British Journal of Pharmacology (2009), 156, 563–574

0007-1188/09
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REVIEW

Endothelium-dependent contractions in SHR: a tale of prostanoid TP and IP receptors

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In the aorta of spontaneously hypertensive rats (SHR), the endothelial dysfunction is due to the release of endothelium-derived contracting factors (EDCFs) that counteract the vasodilator effect of nitric oxide, with no or minor alteration of its production. The endothelium-dependent contractions elicited by acetylcholine (ACh) involve an increase in endothelial $[Ca^{2+}]_i$, the production of reactive oxygen species, the activation of endothelial cyclooxygenase-1, the diffusion of EDCF and the subsequent stimulation of smooth muscle cell TP receptors. The EDCFs released by ACh have been identified as PGH_2 and paradoxically prostacyclin. Prostacyclin generally acts as an endothelium-derived vasodilator, which, by stimulating IP receptors, produces hyperpolarization and relaxation of the smooth muscle and inhibits platelet aggregation. In the aorta of SHR and Wistar-Kyoto rats, prostacyclin is the principal metabolite of arachidonic acid released by ACh. However, in SHR aorta, prostacyclin does not produce relaxations but activates the TP receptors on vascular smooth muscle cells and produces contraction. The IP receptor is not functional in the aortic smooth muscle cells of SHR as early as 12 weeks of age, but its activity is not reduced in platelets. Therefore, prostacyclin in the rule protects the vascular wall, but in the SHR aorta it can contribute to endothelial dysfunction. Whether or not prostacyclin plays a detrimental role as an EDCF in other animal models or in human remains to be demonstrated. Nevertheless, because EDCFs converge to activate TP receptors, selective antagonists of this receptor, by preventing endothelium-dependent contractions, curtail the endothelial dysfunction in diseases such as hypertension and diabetes.
British Journal of Pharmacology (2009) **156**, 563–574; doi:10.1111/j.1476-5381.2008.00060.x; published online 21 January 2009

Keywords: nitric oxide; prostaglandins; EDCF; oxidative stress; superoxide anion; hypertension; endothelium; smooth muscle; platelets; cyclooxygenase

Abbreviations: ACh, acetylcholine; COX, cyclooxygenase; DETCA, diethyldithiocarbamate acid; EDCF, endothelium-derived contracting factor; NOS, nitric oxide synthase; PGIS, prostacyclin synthase; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats

Introduction

Endothelial cells synthesize and release various factors that modulate vascular tone as well as angiogenesis, inflammatory responses, haemostasis and permeability. As a major regulator of local vascular homeostasis, the endothelium maintains the balance between vasodilatation and vasoconstriction, inhibition and promotion of the proliferation and migration of smooth muscle cells, prevention and stimulation of the adhesion and aggregation of platelets as well as thrombogenesis and fibrinolysis. Upsetting this tightly regulated balance leads to endothelial dysfunction. A reduced bioavailability of nitric oxide (NO), an alteration in the production of prostanoids (including prostacyclin, thromboxane A_2 and/or isoprostanes), an impairment of endothelium-dependent hyperpolarization as well as an increased release of endothelin-1 can individually or in association contribute to endothelial dysfunction (Félétou and Vanhoutte, 2006a). The present review focuses on the endothelial function observed in spontaneously hypertensive rats (SHR).

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Received 26 August 2008; revised 29 September 2008; accepted 2 October 2008

Endothelium-dependent contractions in SHR aorta

The endothelium-dependent relaxations are impaired in the aorta of hypertensive rats (Lockette *et al.*, 1986; Luscher and Vanhoutte, 1986). Thus, in contracted aortic rings of SHR, acetylcholine (ACh) induces endothelium-dependent relaxations, but the concentration–response curve to the muscarinic agonist is biphasic and at concentrations higher than 100 $nmol \cdot L^{-1}$ the relaxations become smaller. In quiescent aortic rings of SHR, ACh produces endothelium-dependent

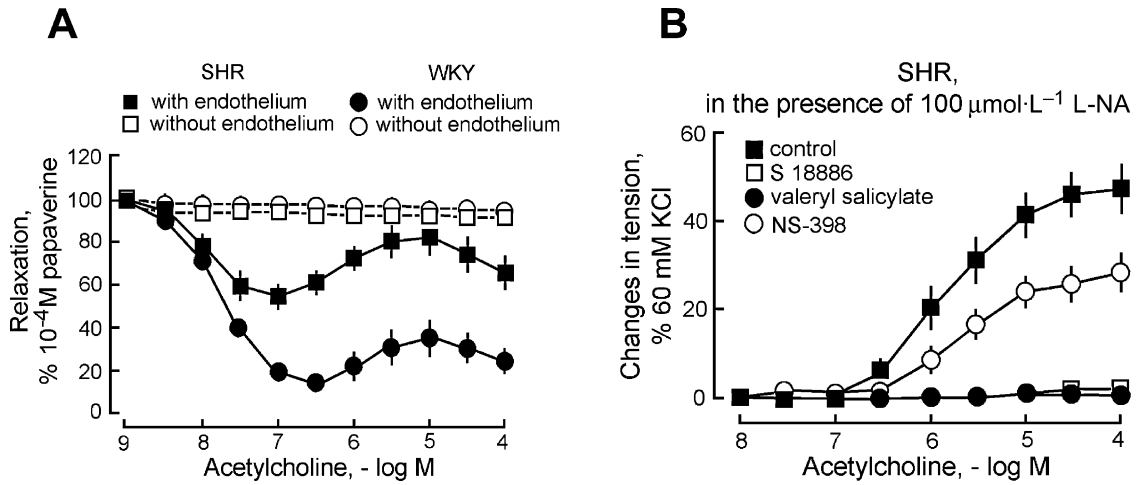

Figure 1 Endothelial dysfunction and endothelium-dependent contractions in SHR aorta. (A) In contracted rings of WKY and SHR aorta, acetylcholine induces endothelium-dependent relaxations, which at higher concentrations are blunted in the arteries of the hypertensive strain. (B) In quiescent aortic rings of SHR, acetylcholine induces endothelium-dependent contractions (in the presence of the nitric oxide synthase inhibitor L-nitro-arginine, L-NA) that are blocked by the antagonist of the TP receptor, S18886, the preferential COX-1 inhibitor, valeryl salicylate, but only partially affected by the preferential COX-2 inhibitor, NS-398. Modified from Yang *et al.* (*Br J Pharmacol*, 2002). COX, cyclooxygenase; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

contractions that are amplified in the presence of inhibitor of NO synthases (NOS; Luscher and Vanhoutte, 1986; Auch-Schwelk *et al.*, 1992; Iwama *et al.*, 1992; Yang *et al.*, 2002) (Fig. 1). These endothelium-dependent contractions are larger in aortae from male than female SHR (Kauser and Rubanyi, 1995), are positively correlated with the severity of hypertension and the aging process and occur in aging normotensive Wistar-Kyoto rats (WKY) (Koga *et al.*, 1988; 1989; Iwama *et al.*, 1992; Ibarra *et al.*, 1995).

