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TOPICAL REVIEW

Endothelium-dependent contractions: when a good guy turns bad!

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Endothelial cells can induce contractions of the underlying vascular smooth muscle by generating vasoconstrictor prostanoids (endothelium-dependent contracting factor; EDCF). The endothelial COX-1 isoform of cyclooxygenase appears to play the dominant role in the phenomenon. Its activation requires an increase in intracellular Ca^{2+} concentration. The production of EDCF is inhibited acutely and chronically by nitric oxide (NO), and possibly by endothelium-dependent hyperpolarizing factor (EDHF). The main prostanoids involved in endothelium-dependent contractions appear to be endoperoxides (PGH_2) and prostacyclin, which activate thromboxane-prostanoid (TP) receptors of the vascular smooth muscle cells. Oxygen-derived free radicals can facilitate the production and/or the action of EDCF. Endothelium-dependent contractions are exacerbated by ageing, obesity, hypertension and diabetes, and thus are likely to contribute to the endothelial dysfunction observed in older people and in essential hypertensive patients.

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Besides playing an essential role in vasodilator responses by releasing endothelium-derived relaxing factor(s) (EDRF(s)) (Furchgott & Zawadzki, 1980), the endothelial cells of certain arteries and veins can also initiate contractions of the vascular smooth muscle that surrounds them (De Mey & Vanhoutte, 1982, 1983). Bioassay studies demonstrated that the transfer of diffusible factors is involved in such endothelium-dependent contractions (Rubanyi & Vanhoutte, 1985; Iqbal & Vanhoutte, 1988; Yang *et al.* 2003). Theoretically, endothelium-dependent contractions could be explained by either the withdrawal of endothelial inhibitory signals (prostacyclin, nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF) or the production of vasoconstrictor substances. Over the years, it has become evident that prostanoids, derived from the endothelial cyclooxygenase, explain most endothelium-dependent contractions (see Vanhoutte *et al.* 2005). Obviously, endothelial cells can produce vasoconstrictor substances other than prostanoids in particular different peptides (Yanagisawa *et al.* 1988; Dhein *et al.* 1997; Saifeddine *et al.* 1998) or the non-peptidic dinucleotide uridine adenosine tetraphosphate (UP_4A) (Jankowski *et al.* 2005). However, it is uncertain whether or not the instantaneous release of these non-prostanoid substances can lead to endothelium-dependent contractions. Thus, the present brief review will focus on cyclooxygenase-derived vasoconstrictor substances (EDCF) initiating endothelium-dependent contractions.

EDCF-mediated responses

Endothelium-dependent contractions to acetylcholine, and other vasoactive substances (e.g. arachidonic acid, ATP, the calcium ionophore A23187), have been reported in a variety of blood vessels from different species (see Furchgott & Vanhoutte, 1989; Lüscher & Vanhoutte, 1990; Vanhoutte *et al.* 2005).

The source of EDCF. The endothelium-dependent contractions of canine veins to arachidonic acid were prevented by non-selective inhibitors of cyclooxygenase (e.g. indomethacin), as were those evoked by acetylcholine in the canine basilar artery or the aorta of the spontaneously hypertensive rat (SHR) (Miller & Vanhoutte, 1985; Lüscher & Vanhoutte, 1986; Katusic *et al.* 1988). This demonstrated the key role of the metabolism of arachidonic acid into prostanoids in the genesis of endothelium-dependent contractions (see Vanhoutte *et al.* 2005). Bioassay studies revealed that it is mainly the cyclooxygenase of the endothelial cells, rather than that of the vascular smooth muscle which

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is responsible (Fig. 1) (Yang *et al.* 2003). Studies in the SHR aorta using preferential and selective inhibitors of the two isoforms of the enzyme (cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2)) and molecular biology techniques (Fig. 2), as well as experiments in the aorta of genetically modified mice prompted the conclusion that the constitutive isoform, COX-1, plays the key role in the occurrence of endothelium-dependent contractions in those blood vessels (Ge *et al.* 1995; Traupe *et al.* 2002; Yang *et al.* 2003; Tang *et al.* 2005*a*; Gluais *et al.* 2006). However, in blood vessels where endothelial COX-2 is present, the prostanoids generated by this isoform can contribute to EDCF-mediated contractions (Camacho *et al.* 1998; Zerrouk *et al.* 1998; Garcia-Cohen *et al.* 2000; Álvarez *et al.* 2005; Blanco-Rivero *et al.* 2005; Hirao *et al.* 2008; Shi & Vanhoutte, 2008).

