vasodilator effect of prostacyclin (Meeking *et al.*, 2000). Compared with patients with non-documented diabetes, coronary arterioles from diabetic patients show a significant up-regulation of COX-2 expression, which contributes to enhanced bradykinin-induced vasodilatations (Szerafin *et al.*, 2006). The availability of selective COX-2 inhibitors and genetically engineered mice allow a more in-depth investigation on the emerging role of COX-2 as an inflammatory mediator releasing vasoconstrictors in hypertension, diabetes and aging.

Hypertension and diabetes. Vasculopathies are the leading causes of morbidity and mortality in hypertensive and diabetic patients. In addition to COX-1, COX-2 also can generate vasoconstrictor prostanoids in the SHR endothelial cells. In both WKY and SHR endothelial cells, the induction of COX-2, not only in the aorta but also in resistance arteries, is accelerated by aging and can be associated with the generation of endothelium-derived contractile prostanoids. (Heymes *et al.*, 2000; Alvarez *et al.*, 2005; Blanco-Rivero *et al.*, 2005; Shi *et al.*, 2008; Virdis *et al.*, 2009). For instance, in WKY rats, the impairment of the aortic endothelium-dependent relaxations, observed after a chronic treatment with fenofibrate, has been attributed to the endothelial release of COX-2-derived PGE_2 acting on smooth muscle TP receptors (Blanco-Rivero *et al.*, 2007). In L-NAME-hypertensive rats, the up-regulation of COX-2 enhances the release of EDCFs (Qu *et al.*, 2010). Additionally, the release of COX-2-derived $PGF_{2\alpha}$ and 8-isoprostane augments α-adrenoceptor-induced contractions in SHR arteries (Alvarez *et al.*, 2005), and COX-2 also mediates vasoconstrictions in response to *tert*-butyl hydroperoxide, a product of lipid peroxidation (Garcia-Cohen *et al.*, 2000). Elevated COX-2 expression contributes to deoxycorticosterone acetate salt–induced hypertension, which is reversed by intra-peritoneal administration of the preferential COX-2 inhibitor NS-398 (Adeagbo *et al.*, 2005). Recent findings of Wong *et al.* (2010c) further support the pathological role of COX-2 in endothelial dysfunction in hypertension. Bone morphogenic protein 4 (BMP4) and COX-2 are elevated in the renal arteries of hypertensive patients. BMP4, by increasing NADPH oxidase–derived reactive oxygen species and activating p38 mitogen-activated protein kinase, up-regulates COX-2 in the murine aorta, resulting in the impairment of endothelium-dependent relaxations and appearance of endothelium-dependent contractions (Figure 6). A similar association is observed in SHR intrarenal arteries and renal arteries from hypertensive patients, in which both noggin (BMP4 antagonist) and celecoxib (selective COX-2 inhibitor) normalize the relaxations and/or abolish endothelium-dependent contractions.

While ROS up-regulate the expression and activity of COX-2, the latter in turn can increase oxidative stress in the vascular wall. Thus, COX-2-derived $PGF_{2\alpha}$ and superoxide anions underpin the diminished acetylcholine-induced relaxations in perfused mesenteric arteries of female Wistar rats with alloxan-induced diabetes (Akamine *et al.*, 2006). Mesenteric arteries of type 2-diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats exhibit attenuated endothelium-dependent relaxations and enhanced acetylcholine-induced contractions, attributed to the elevated expression of COX-1 and COX-2 and the release of thromboxane A_2 and PGE_2 (Matsumoto *et al.*, 2007); these abnormal responses are reversed in part by chronic oral treatment with eicosapentaenoic acid (omega-3 fatty acid), metformin (oral anti-diabetic drug), pyrrolidine dithiocarbamate (thiol antioxidant) or ozagrel (thromboxane synthase inhibitor). The reversals are due to the suppression of extracellular signal-regulated kinase (ERK) and nuclear factor-κB (NF-κB)–mediated COX-2 expression (Matsumoto *et al.*, 2008; Matsumoto *et al.*, 2009a,b,c).

Nephropathies and renovascular abnormalities are common in diabetic patients (Mogensen and Schmitz, 1988;

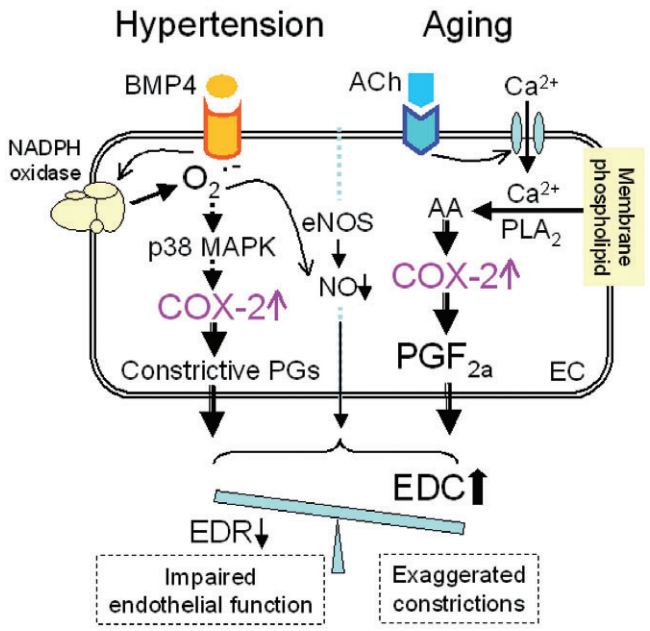

Figure 6

Exaggerated role of COX-2 in hypertension and aging. In hypertension, BMP-4 expression is elevated, resulting in the up-regulation of COX-2 expression and activity via NAPDH oxidase-mediated generation of reactive oxygen species and the subsequent activation of p38 MAPK. During aging, COX-2 expression, release of and vascular contractility to the COX-2-derived $PGF_{2\alpha}$ are augmented. Under these conditions, NO bioavailability is diminished, thus favouring the emergence of the exaggerated endothelium-dependent contractions. AA, arachidonic acid; ACh, acetylcholine; BMP4, bone morphogenic protein 4; COX-2, cyclooxygnease-2; EC, endothelial cells; EDC, endothelium-dependent contraction; EDR, endothelium-dependent relaxation; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; PG, prostaglandin; PLA_2, phospholipase A_2.