Inhibitors of cyclooxygenase (COX) inhibit the endothelium-dependent contractions and fully restore the impaired endothelium-dependent relaxations, indicating that there is no or little alteration in NO production (Luscher and Vanhoutte, 1986), a conclusion strengthened by perfusion-superfusion bioassay studies (Hoeffner and Vanhoutte, 1989). Further bioassay studies using layered 'sandwich' preparations demonstrated that endothelium-dependent contractions to ACh involve the endothelial release of diffusible contractile COX derivatives, which oppose the relaxing effect of NO (Yang *et al.*, 2003a) (Fig. 2).

The generation of endothelium-derived contracting factor (EDCF) is observed not only in response to endothelial muscarinic M3 receptor stimulation (Boulanger *et al.*, 1994) but also in response to ATP (Koga *et al.*, 1989; Mombouli and Vanhoutte, 1993;Yang *et al.*, 2004), VEGF (Liu *et al.*, 2001), as well as in response to receptor-independent stimuli, for instance the calcium ionophore, A 23187 (Yang *et al.*, 2004; Tang *et al.*, 2007). EDCF contributes to the contractile responses of endothelin (Taddei and Vanhoutte, 1993a,b) and in the presence of inhibitor of NOS, a tonic generation of EDCF is observed in SHR aorta and in that of aging WKY (Abeywardena *et al.*, 2002).

Mechanisms underlying endothelium-dependent contractions

Endothelium-dependent contractions can be elicited by receptor-dependent mechanisms and by A 23187, which allows the free entry of extracellular calcium into endothelial cells, indicating that an increase in intracellular calcium is necessary for the production of endothelium-dependent contractions (Yang *et al.*, 2004; Gluais *et al.*, 2006; Tang *et al.*, 2007). Indeed, ACh causes a rapid increase in cytosolic calcium concentration in endothelial cells of SHR and to a lesser extent in that of WKY. This rise of calcium was not affected by inhibiting COX or by the combination of tiron (a superoxide scavenger) plus diethyldithiocarbamate acid (DETCA; a superoxide dismutase inhibitor) (Tang *et al.*, 2007). However, endothelium-dependent contractions are reduced by the acute exposure to the combination of tiron plus DETCA or by a chronic treatment with dimethylthiourea (an *in vivo* depletor of free radicals) (Yang *et al.*, 2002). Furthermore, the production of superoxide anions selectively enhances endothelium-dependent contractions (Yang *et al.*, 2003b) and under bioassay conditions, the transfer of EDCF from the donor tissue to the bioassay preparation is diminished by the combination of superoxide dismutase plus catalase (Yang *et al.*, 2003a). Confocal microscopy shows that ACh causes a rapid increase in reactive oxygen species in endothelial cells of SHR aorta but not in that of WKY. This burst in reactive oxygen species generation is prevented by COX inhibition or by the combination of tiron plus DETCA (Tang *et al.*, 2007). In contrast to ACh, the increase in endothelial intracellular calcium, the generation of reactive oxygen species and the amplitude of endothelium-dependent contractions elicited by A 23187 are of similar amplitude in WKY and SHR aorta (Gluais *et al.*, 2006; Tang *et al.*, 2007).

These results indicate that an abnormal accumulation of calcium in SHR endothelial cells is a prerequisite to initiate the release of EDCF, and this can be mimicked in that of WKY when stimulated by the calcium ionophore. The sequence of events occurring during endothelium-dependent contractions firstly requires the accumulation of calcium, which then most likely induces the phospholipase A2-dependent mobilization of arachidonic acid (Luscher and Vanhoutte, 1986),