Pivotal role of TP receptors. Most cyclooxygenase-dependent, endothelium-dependent contractions are abolished by TP-receptor antagonists (Tesfamariam *et al.* 1989; Auch-Schwelk *et al.* 1990; Kato *et al.* 1990; Mayhan, 1992; Yang *et al.* 2002, 2003; Zhou *et al.* 2005). Bioassay experiments demonstrate that the TP receptors involved are those located in the vascular smooth muscle cells (Yang *et al.* 2003). The contraction of the latter upon TP-receptor activation is due to the combination of an increased entry of Ca^{2+} resulting from the opening of both receptor-operated and voltage-gated Ca^{2+} channels and Rho-kinase-mediated sensitization of the myofilaments (Okon *et al.* 2002; Huang *et al.* 2004; O'Rourke *et al.* 2006).

Impact of ageing. Endothelium-dependent contractions become more prominent in arteries of older, compared to younger animals (Koga *et al.* 1989; Iwama *et al.* 1992; Abeywardena *et al.* 2002). This increased response is accompanied by an increased expression of COX-1 (Tang & Vanhoutte, 2008*b*). When COX-2 is induced by the ageing process, this isoform of the enzyme can contribute in part to endothelium-dependent contractions (Shi *et al.* 2008). The ability of prostacyclin to induce

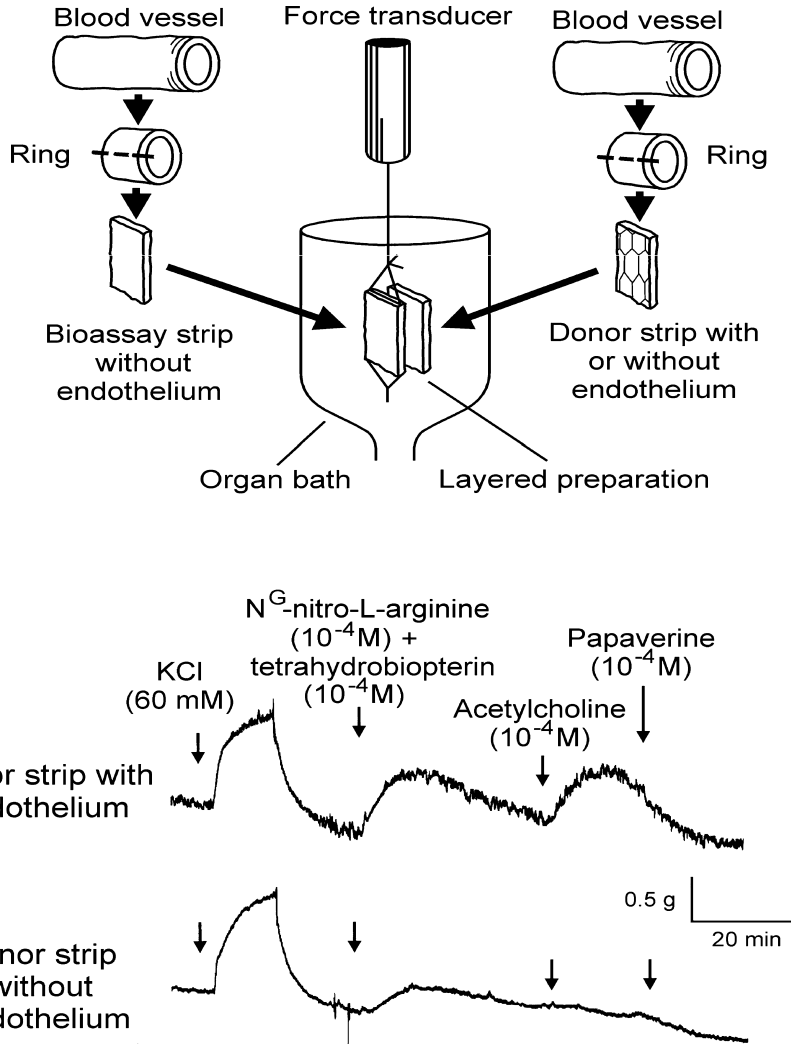

Figure 1
Upper panel: a donor strip is stitched onto the bioassay tissue creating a 'sandwich'-like layered preparation. Isometric tension is recorded from the bioassay strip and the donor tissue does not directly contribute to the recorded response. Lower panel: acetylcholine-induced contractions only occurred when the donor strip contained endothelium. The experiment was performed in the presence of nitro-L-arginine and tetrahydrobiopterin to optimize the EDCF-mediated response (reproduced from Vanhoutte *et al.* 2005, with permission).

