COX-mediated endothelium-dependent contractions: from the past to recent discoveries

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Abstract

Endothelial cells release various substances to control the tone of the underlying vascular smooth muscle. Nitric oxide (NO) is the best defined endothelium-derived relaxing factor (EDRF). Endothelial cells can also increase vascular tone by releasing endothelium-derived contracting factors (EDCF). The over-production of EDCF contributes to the endothelial dysfunctions which accompanies various vascular diseases. The present review summarizes and discusses the mechanisms leading to the release of EDCFs derived from the metabolism of arachidonic acid. This release can be triggered by agonists such as acetylcholine, adenosine nucleotides or by stretch. All these stimuli are able to induce calcium influx into the endothelial cells, an effect which can be mimicked by calcium ionophores. The augmentation in intracellular calcium ion concentration initiates the release of EDCF. Downstream processes include activation of phospholipase A_2 (PLA_2), cyclooxygenases (COX) and the production of reactive oxygen species (ROS) and vasoconstrictor prostanoids (endoperoxides, prostacyclin, thromboxane A_2 and other prostaglandins) which subsequently diffuse to, and activate thromboxane-prostanoid (TP) receptors on the vascular smooth muscle cells leading to contraction.

Keywords: cyclooxygenase; EDCF; endothelium; gap junctions; phospholipase A_2; prostanoids; reactive oxygen species; TP-receptors.

Introduction

Following the first report by Furchgott and Zawadzki (1980) that in response to acetylcholine, endothelial cells release a vasodilator substance [endothelium-derived relaxing factor (EDRF)] later identified as nitric oxide (NO), a number of other inhibitory endothelial signals [endothelium-derived hyperpolarizing factors (EDHF)] have been shown to contribute to relaxations of the underlying vascular smooth muscle cells (Furchgott and Vanhoutte, 1989; Luescher and Vanhoutte, 1990; Vanhoutte, 1993; Gluais et al., 2005a; Gluais et al., 2005b; Feletou and Vanhoutte, 2006; Shi et al., 2006; Michel et al., 2008). In addition, it soon became apparent that under certain circumstances the endothelium can also produce diffusible substances [endothelium-derived contracting factors (EDCF)] which activate the contractile process in the underlying vascular smooth muscle cells (De Mey and Vanhoutte, 1982). Besides receptors-mediated agonists such as thrombin, acetylcholine and adenosine nucleotides (ADP and ATP) (Luescher and Vanhoutte, 1986; Katusic et al., 1988; Mombouli and Vanhoutte, 1993), stretch can also elicit endothelium-dependent contractions, at least in canine cerebral arteries (Katusic et al., 1987). The early

observation that such endothelium-dependent contractions could be prevented by inhibitors of cyclooxygenase suggested that down-stream products of this enzyme, i.e. prostanoids, were likely candidates as EDCF (Miller and Vanhoutte, 1985; Luescher and Vanhoutte, 1986; Auch-Schwelk et al., 1989; Yang et al., 2002; Yang et al., 2003a). Although endothelial cells can produce vasoconstrictors including endothelin-1 and angiotensin II, there is lack of convincing evidence showing a direct link between these substances and instantaneous changes in tension that can be attributed to the release of EDCF. Thus, the present article focuses on the mechanisms leading to the production of endothelial and cyclooxygenase-derived vasoconstrictors, and updates earlier reviews on this topic (Feletou et al., 2009; Shi and Vanhoutte, 2009; Tang and Vanhoutte, 2009).

Endothelial calcium concentration

An increase in intracellular calcium concentration in the endothelial cells is the triggering event leading to the release of EDCF. This conclusion is based on the following observations: (a) Activation of cell membrane receptors by agonists such as acetylcholine [activating endothelial M3-muscarinic receptors (Boulanger et al., 1994)], ADP and ATP [activating purinoceptors (Koga et al., 1989; Mombouli and Vanhoutte, 1993)], which are known to induce the release of calcium from the

sarcoplasmic reticulum (Liu et al., 2009), initiate the production of EDCF; (b) Reduction in the extracellular calcium concentration decreases endothelium-dependent contractions (Okon et al., 2002); (c) Calcium ionophores such as A23187 elicit endothelium-dependent contractions (Katusic et al., 1988; Gluais et al., 2006; Shi et al., 2007a; Tang et al., 2007; Wong et al., 2008); (d) Endothelium-dependent contractions induced by acetylcholine in the rat aorta are accompanied by an increase in cytosolic endothelial calcium concentration (Tang et al., 2007; Wong et al., 2008) and this increment is greater in preparations of spontaneously hypertensive rats (SHR) compared to those of age-matched normotensive Wistar-Kyoto rats (WKY), in line with the larger EDCF-mediated responses in the former (Luescher and Vanhoutte, 1986; Yang et al., 2003a; Tang et al., 2007). On the other hand, no significant difference in the increase of calcium concentration in the two strains was observed if the aortae were exposed to A23187 (Tang et al., 2007).