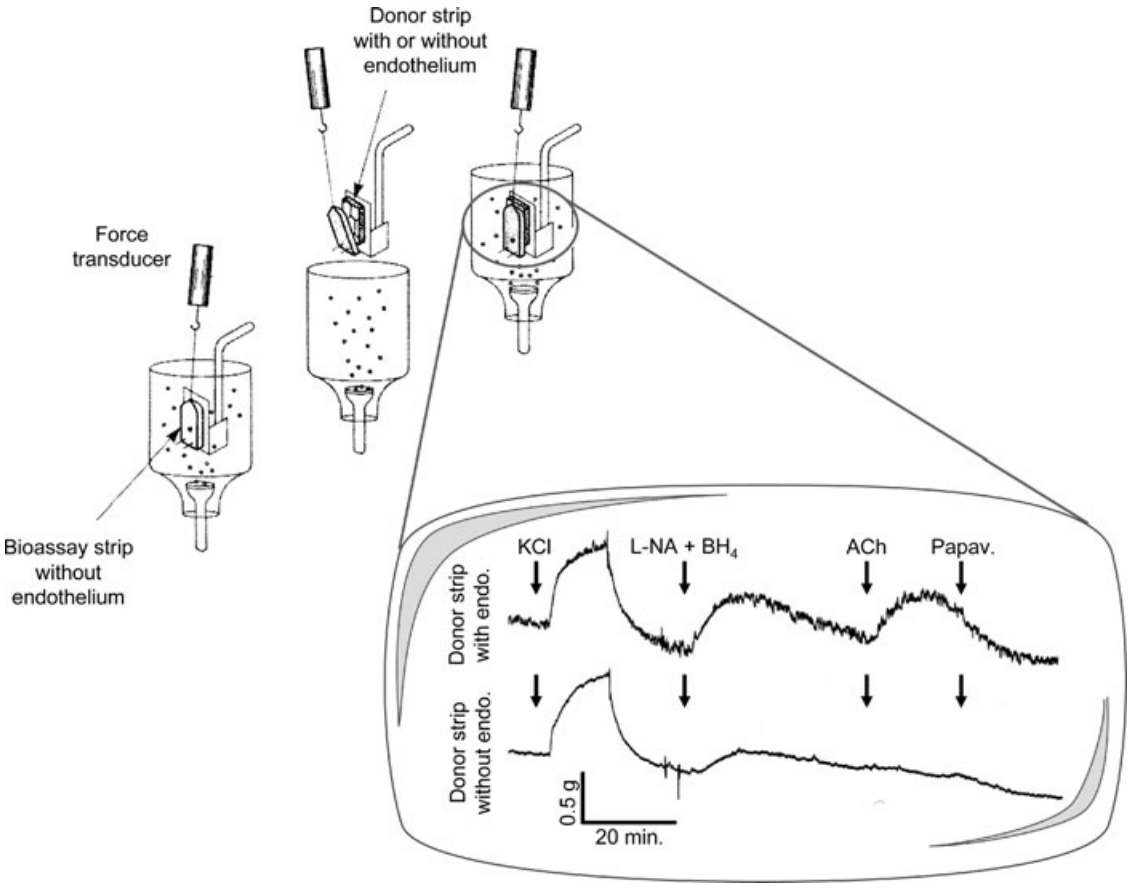

Figure 2 Bioassay of endothelium-derived contracting factor in SHR aortic rings: layered or 'sandwich' preparation. Acetylcholine (ACh) produces contraction of the bioassay strip (without endothelium) only if endothelial cells are present on the donor aortic strip. L-nitro-arginine (L-NA) and tetrahydrobiopterin (BH_4) are present to optimize endothelium-derived contracting factor-mediated responses. Papaverine (Papav.) produces complete relaxation of the bioassay strip. Modified from Vanhoutte *et al.* (*Br J Pharmacol*, 2005).

COX activation and the production of reactive oxygen species along with that of EDCF(s) (Tang *et al.*, 2007). Reactive oxygen species can diffuse towards the vascular smooth muscle cells and produce contraction (Auch-Schwelk *et al.*, 1989; Katusic and Vanhoutte, 1989; Suzuki and Ford, 1992; Yang *et al.*, 2002) and be involved in a positive feedback loop on the endothelial cells by further activating COX (Harlan and Callahan, 1984).

Identification of EDCFs

Cyclooxygenases and prostaglandin synthases. COX are the first enzymes involved in the biosynthetic pathway leading to prostanoid formation. A constitutive (COX-1) and an inducible isoforms (COX-2) have been cloned and characterized (De Witt, 1988; Merlie *et al.*, 1988; Hla and Neilson, 1992; O'Banion *et al.*, 1992; Yokoyama *et al.*, 2002). COX-2 can be induced by several stimuli associated with cell activation and inflammation. In endothelial cells, COX-1 is expressed constitutively but can also be over-expressed, for instance by shear stress (Vane *et al.*, 1998; Doroudi *et al.*, 2000; Davidge, 2001). Both endothelial and vascular smooth muscle cells contain COX, however endothelial cells contain 20 times more of the enzyme than smooth muscle cells (DeWitt *et al.*, 1983). Endothelial cells express preferentially COX-1 versus COX-2 (Onodera *et al.*, 2000; Kawka *et al.*, 2007). In SHR endothelial cells the mRNA and protein expression of COX-1 are enhanced when compared with that of WKY, and in both strains they are augmented by aging (Ge *et al.*, 1995; Tang and Vanhoutte, 2008). Endothelium-dependent contractions to ACh are blocked by specific inhibitors of COX-1 and minimally affected by specific inhibitors of COX-2 (Ge *et al.*, 1995; Yang *et al.*, 2002; 2003a,b; Gluais *et al.*, 2006) (Fig. 1). In agreement with a preponderant role for COX-1 in endothelium-dependent contractions, these responses are abolished in aorta taken from COX-1 knockout mice while they are maintained in aortic rings of COX-2 knockout animals (Tang *et al.*, 2005). However, in some instances, COX-2-derived contractile prostanoids are produced by WKY and SHR aortic endothelial cells (Camacho *et al.*, 1998; Zerrouk *et al.*, 1998; Garcia-Cohen *et al.*, 2000; Alvarez *et al.*, 2005; Blanco-Rivero *et al.*, 2005).

Various biologically active eicosanoids are formed from the short lasting but biologically active endoperoxide (PGH_2), through the action of a set of synthases namely PGD, PGE, PGF, PGI and thromboxane synthases (Tsuboi *et al.*, 2002). The expression of prostacyclin synthase (PGIS) is by far the most abundant of these synthases expressed in the rat aortic endothelial cells (Tang and Vanhoutte, 2008), and a greater co-distribution of PGIS with COX-1 is observed when