relaxation is lost in the aorta of 15-week-old and older WKY (Levy, 1980; Rapoport & Williams, 1996; Gluais *et al.* 2005). This inability is due to a dysfunction of the IP receptors, since the relaxation to isoproterenol (isoprenaline; a β-adrenoceptor agonist which also evokes cAMP-dependent dilatations) is maintained in those arteries (Rapoport & Williams, 1996).

Hallmark of vascular disease. *Spontaneous hypertension.* The endothelium-dependent relaxations to acetylcholine are blunted in the aorta of the SHR, and this is due to the concomitant release of EDCF rather than a reduced production of EDRF (Lockette *et al.* 1986; Lüscher & Vanhoutte, 1986; Lüscher *et al.* 1987*b*). The endothelium-dependent contractions to acetylcholine are more pronounced in the quiescent aorta of the adult SHR than in that of normotensive Wistar-Kyoto rats (WKY) (Lüscher & Vanhoutte, 1986) and this is accompanied by an increased expression/presence of COX-1 in the endothelial cells (Ge *et al.* 1995; Tang & Vanhoutte, 2008*b*). The overexpression of COX-1 is not observed in aortae of pre-hypertensive SHR, while the isoform is more prominent in arteries from ageing normotensive rats (Ge *et al.* 1999; Tang & Vanhoutte, 2008*b*). These findings then prompt the conclusion that the overexpression of COX-1 in arteries from adult hypertensive rats reflects a premature ageing of the endothelium rather than a genetic predisposition. The mRNA and protein expression of TP receptors do not differ between aortae of WKY and SHR (Tang & Vanhoutte, 2008*b*; Tang *et al.* 2008), indicating that alteration in their density is not a contributing factor in the augmented endothelium-dependent contractions observed in the aorta of the hypertensive rat. Despite the unaltered density of TP receptors, the aorta of the SHR is hyper-responsive to the vasoconstrictor effect of endoperoxides (Ge *et al.* 1995). This hyper-responsiveness is present early on in the hypertensive strain and is thus not a consequence of the chronic exposure of the vascular wall to the high arterial blood pressure (Ge *et al.* 1999).

Obesity and diabetes. Obesity potentiates the occurrence of EDCF-mediated responses in mouse arteries, possibly because of an up-regulation of the expression of TP receptors (Traupe *et al.* 2002; Gollasch, 2002). The endothelium-dependent relaxations to acetylcholine are blunted in a number of arteries from diabetic animals (see Tesfamariam, 1994; De Vriese *et al.* 2000). This is due in part to the concomitant release of EDCF and can be attributed to the exposure of the endothelial cells to high glucose, resulting in increased oxidative stress and overexpression of both COX-1 and COX-2 (Tesfamariam *et al.* 1990, 1991; Xu *et al.* 2006; Shi *et al.* 2007*a*,*b*, 2008; Michel *et al.* 2008*b*; Shi & Vanhoutte, 2008).