Phospholipase A_2

The increase in endothelial concentration of the activator ion elicited by agonists such as acetylcholine involves two steps, release of calcium from the sarcoplasmic reticulum followed by influx of extracellular calcium. Acetylcholine binds to the G

proteins-coupled muscarinic receptors on the endothelial cell membrane and activates phospholipase C. The latter produces inositol triphosphate which in turn causes the release of calcium from intracellular stores. The resulting calcium-depletion process leads to the production of a messenger termed calcium influx factor [CIF; (Randriamampita and Tsien, 1993)] which displaces the inhibitory calmodulin from the calcium-independent phospholipase A_2 [$iPLA_2$; (Wolf and Gross, 1996; Wolf et al., 1997; Trepakova et al., 2000; Smani et al., 2004)]. Activation of $iPLA_2$ is an initiating event in the generation of EDCF induced by acetylcholine in the rat aorta (Wong et al., 2010). Activated $iPLA_2$ produces lysophospholipids which facilitate the opening of store-operated calcium channels (SOCs) leading to the influx of extracellular calcium into the endothelial cells (Trepakova et al., 2001; Smani et al., 2004). This large influx of calcium ions then activates the calcium-dependent phospholipase A_2 ($cPLA_2$) which converts membrane phospholipids to arachidonic acids, the precursor of prostanoids (**Figure 1**). That the calcium-dependent form of phospholipase A_2 is crucial for the ultimate production of EDCF is demonstrated by the observation that a specific inhibitor of $iPLA_2$ does not affect A23187-induced endothelium-dependent contractions, while quinacrine, which inhibits both forms of the enzyme, abolishes the response to both acetylcholine and A23187 (Luescher and Vanhoutte, 1986; Wong et al., 2010).

Vitamin D and EDCF

High concentrations of vitamin D appear to have an acute protective effect on endothelial cells by reducing the production of EDCF. Indeed, the *in vitro* administration of 1,25-dihydroxyvitamin D_3 [the most active metabolite of vitamin D (Holick, 2003)] reduces EDCF-mediated responses induced by acetylcholine but not by the calcium ionophore A23187 in aorta of both SHR and WKY (Wong et al., 2008), suggesting that vitamin D acutely reduces EDCF production by an action upstream of the increase in calcium concentration and thus interferes with the calcium surging process (**Figure 1**) (Wong et al., 2008).

Cyclooxygenase

The two isoforms of cyclooxygenase (COX), COX-1 and COX-2, have a comparable ability to catalyze the transformation of arachidonic acid into prostaglandins (**Figure 2**) (Garavito and DeWitt, 1999). Both isoforms can play a key role in the generation of EDCF depending on the species, the blood vessel studied and the health conditions of the donor (Furchgott and Vanhoutte, 1989; Luescher and Vanhoutte, 1990; Kauser and Rubanyi, 1995; Shi et al., 2007a; Tang and Vanhoutte, 2009; Vanhoutte, 2009; Vanhoutte et al., 2009; Wong et al., 2009). COX-1 is constitutively

expressed in most tissues while COX-2 is inducible (Vane et al., 1998; Davidge, 2001). Early studies demonstrated that non-selective COX inhibitors abolish endothelium-dependent contractions (Miller and Vanhoutte, 1985; Katusic et al., 1988), an observation that has been repeated over the years. Selective inhibitors of COX-1, but not those of COX-2, abrogate endothelium-dependent contractions in the rat aorta (Ge et al., 1995; Ospina et al., 2003; Yang et al., 2003a; Yang et al., 2004a). In that preparation, COX-1 is expressed in both endothelial and vascular smooth muscle cells, but the over-expression of this isoform seen in the SHR aorta is confined to the endothelial cells (Tang and Vanhoutte, 2008b). Likewise, bioassay studies demonstrate that only the activation of endothelial COX contributes to the generation of diffusible EDCF in the SHR aorta (Yang et al., 2003a). Endothelium-dependent contractions are present in the aorta of COX-2, but not in that of COX-1 knock-out mice (Tang et al., 2005b). Taken in conjunction, these findings demonstrated that COX-1 is the preferential constitutive isoform of cyclooxygenase which mediates endothelium-dependent contractions in large arteries of rat and mice. However, with aging or disease, COX-2 can be induced and then contributes to EDCF-mediated responses (Camacho et al., 1998; Blanco-Rivero et al., 2005; Shi et al., 2008; Matsumoto et al., 2009). By contrast, constitutively expressed COX-2 plays a dominant role in the endothelium-dependent contraction of the hamster aorta

irrespective of age (Wong et al., 2009).

Prostanoids

Cyclooxygenase converts arachidonic acid into endoperoxides (PGH_2), the intermediate of the prostanoid biosynthesis, which can either act as an EDCF *per se* (Asano et al., 1994; Ge et al., 1995) or be further transformed into prostacyclin (PGI_2), thromboxane A_2 and various other prostaglandins including prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by their respective synthases (**Figure 2**) (Gluais et al., 2005c; Gluais et al., 2006; Gluais et al., 2007; Tang and Vanhoutte, 2008b).