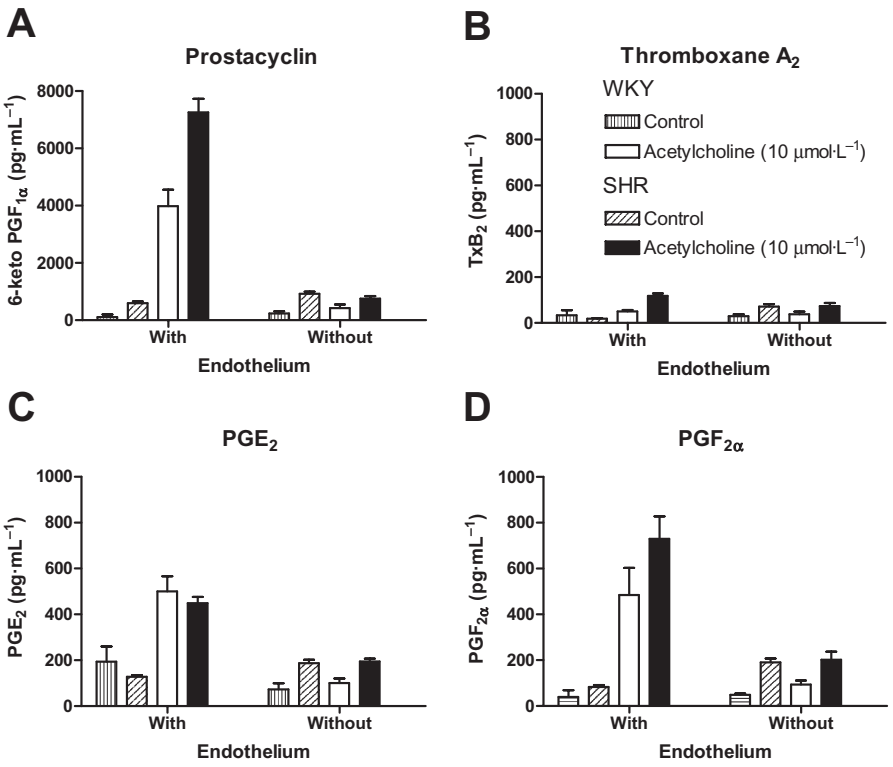

Figure 3 Acetylcholine-induced prostaglandin release in WKY and SHR aorta. Acetylcholine produces the endothelium-dependent release of prostacyclin (A, as measured as its stable metabolite 6-keto-$PGF_{1\alpha}$), thromboxane A_2 (B, as measured as its stable metabolite thromboxane B_2), PGE_2 (C) and $PGF_{2\alpha}$ (D). Prostacyclin is by far the most abundant prostaglandin released, and its generation is markedly higher in SHR than in WKY aortic rings. Modified from Gluais *et al.* (*Br J Pharmacol*, 2005). SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

compared with COX-2 (Kawka *et al.*, 2007) explaining why the majority of the endothelial COX-1-derived endoperoxides are transformed into prostacyclin (Gluais *et al.*, 2005; 2006; Tang and Vanhoutte, 2008) (Fig. 3). The expression of PGIS and thromboxane synthase is higher in SHR aortic endothelial cells than in those of WKY (Tang and Vanhoutte, 2008).

Acetylcholine-induced endothelium-dependent contractions and thromboxane A_2. Prostaglandins interact with specific seven transmembrane, G protein-coupled receptors, which are classified in five subtypes DP, EP, FP, IP and TP in function of their sensitivity to the five primary prostanoids, prostaglandins D_2, E_2, $F_{2\alpha}$, I_2 and thromboxane A_2 respectively (Tsuboi *et al.*, 2002; Alexander *et al.*, 2008). These receptors are all expressed in WKY and SHR aortae, although at low levels (Tang and Vanhoutte, 2008). ACh-induced endothelium-dependent contractions are blocked by antagonists of the TP receptors. However, inhibitors of thromboxane synthase do not affect these endothelium-dependent contractions indicating that thromboxane A_2 is not the EDCF released following muscarinic receptor activation (Luscher and Vanhoutte, 1986; Koga *et al.*, 1989; Auch-Schwelk *et al.*, 1990; Kato *et al.*, 1990; Ge *et al.*, 1995; Tesfamariam and Ogletree, 1995; Yang *et al.*, 2002; 2003a,b; 2004; Gluais *et al.*, 2005; 2006) (Fig. 4).

Acetylcholine-induced endothelium-dependent contractions and PGH_2. Thromboxane A_2 is the most potent agonist at TP receptors but is not its exclusive ligand. In fact, in WKY and SHR aorta numerous prostanoids produce contraction by activating TP receptors, with the following order of potency 9,11-dideoxy-9α,11α-epoxymethano prostaglandin $F_{2\alpha}$ (U 46619) >> 8-isoprostane = $PGF_{2\alpha}$ = PGH_2 > PGE_2 = PGD_2 > PGI_2. Among those agonists, only PGH_2 and prostacyclin evoke transient contractions, possibly because of their short half-life in aqueous solutions (3–4 min, Dickinson and Murphy, 2002), that mimic endothelium-dependent contractions (Gluais *et al.*, 2005). PGH_2 is the second most potent agonist at TP receptors, and there is an augmented sensitivity of the SHR smooth muscle cells towards this endoperoxide when compared with that of WKY (Ge *et al.*, 1995; 1999). Therefore, PGH_2 has been considered as a suitable candidate for an EDCF (Auch-Schwelk *et al.*, 1990; Kato *et al.*, 1990; Ge *et al.*, 1995; Gluais *et al.*, 2005; 2006; 2007). However, in SHR aortic endothelial cells, the massive expression of PGIS (Tang and Vanhoutte, 2008) and its close association with COX-1 (Kawka *et al.*, 2007) are not in favour of a large PGH_2 spillover. The levels of PGH_2 are difficult to measure and still need to be better assessed in order to evaluate more precisely the contribution of this endoperoxide to endothelium-dependent contractions.

PGH_2 is spontaneously or enzymatically transformed in the more stable isomer PGE_2 and in presence of mild reducing agent or enzymatically into $PGF_{2\alpha}$. Although PGE_2, via EP receptor activation, is an endothelium-derived contractile factor in a rat model of diabetes (Shi *et al.*, 2007), in the SHR aorta the involvement of this prostaglandin either via EP or TP receptor activation has been ruled out (Tang *et al.*, 2008). However, when PGIS is inhibited, a compensatory increase in