The nature of EDCF. *When prostacyclin turns bad.* Cyclooxygenase transforms arachidonic acid into endoperoxides which *per se* cause contraction of vascular smooth muscle. Indeed, endoperoxides are released during endothelium-dependent contractions of the SHR aorta and thus can be regarded as EDCF (Ito *et al.* 1991; Asano *et al.* 1994; Ge *et al.* 1995; Vanhoutte *et al.* 2005; Hirao *et al.* 2008). Endoperoxides are converted further into prostacyclin, thromboxane A_2, prostaglandin D_2, prostaglandin E_2 and/or prostaglandin $F_{2\alpha}$ by their respective synthases (Bos *et al.* 2004). Of those enzymes, the prostacyclin synthase gene is by far the most abundantly expressed in endothelial cells, and more so in the SHR than in the WKY endothelium (Tang & Vanhoutte, 2008*b*). The protein expression of the enzyme augments with age and by hypertension (Numaguchi *et al.* 1999). Acetylcholine causes a greater release of prostacyclin in the aorta of SHR than in that of the WKY (Gluais *et al.* 2005). The prostanoid no longer evokes relaxations in arteries from ageing or hypertensive rats, and induces larger contractions in the latter (Rapoport & Williams, 1996; Gluais *et al.* 2005). These are the main reasons to accept that, in the SHR aorta, endoperoxides and prostacyclin are the main mediators of the endothelium-dependent contractions evoked by acetylcholine (Ge *et al.* 1995; Blanco-Rivero *et al.* 2005; Gluais *et al.* 2005). In other blood vessels, or even in the SHR aorta exposed to other agonists (ADP, A23187, endothelin-1, nicotine), thromboxane A_2 may contribute (Katusic *et al.* 1988; Shirahase *et al.* 1988; Auch-Schwelk & Vanhoutte, 1992; Taddei & Vanhoutte, 1993; Gluais

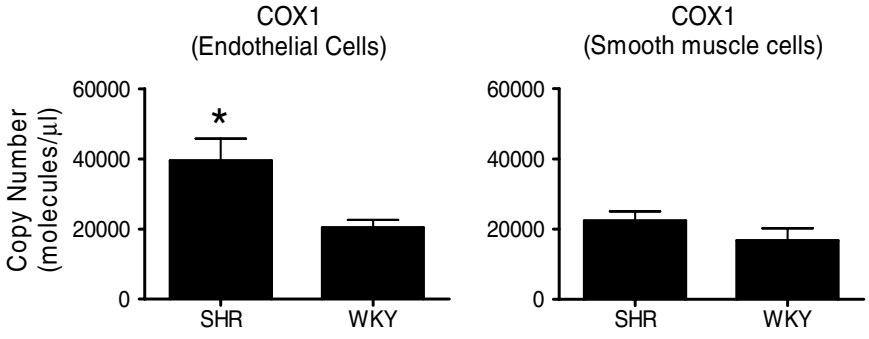

Figure 2
The mRNA expression of COX-1, measured by RT-PCR in freshly isolated endothelial cells, was significantly higher in 36-week-old SHR compared to 36-week-old WKY ($n = 6$). There was no difference in gene expression of COX-1 in smooth muscle cells between 36-week-old WKY and SHR ($n = 8$). Data are means ± S.E.M. *$P < 0.05$ (data from Tang & Vanhoutte, 2008*b*, reproduced with permission).

et al. 2006, 2007). The contribution of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ to endothelium-dependent contractions is marginal in most cases. However, when prostacyclin synthase is inhibited (pharmacologically or by peroxynitrite-dependent tyrosine nitration), or after photochemical endothelial injury, these two prostanoids can contribute to EDCF-mediated responses (Zou *et al.* 1999, 2002; Bachschmid *et al.* 2003; Gluais *et al.* 2005; Hirao *et al.* 2008).

Calcium, the trigger for release. In certain vascular beds, a tonic release of EDCF may participate in the regulation of vasomotor tone (Iwatani *et al.* 2008). The release of EDCF can be triggered by vasoactive agonists acting at the cell membrane, such as acetylcholine (activating endothelial M3-muscarinic receptors (Boulanger *et al.* 1994) or ADP (activating purinoceptors; Koga *et al.* 1989; Mombouli & Vanhoutte, 1993). Endothelium-dependent contractions of basilar arteries also can be elicited by sudden stretch (Katusic *et al.* 1987), which raises the possibility of a role in autoregulation of the cerebral circulation. Endothelium-dependent contractions are reduced when the external Ca^{2+} concentration is lowered, and can be evoked by calcium ionophores (Katusic *et al.* 1988; Okon