Kamgar *et al.*, 2006). Both type 1 and type 2 diabetic rats exhibit exaggerated renal cortical expression and activity of COX-2, accompanied by pathological haemodynamic alterations (Komers *et al.*, 2001; 2005). Preliminary data in human renal arteries from diabetic subjects reveal acetylcholine- and $PGF_{2\alpha}$-induced vasoconstrictions, whereby the muscarinic agonist stimulates the celecoxib-sensitive liberation of $PGF_{2\alpha}$ (Wong *et al.*, 2009), indicating a pivotal role of COX-2 in diabetic renovascular pathologies in humans.

Aging. COX-2 can play a constitutive role in the regulation of vascular tone. Thus, L-NAME-treated aortic rings from young hamsters show COX-2- and TP receptor–mediated endothelium-dependent contractions. In that preparation, endothelial COX-2 is constitutively expressed and generates $PGF_{2\alpha}$ (Wong *et al.*, 2009). With aging, diminished NO-mediated endothelium-dependent relaxations favour the appearance of COX-dependent vasoconstrictions. Studies on aortae from old hamsters show that aging not only enhances COX-2-dependent endothelium-dependent contractions but also the expression of the isoform as well as the release of and the vascular responsiveness to $PGF_{2\alpha}$ (Wong *et al.*, 2009). Due to a reduced NO bioavailability (as reflected by attenuated endothelium-dependent relaxations) in the aorta of the aged hamster, endothelium-dependent contractions can be observed under physiological conditions, that is in the absence of pharmacological inhibition of eNOS (Wong *et al.*, 2009). The preferential COX-2 inhibitor NS-398 also restores normal endothelium-dependent relaxations and abolishes acetylcholine-induced contractions in small mesenteric arteries from aged rats (Alvarez de Sotomayor *et al.*, 2007). Apparently, both COX-1 and COX-2 contribute to the exaggerated endothelium-dependent contractions to the calcium ionophore A23187 in femoral arteries from aged rats (Shi *et al.*, 2008). Indeed, mRNA levels of COX-1, COX-2, thromboxane synthase, PGF synthase, haematopoietic-type PGD synthase and membrane PGE synthase-2 are all augmented in endothelial cells from aged rats (Tang *et al.*, 2008), further indicating an increasing importance of the arachidonic acid–COX cascade during aging.

COX-2 derived PGF2α acting on TP receptor. Although $PGF_{2\alpha}$ is the natural agonist of the FP receptor, TP receptors appear to be the preferential cellular target of $PGF_{2\alpha}$ in inducing vasoconstrictions, possibly because of different relative expression levels of these receptors in the vasculature. Indeed, while TP receptors are well expressed in the hamster aorta and human renal arteries, FP receptors are nearly undetectable (Wong *et al.*, 2009). As a consequence and because of the chemical similarity between various prostanoids, when $PGF_{2\alpha}$ is released in sufficient quantities, TP receptor activation ensues, leading to vasoconstriction.

Conclusions and perspectives

Aging and cardiovascular diseases are associated with multiple endothelial dysfunctions, which often involve COX activation and TP receptor stimulation. Generally, the production of EDCFs does not significantly influence systemic arterial blood pressure, but TP activation amplifies the endothelial dysfunction (Félétou *et al.*, 2009).

In humans, the production of COX-derived EDCF is a characteristic of the aging blood vessels, with essential hypertension merely causing an earlier onset and an acceleration of this endothelial dysfunction (Taddei *et al.*, 1993; 1995; 1997a,b). In primary hypertensive patients, selective inhibition of COX-1 partially reverses the impairment of vasodilator responses to acetylcholine, while the selective inhibition of COX-2, which does not produce adverse effects in the forearm of healthy subjects (Verma *et al.*, 2001), further reduces the increase in forearm blood flow produced by the muscarinic agonist (Bulut *et al.*, 2003). These results indicate not only that COX-1-derived vasoconstrictor PGs contribute to the endothelial dysfunction, but that the production of vasodilator PGs by COX-2 is of minor importance in subjects with normal endothelial function but becomes relatively more important in hypertensive patients with endothelial dysfunction, presumably playing a beneficial compensatory role. By contrast, in patients with coronary artery disease, the impaired acetylcholine- and flow-induced forearm vasodilatation is restored by the administration of a TP receptor antagonist (Belhassen *et al.*, 2003). The fact that the patients

in this latter study were already treated with aspirin suggests that COX-2 activity, rather than COX-1, should be the main source of the vasoconstrictor prostanoids involved in this endothelial dysfunction. Indeed, in patients with severe coronary artery disease, COX-2 inhibition improved flow-mediated dilatation (Chenevard *et al.*, 2003). These results indicate that, as it has been observed in animal models, COX-1, COX-2 or both isoforms can contribute to endothelial dysfunctions. Since in most cases, the activation of TP receptors is the common downstream effector, selective antagonists of this receptor could be of therapeutic interest in the treatment of cardiovascular disorders.

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Conflict of interest

Michel Félétou is an employee of a pharmaceutical company, the 'Institut de Recherches Servier', currently developing a TP receptor antagonist. Paul M. Vanhoutte is a former employee and is a consultant for this company.

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