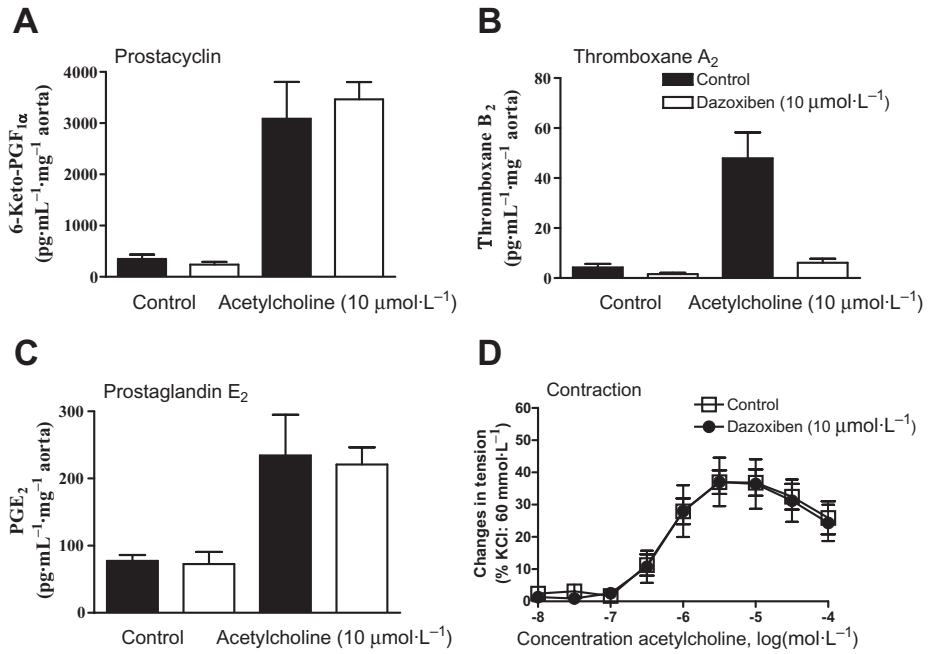

Figure 4 Thromboxane A_2 generation and acetylcholine-induced endothelium-dependent contractions. Dazoxiben, the thromboxane synthase inhibitor, does not affect acetylcholine-induced release of prostacyclin (A), PGE_2 (C), $PGF_{2\alpha}$ (data not shown), but abolishes that to thromboxane A_2 (B). However, acetylcholine-induced endothelium-dependent contraction in SHR aortic rings is not influenced by dazoxiben (D). Modified from Gluais *et al.* (*Br J Pharmacol,* 2005).

the production of PGE_2 and $PGF_{2\alpha}$ is observed, and then these prostaglandins act as EDCF (Gluais *et al.*, 2005).

Acetylcholine-induced endothelium-dependent contractions and 8-isoprostane. 8-isoprostane (8-*epi*$PGF_{2\alpha}$) is generally produced from the oxidative modification of polyunsaturated fatty acids via a free radical-catalysed mechanism (Morrow *et al.*, 1990). However, under some circumstances, 8-isoprostane could be a direct product of COX or an indirect consequence of superoxide anion production by COX-mediated metabolism (Watkins *et al.*, 1999). In both WKY and SHR aortic rings, 8-isoprostane is a potent constrictor (Gluais *et al.*, 2005), supporting the hypothesis that an isoprostane could contribute to EDCF-mediated responses (Janssen, 2002). However, proper measurement of 8-isoprostane generation failed to detect significant ACh-stimulated and endothelium-dependent release of this prostanoid (Gluais *et al.*, 2005). Therefore, in the SHR aorta, 8-isoprostane is unlikely to be an EDCF released by ACh.

Acetylcholine-induced endothelium-dependent contractions and PGI_2. ACh produces the endothelium-dependent release of prostacyclin, PGE_2, $PGF_{2\alpha}$ and thromboxane A_2 in the aorta of both WKY and SHR. The release of prostacyclin is 10 to 100 times larger than that of the other prostaglandins, while the generation of thromboxane A2 is the smallest. Furthermore, the release of prostacyclin is much larger in the aorta of SHR than in that of WKY (Gluais *et al.*, 2005). In the SHR aorta and that of aging WKY, prostacyclin paradoxically is not a relaxing but a contracting prostaglandin (Gluais *et al.*, 2005; Gomez *et al.*, 2008) (Figs 5 and 6). Whether or not, the reduction in the relaxing response to prostacyclin in SHR and the decrease of this response during aging is associated with parallel changes in the expression of the IP receptor gene remains controversial (Numaguchi *et al.*, 1999; Tang and Vanhoutte, 2008). Nevertheless, the IP receptor dysfunction is specific of vascular smooth muscle cells because IP receptor-dependent inhibition of platelet activation is not altered in SHR or by aging (Gomez *et al.*, 2008). Therefore, endothelium-dependent contractions elicited by ACh in the aorta of SHR and aging WKY are likely to involve at least in part the release of prostacyclin.

This conclusion is based on the following observations: (i) in WKY and SHR, prostacyclin is a contracting but not a relaxing factor (Figs 5 and 6); (ii) prostacyclin is a more potent contracting agent in SHR than in WKY (Fig. 6); (iii) the contractions evoked by prostacyclin mimic the endothelium-dependent contractions produced by ACh both in term of duration and amplitude (Fig. 5); (iv) prostacyclin- and the endothelium-dependent contractions both involve activation of TP receptors (Figs 1 and 5); (v) prostacyclin is the most abundant prostaglandin released by ACh and is of endothelial origin (Fig. 3); (vi) the release of prostacyclin is two times larger in SHR than in WKY (Fig. 3); (vii) the time course of the release of prostacyclin is compatible with the time course of the observed endothelium-dependent contractions; (viii) the release of prostacyclin correlates with the amplitude of the endothelium-dependent contractions over the full concentration range of ACh in both WKY and SHR (Fig. 7); (ix) the endothelium-dependent contractions and the release of prostacyclin are affected similarly by COX inhibitors; (x) The expression of PGIS is by far the most abundant of the prostaglandin synthases expressed in the rat aortic endothelial cells and PGIS and COX-1 co-segregate;

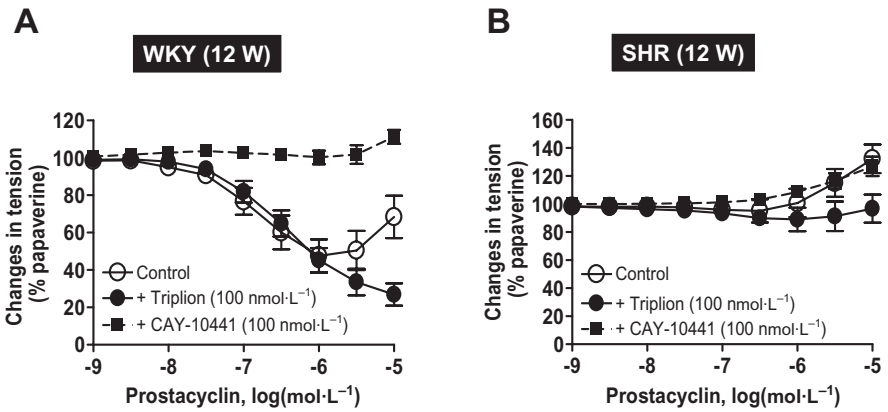

Figure 5 Prostacyclin induces relaxation in WKY aortic rings but not in those of SHR. (A) In aortic rings with endothelium of young 12-week-old WKY, prostacyclin induces relaxations that are inhibited by the specific IP receptor antagonist CAY 10441. The presence of the TP receptor antagonist, Triplion®, enhances the relaxations to prostacyclin. (B) In aortic rings with endothelium of young 12-week-old SHR, prostacyclin does not provoke relaxations but only contractions at higher concentrations. These contractions are blocked by the TP receptor antagonist, Triplion®. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats. Modified from Gomez *et al.* (*AM J Physiol* 2008).