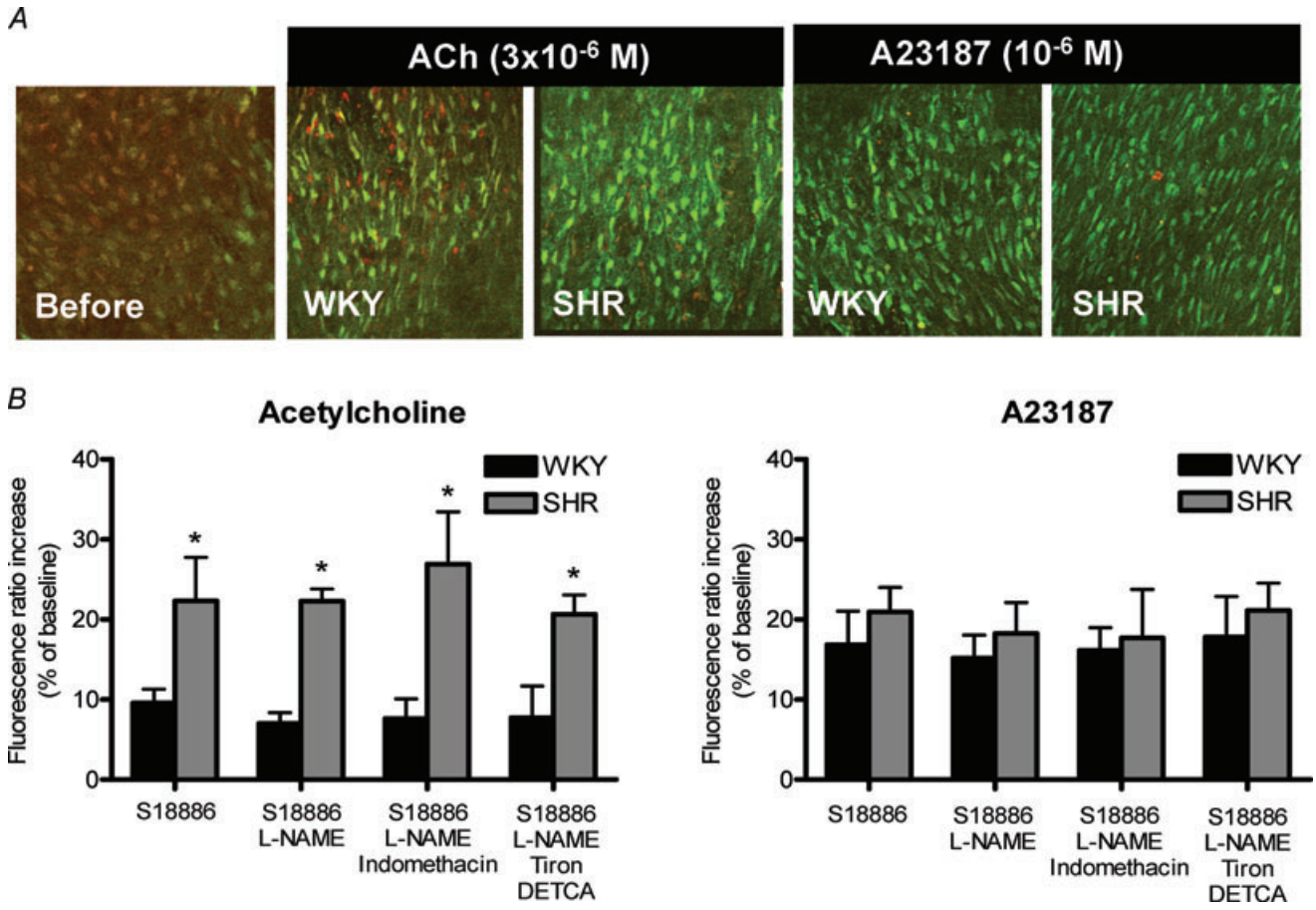

Figure 3
A, representative merged images taken by confocal microscopy showing responses to infusion of acetylcholine (3×10^{-6} M) or A23187 (10^{-6} M) on cytosolic calcium of aortic endothelial cells from WKY and SHR. The addition of acetylcholine caused a rapid increase in intracellular calcium in aortic endothelial cells of both WKY and SHR (indicated by an increase in green fluorescence and a decrease of red fluorescence), which was greater in the latter. The calcium ionophore A23187 caused a comparable increase in intracellular calcium in preparations from WKY and SHR. *B*, the increase in fluorescence ratio in endothelial cells from WKY and SHR in response to acetylcholine (3×10^{-6} M) (left) or A23187 (10^{-6} M) (right) is expressed in percentages of the baseline values. Data are shown as means ± S.E.M.; $n = 5$. $^*P < 0.05$ WKY *versus* SHR. Acetylcholine caused greater calcium increase in aortic endothelial cells of SHR than WKY, while A23187 caused a comparable response in the two strains. The increase of calcium was not affected by treatment with indomethacin, tiron plus diethyldithlocarbonate acid (DETCA; an inhibitor of superoxide dimutase) or N^G-nitro-L-argine methylestes (L-NAME; an inhibitor of nitric oxide synthase) (reproduced from Tang *et al.* 2007, with permission). The experiment was performed in the presence of S18886, a TP-receptor antagonist, to prevent contraction of the smooth muscle.

et al. 2002; Gluais *et al.* 2006; Shi *et al.* 2007*a*,*b*, 2008; Tang *et al.* 2007). They are accompanied by an increase in endothelial cytosolic Ca^{2+} concentration (Fig. 3) (Tang *et al.* (2007). The increase in intracellular endothelial Ca^{2+} concentration caused by acetylcholine is greater in the aorta of the SHR than in that of the WKY, which is in line with the absence of endothelium-dependent contraction in the latter (Tang *et al.* 2007). By contrast, the increase in Ca^{2+} concentration is comparable in endothelial cells of the two strains when exposed to A23187, which causes contractions in aortae of both SHR and WKY (Tang *et al.* 2007). These observations suggest that the increase in intracellular Ca^{2+} concentration is the initial trigger for endothelium-dependent contractions. The increased Ca^{2+} then presumably activates phospholipase A_2 which makes arachidonic acid available for metabolism by the endothelial cyclooxygenase.

Modulation by EDRF(s). Many blood vessels exhibit a basal release of NO, which is augmented by increases in shear stress (Rubanyi *et al.* 1986). Hence, it is not surprising that if their smooth muscle possesses myogenic tone or is contracted by vasoconstrictor agents, a sudden reduction in the activity of endothelial NO synthase (NOS), for example by the administration of NOS inhibitors, results in endothelium-dependent contractions *in vitro* or vasoconstrictions *in vivo* (Rees *et al.* 1989). Thus, in the intact organism, inhibition of NOS (either by pharmacological agents or by gene deletion) causes an increase in arterial blood pressure (Rees *et al.* 1989; Huang *et al.* 1995), although part of the response is due to withdrawal of the inhibitory effect of NO on the release of angiotensin II and endothelin-1 (see Vanhoutte, 2000; Félétou *et al.* 2008) rather than to the absence of the direct inhibitory effect of the endothelial mediator on vascular smooth muscle cells. Likewise, the continuous presence of signals resulting in EDHF-mediated responses may contribute to vascular tone, and the genetic deletion of these signals may also result in an increase in arterial blood pressure (see Félétou & Vanhoutte, 2006*a*,*b*; Félétou & Vanhoutte, 2007). In addition, a reduction in the release of EDRF will facilitate or permit the occurrence of endothelium-dependent constrictor responses.

Reduction in NO production. Inhibitors of NOS cause a marked acute potentiation of EDCF-mediated responses of the rat aorta (Auch-Schwelk *et al.* 1992; Yang *et al.* 2002). Previous exposure to NO, whether released from the endothelium (by acetylcholine or the calcium ionophore A23187) or provided by NO donors, results in a prolonged inhibition of endothelium-dependent contractions (Tang *et al.* 2005*b*). Therefore, most experiments (at least in the authors' laboratory) investigating EDCF-mediated responses are performed in the presence of an inhibitor of NOS, to optimize endothelium-dependent, cyclooxygenase-dependent contractions. In addition to unmasking EDCF-mediated responses, a reduction in endothelial NO production can sensitize the underlying vascular smooth muscle to hypoxia. When isolated arteries and veins are suddenly made hypoxic, this results in a distinct endothelium-dependent contraction (De Mey & Vanhoutte, 1982, 1983; Katusic & Vanhoutte, 1986; Iqbal & Vanhoutte, 1988; Gräser & Vanhoutte, 1991; Hoshino *et al.* 1994; Pearson *et al.* 1996). The hypoxia-induced endothelium-dependent contraction involves a diffusible factor (Rubanyi & Vanhoutte, 1985), which does not require the activity of cyclooxygenase. It is absent in preparations incubated with inhibitors of endothelial NOS but can be induced in preparations without endothelium by exogenous NO donors (Gräser & Vanhoutte, 1991; Pearson *et al.* 1996), which suggests the involvement of a critical concentration of NO. The hypoxic response of coronary arteries is potentiated *in vitro* and *in vivo* by previous ischaemia–reperfusion injury (Pearson *et al.* 1996) which makes the phenomenon highly relevant as a contributor to coronary vasospasm. However, the exact mechanism by which a reduction in NO production underlies this type of endothelium-dependent contraction remains elusive.