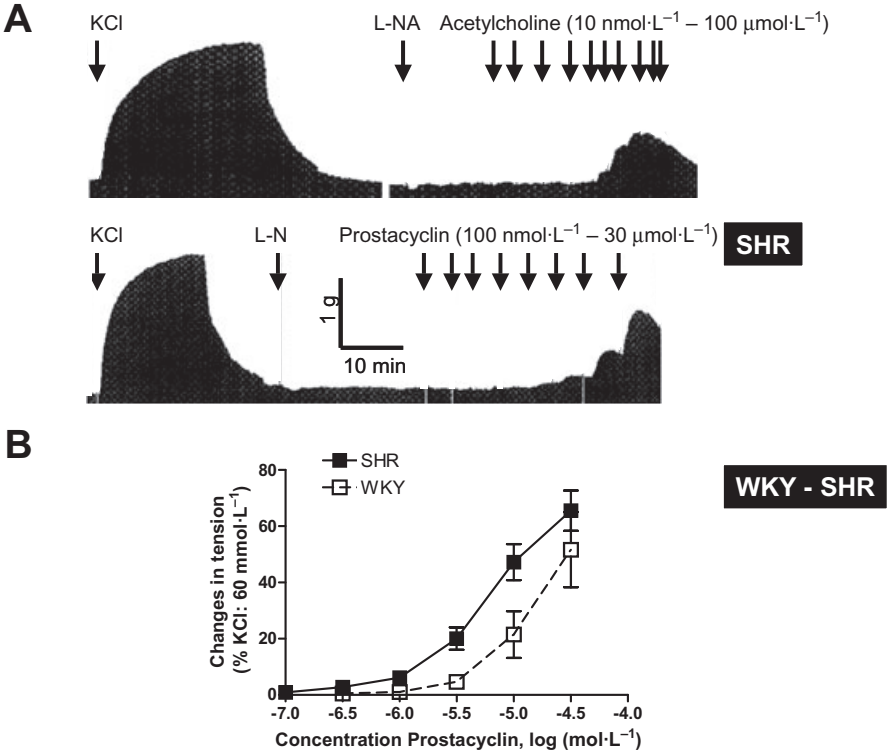

Figure 6 Prostacyclin-induced contractions and acetylcholine-induced endothelium-dependent contractions. (A) Original trace showing acetylcholine-induced concentration and endothelium-dependent contractions in SHR aortic rings (presence of L-nitro-arginine, L-NA). The modest amplitude of the contractions and their transient nature can be visualized in comparison with the reference contraction produced by 60 mmol·L^{-1} KCl (upper trace). In aortic rings with endothelium and in the presence of L-NA (or in rings without endothelium, data not shown), prostacyclin induces transient concentration-dependent contractions (lower trace). (B) SHR aortic rings are more sensitive than that of WKY to the contractile effect of prostacyclin. In both strains, these contractions are blocked by TP receptor antagonists (data not shown). Modified from Gluais *et al.* (*Br J Pharmacol*, 2005). SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

and finally (xi) the inhibition of prostacyclin synthesis enhances the ACh-induced endothelium-dependent contractions. Paradoxically, this latter observation also supports the hypothesis that prostacyclin contribute to endothelium-dependent contractions because the inhibition of PGIS may enhance PGH_2 spillover, a more potent TP receptor agonist than prostacyclin itself (Rapoport and Williams, 1996; Gluais *et al.*, 2005). The hypothesis that prostacyclin is an EDCF is in agreement with the conclusion that prostacyclin is the main factor accounting for endothelial dysfunction in the aorta of WKY and SHR treated with aldosterone (Blanco-Rivero *et al.*, 2005).

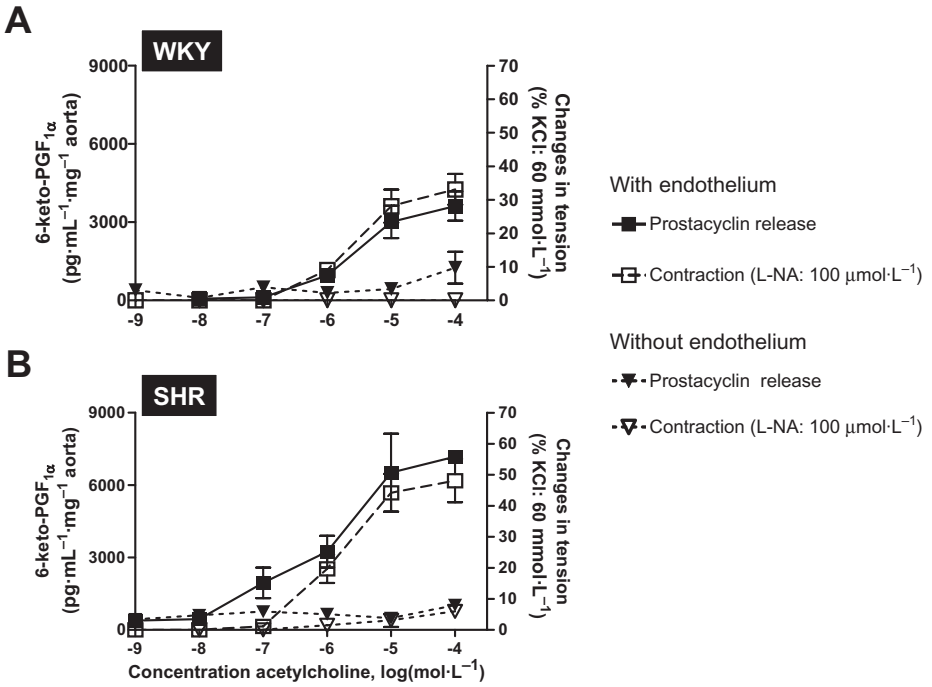

Figure 7 Acetylcholine-induced concentration-dependent and endothelium-dependent prostacyclin release and contractions. (A) WKY aortic rings with and without endothelium. (B) SHR aortic rings with and without endothelium. Prostacyclin release is represented on the left Y-scale and endothelium-dependent contractions on the right Y-scale (presence of L-nitro-arginine, L-NA). Modified from Gluais *et al.* (*Br J Pharmacol*, 2005). SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