Reduction in EDHF-mediated responses. In the renal artery of WKY, inhibitors of EDHF-mediated responses potentiate the endothelium-dependent component of the contraction elicited by acetylcholine, suggesting that the absence of endothelium-dependent hyperpolarizations favours the production or the action of EDCF (Michel *et al.* 2008*a*). This is not seen in the renal artery of the SHR, presumably because the EDHF-mediated responses are already blunted in arteries of the hypertensive strain (Fujii *et al.* 1992; Hayakawa *et al.* 1995; Dohi *et al.* 1996; Hutri-Kahonen *et al.* 1997; Bussemaker *et al.* 2003; Michel *et al.* 2008*a*).

Modulation by oxygen-derived free radicals. To estimate the actual involvement of oxygen-derived free radicals (ROS) in cyclooxygenase-, endothelium-dependent contractions is beyond the scope of this focused review, as it appears variable depending on the species, the blood vessel and sometimes the laboratory involved. For example, superoxide dismutase (SOD), that does not permeate cells, abolishes endothelium-dependent contractions in the canine basilar artery (implying a pivotal role for superoxide anions as intercellular messengers; Katusic & Vanhoutte, 1989) and reduces them in layered 'sandwich' preparations (Yang *et al.* 2003) but not in intact rings (Auch-Schwelk *et al.* 1989) of SHR aorta. Tiron, a cell-permeable scavenger of superoxide anions, reduces endothelium-dependent contractions to acetylcholine in the SHR aorta in studies carried out in Paris (Yang *et al.* 2002) but not in Hong Kong (Tang & Vanhoutte, 2008*a*). In the same preparation, acetylcholine

causes a burst of endothelial free radical production, which is larger in the endothelium of the SHR than in that of the WKY (Tang *et al.* 2007). Since the burst of ROS is prevented by indomethacin, cyclooxygenase appears to be the main source of free radicals under these conditions, and their production is a secondary event (Tang *et al.* 2007). However, once produced, the free radicals can amplify the EDCF-mediated response. They probably do so in part by activating/facilitating the production of vasoconstrictor prostanoids in the vascular smooth muscle cells (Auch-Schwelk *et al.* 1989; Yang *et al.* 2002, 2003; Álvarez *et al.* 2008), possibly reaching the latter through the shielded channels constituted by the myo-endothelial gap junctions (Tang & Vanhoutte, 2008*a*). Whether or not the ROS, liberated by the endothelial cyclooxygenase, can activate the enzyme through a positive feedback mechanism is still uncertain. In the case of diabetes, the production of ROS may play a more crucial role in triggering and amplifying EDCF-mediated responses (Shi *et al.* 2007*b*, 2008; Shi & Vanhoutte, 2008). Obviously, the scavenging action of superoxide anions on NO, by reducing the bioavailability of the latter (Rubanyi & Vanhoutte, 1986; Gryglewski *et al.* 1986; Auch-Schwelk *et al.* 1992; Cosentino *et al.* 1994; Tschudi *et al.* 1996; DeLano *et al.* 2006; Miyagawa *et al.* 2007; Macarthur *et al.* 2008) will also favour the occurrence of endothelium-dependent contractions.

Human relevance. The observations that indomethacin potentiates the relaxations to acetylcholine in isolated renal arteries of aged patients (Lüscher *et al.* 1987*a*) and the vasodilator response to the muscarinic agonist in the forearm of people with essential hypertension (Taddei *et al.* 1995, 1997*a*,*b*) suggest that endothelium-derived vasoconstrictor prostanoids also contribute to endothelial dysfunction in the human. This conclusion is supported by the finding that the TP-receptor inhibitor terutroban improves endothelial function in patients with coronary disease (Belhassen *et al.* 2003). To judge from the

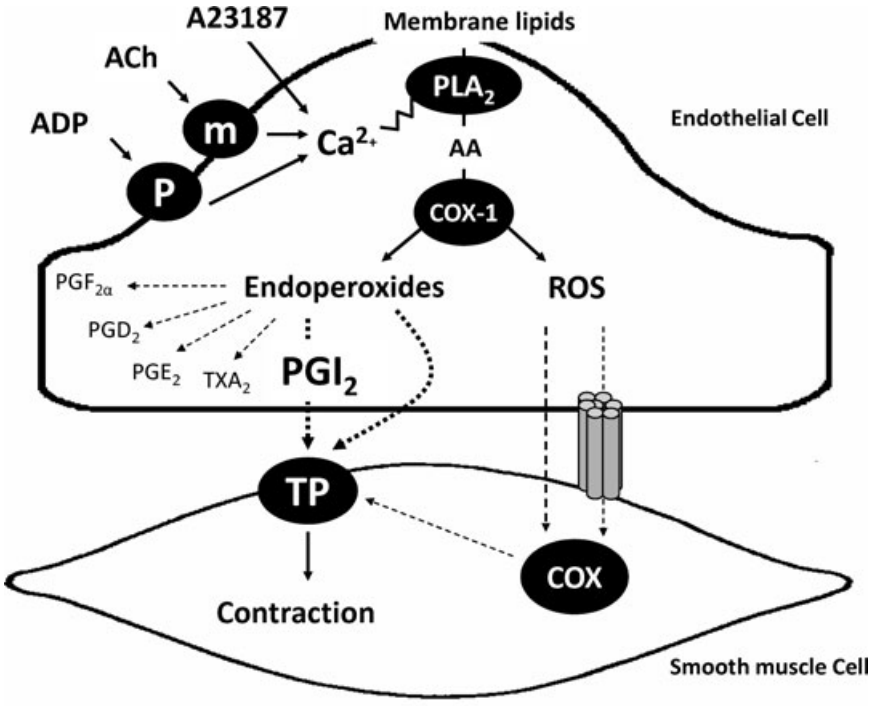

Figure 4
The chain of events leading to the occurrence of endothelium-dependent contractions first involves an abnormal increase in intracellular calcium (which can be evoked by receptor-dependent agonists, such as acetylcholine or ADP, or mimicked with calcium-increasing agents, such as the calcium ionophore A23187) that presumably activates phospholipase A_2 to release arachidonic acid. The endothelial COX-1 isoform metabolizes the fatty acid into endoperoxides which *per se* are EDCF or are transformed predominantly into prostacyclin that subsequently causes contraction by activating the TP receptors of the underlying vascular smooth muscle cells. Reactive oxygen species generated in the endothelium may reach the smooth muscle layer by passive diffusion or through myoendothelial gap junctions and serve to amplify TP receptor-mediated contractions by activating the cyclooxygenase of the vascular smooth muscle. AA, arachidonic acid; ACh, acetycholine; ADP, adenosine diphosphate; m, muscarinic receptors; P, purinergic receptors; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGI_2, prostacyclin; PLA_2, phospholipase A_2; ROS, reactive oxygen species; TXA_2, thromboxane A_2.

comparison of the effect of indomethacin in different age groups, the contribution of vasoconstrictor prostanoid augments with advancing age (Taddei *et al.* 1995, 1997*b*), as it does in animal blood vessels.

Conclusion

The sequence of events (Fig. 4) that leads to endothelium-dependent contractions first requires an increase in endothelial Ca^{2+} concentration, which activates endothelial COX-1, leading to the production of EDCF(s). The major prostanoids involved in EDCF-mediated contractions are endoperoxides, prostacyclin and, to a lesser extent, thromboxane A_2. They activate TP receptors of the vascular smooth muscle cells which initiate the contractile process. Reactive oxygen species may stimulate cyclooxygenase both in the endothelium and in the vascular smooth muscle, with subsequent activation of the TP receptors by the produced prostanoids. Dysfunction in calcium handling is the leading causal factor for the exacerbated occurrence of endothelium-dependent contractions in the aorta of the SHR. An increased expression of endothelial COX-1, prostacyclin synthase, thromboxane synthase and enhanced TP receptor sensitivity are not prerequisites for but intensify the magnitude of endothelium-dependent contractions.

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