EDCFs released in response to other stimuli. In response to other stimuli, such as ATP or the calcium ionophore A 23187, thromboxane synthase inhibitors partially inhibit endothelium-dependent contractions indicating that thromboxane A_2 contributes to these responses, prostacyclin and/or PGH_2 being the other contributors (Gluais *et al.*, 2006; 2007) (Fig. 8). Similarly, in response to endothelin, the endothelial generation of thromboxane A_2 contributes to the contractile response (Taddei and Vanhoutte, 1993a,b).

Different stimuli, that is, ATP, ACh and A 23187, induce a very different pattern in prostaglandin release. Subsequently, the endothelium-dependent contractions do not involve the same COX derivatives, although the final effector remains the prostanoid TP receptor on the vascular smooth muscle cells (Yang *et al.*, 2004). These differences are not linked to agonist stimulating G protein-coupled receptors versus receptor-independent mechanism because thromboxane A_2 contributes to both ATP- and A 23187-induced endothelium-dependent contractions but not in those evoked by ACh. These differences are unexplained at present but could be linked to differences in the dynamic of the increase in endothelial intracellular calcium evoked by these different stimuli (Gordon and Martin, 1983; Carter *et al.*, 1988). (Fig. 9)

Endothelial dysfunction in other SHR vascular beds

The endothelial dysfunction observed in the mesenteric (Lüscher *et al.*, 1990; Fu-Xiang *et al.*, 1992; Takase *et al.*, 1994; Lang *et al.*, 1995; Hutri-Kahonen *et al.*, 1997; Dantas *et al.*, 1999; Xavier *et al.*, 2008), renal (Lüscher *et al.*, 1988; Dai *et al.*, 1992; Fu-Xiang *et al.*, 1992; Ito and Carretero, 1992; Dohi *et al.*, 1996; Kagota *et al.*, 1999) and skeletal muscle (Huang *et al.*, 1993; Lübbe *et al.*, 1993; Huang and Koller, 1996; Mori *et al.*, 2006) vascular beds is qualitatively similar to that reported for the aorta. The generation of EDCFs similar to those identified in the aorta contributes to the altered endothelium-dependent relaxations/vasodilatations.

In these resistance arteries, in contrast to the aorta, endothelium-dependent hyperpolarizations (EDHF-mediated responses) participate to endothelium-dependent relaxations (Félétou and Vanhoutte, 2006b). Most studies show a marked attenuation of the EDHF-mediated component in SHR arteries (Fujii *et al.*, 1992; 1993; Hayakawa *et al.*, 1993; 1995; Mantelli *et al.*, 1995; Dohi *et al.*, 1996; Hutri-Kahonen *et al.*, 1997; Bussemaker *et al.*, 2003). The decrease in EDHF-mediated response has been associated with, but not yet causally linked, to a change in the expression profile of gap junctions in endothelial cells (Busse *et al.*, 2002; Félétou and Vanhoutte, 2004; Griffith, 2004). Indeed, the expression of connexins 37 and 40 is lower in arteries of the SHR than in that of the WKY (Kansui *et al.*, 2004; Rummery and Hill, 2004). Additionally, alterations in the expression or function of endothelial calcium-activated potassium channels may lead to the preferential activation of calcium-activated chloride channels and endothelium-dependent depolarization instead of endothelium-dependent hyperpolarization (Corriu *et al.*, 1996; Coleman *et al.*, 2001; Goto *et al.*, 2007). The production of NO is generally not altered, although in the mesenteric artery, a decrease in its bioavailibity due to the generation of oxidative stress may occur (Tschudi *et al.*, 1996; DeLano *et al.*, 2006; Macarthur *et al.*, 2008). In renal arteries of the WKY,

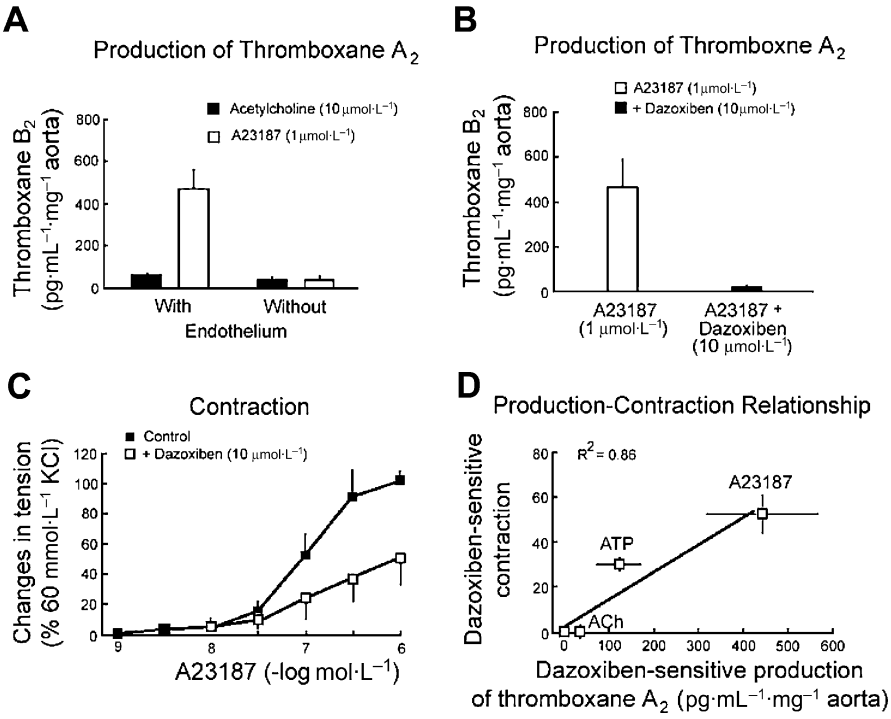

Figure 8 Thromboxane A_2 and endothelium-dependent contractions in SHR aortic rings. (A) The calcium ionophore, A23187, produces a significantly larger endothelial release of thromboxane A_2 than acetylcholine. (B) The endothelial release of thromboxane A_2 by A23187 is blocked by dazoxiben, the thromboxane synthase inhibitor. (C) Dazoxiben partially inhibits the endothelium-dependent contraction to A23187 (in the presence of L-nitro-arginine), indicating the contribution of thromboxane A_2 in the endothelium-dependent contractions elicited by the calcium ionophore. (D) In SHR aortic rings with endothelium, the dazoxiben-sensitive production of thromboxane A_2 is significantly correlated to the dazoxiben-sensitive component of the endothelium-dependent contractions. SHR, spontaneously hypertensive rats.

inhibitors of EDHF-mediated responses favour endothelium-dependent contractions (Michel *et al.*, 2008b).

EDCF-mediated responses are not ubiquitous in SHR arteries. Thus, in the carotid and cerebral arteries of that strain, the endothelium-dependent relaxations to ACh are attenuated, but this involves an impairment of the NO component without generation of EDCF and without alteration of the EDHF-mediated responses (Hongo *et al.*, 1988; Lüscher *et al.*, 1988; Mayhan, 1990; Sobey *et al.*, 1999; Dina *et al.*, 2004; Iaccarino *et al.*, 2004). Likewise, in SHR coronary arteries when compared with those of WKY, the endothelium-dependent relaxations are not or minimally affected and are not associated with the production of EDCF (Tschudi *et al.*, 1994; 1995; Nava *et al.*, 1995; Bund, 1998; Garcia and Bund, 1998).

Conclusions and perspectives

In the SHR, prostacyclin, PGH_2, thromboxane A_2 and depending on the circumstances, PGE_2 and $PGF_{2\alpha}$ can act as EDCFs and all converge towards the TP receptor (Fig. 9). The generation of EDCFs has been demonstrated in human essential hypertension and also in various other animal models of cardiovascular diseases, in particular diabetes (Taddei *et al.*, 2001; Vanhoutte *et al.*, 2005; Verbeuren, 2006a,b; Xu *et al.*, 2006; Cheng *et al.*, 2007; Matsumoto *et al.*, 2007; 2008; Shi *et al.*, 2007; Michel *et al.*, 2008a). In apo E-deficient mice blockade of TP receptors but not aspirin inhibits atherogenesis (Cayatte *et al.*, 2000). In patients with coronary artery disease, a TP receptor blocker improves endothelial function beyond the simple inhibition of COX (Belhassen *et al.*, 2003), indicating that eicosanoids other that the above-mentioned arachidonic acid metabolites, possibly isoprostanes, activate TP receptor and are involved in these pathologies. In the SHR, a functional impairment of IP receptors of the vascular smooth muscle is likely to contribute to the endothelial dysfunction. Mice knockout for the IP receptor (Xiao *et al.*, 2001; Cheng *et al.*, 2002) and human patients with a dysfunctional prostacyclin IP receptor mutation (Arehart *et al.*, 2008) show accelerated atherothrombosis indicating that an imbalance between vasoconstrictor/relaxing and thrombogenic/anti-thrombogenic prostaglandins is of major importance in the generation of cardiovascular disease.

Conflicts of interest

MF and TJV are employees of the Institut de Recherches Servier, a pharmaceutical company that is currently involved in the clinical development of a TP receptor antagonist (S 18886 or Terutroban, or Triplion®). PMV is a consultant for the group Servier and a former employee of this company.

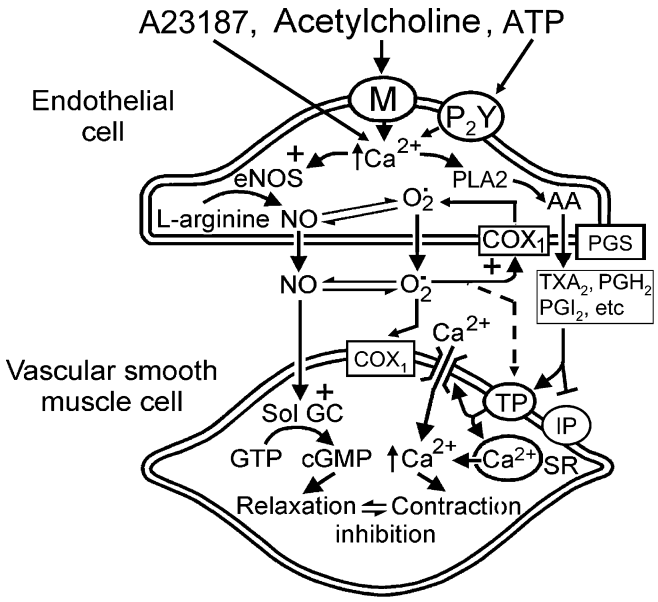

Figure 9 Mechanisms of endothelium-dependent contractions in WKY and SHR aortic rings. The sequence of events occurring during endothelium-dependent contractions firstly requires the intracellular accumulation of calcium, which then most likely induces the phospholipase A2-dependent mobilization of arachidonic acid, COX-1 activation and the production of reactive oxygen species along with that of EDCF(s). Reactive oxygen species inactivate NO, can be involved in a positive feedback loop on the endothelial cells by further activating COX-1 and can diffuse towards the vascular smooth muscle cells and produce contraction. PGH_2 along with the various prostaglandins produced by the PG-synthases, prostacyclin (PGI_2), thromboxane A2 (TXA_2) and possibly under some circumstances PGE_2 or $PGF_{2\alpha}$, converge towards the TP receptors located on the vascular smooth muscle cells and produce contraction. In the SHR, a functional impairment of the smooth muscle IP receptors contributes to the endothelial dysfunction. COX, cyclooxygenase; EDCF, endothelium-derived contracting factor; NOS, nitric oxide synthase; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

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