Although PGH_2 has a relatively short half-life and is unstable (Auch-Schwelk et al., 1990), it can be a vasoconstrictor EDCF (Ge et al., 1995; Gluais et al., 2006; Gluais et al., 2007; Tang and Vanhoutte, 2008b) by activating TP receptors of vascular smooth muscle (Auch-Schwelk et al., 1990; Ito et al., 1991; Ge et al., 1995). This conclusion is supported by two observations: (a) The aorta of SHR releases more PGH_2 than that of WKY when exposed to acetylcholine (Ge et al., 1995). (b) Similarly to the acetylcholine-induced EDCF-mediated responses, PGH_2-induced contractions in aortae without endothelium are transient and are larger in SHR compared to WKY (Ge et al., 1995; Gluais et al., 2005c). In addition, when tyrosine nitration caused by

the local production of peroxynitrite inhibits the activity of prostacyclin synthase (Zou et al., 2002), PGH_2 may become even more important in the process.

Prostacyclin is the major cyclooxygenase-derived metabolite of arachidonic acid in endothelial cells (Moncada et al., 1976). During endothelium-dependent contractions of rodent aortae in response to acetylcholine, its production is markedly larger than that of other prostaglandins and, together with PGH_2, prostacyclin becomes a major EDCF (Ge et al., 1995; Blanco-Rivero et al., 2005; Gluais et al., 2005c; Feletou et al., 2009; Tang and Vanhoutte, 2009). This conclusion is in line with the findings that the gene expression of PGI synthase in the rat aortic endothelial cells is greatly augmented by aging and spontaneous hypertension (Tang and Vanhoutte, 2008b).

During ADP- and A23187-induced endothelium-dependent contractions, the release of thromboxane A_2 is augmented and an inhibitor of thromboxane A_2 can reduce these contractions, unlike those to acetylcholine (Auch-Schwelk et al., 1990; Gluais et al., 2006; Gluais et al., 2007). Therefore, thromboxane A_2 can be regarded as a key EDCF during the EDCF-mediated responses elicited by these agents. Likewise, in certain blood vessels (hamster aorta) or with aging and disease (such as diabetes), an augmented contribution of PGE_2 and $PGF_{2\alpha}$ to EDCF-mediated contractions may become obvious (Matsumoto et al., 2009a; Wong et al., 2009). This can be explained best by the increased generation of these prostaglandins under conditions of enhanced

oxidative stress (Gryglewski et al., 1986), in particular as a consequence of the augmented formation of peroxynitrite which inhibits PGI synthase (Zou et al., 1999; Zou et al., 2002) and diverts arachidonic acid towards PGE_2 and $PGF_{2\alpha}$ synthases (Gluais et al., 2005). Obviously, the involvement of individual prostanoids in EDCF-mediated responses varies depending on the species, the blood vessels studied, the endothelium-dependent agonist used, and the age and disease state of the donor.

Reactive oxygen species

Reactive oxygen species (ROS) are generated during a number of normal metabolic activities, but their overproduction leads to oxidative stress which is commonly observed in hypertension, diabetes and atherosclerosis (Liu et al., 2005; Shi et al., 2007b; Shi and Vanhoutte, 2009). During the generation of prostanoids by COX, ROS are formed as by-products. ROS of relevance for endothelium-dependent responses include superoxide anions (O_2^-), hydroxyl radicals (· OH) and hydrogen peroxide (H_2O_2) (**Figure 3**). ROS either directly act as EDCF (Katusic and Vanhoutte, 1989; Katusic et al., 1993) or indirectly potentiate EDCF-mediated responses by reducing the bioavailability of NO (Rubanyi and Vanhoutte, 1986; Huie and Padmaja, 1993; Paolocci et al., 2001) and activating COX in the vascular smooth muscle cells (Auch-Schwelk et al., 1989; Hibino et al., 1999; Yang et al., 2002). This conclusion is

based on the following observations: (a) An increased ROS production accompanies acetylcholine- or A23187-induced endothelium-dependent contractions (Tang et al., 2007); (b) Tiron (which scavenges superoxide anions intracellularly) or catalase (which converts hydrogen peroxide to water and oxygen) plus deferoxamine (which prevents the formation of hydroxyl radicals) reduce endothelium-dependent contractions in the SHR aorta (Yang et al., 2003b) and the femoral artery of diabetic rats (Shi et al., 2007b), suggesting that superoxide anions and hydrogen peroxide augment or even mediate part of the response; (c) ROS formed by the xanthine plus xanthine oxidase reaction elicit contractions of SHR aortae without endothelium which are prevented by both COX inhibitors and TP receptor antagonists, suggesting that the oxygen-derived free radicals stimulate COX in the vascular smooth muscle to produce prostanoids which in turn activate their TP receptors (Auch-Schwelk et al., 1989; Yang et al., 2003b). (d) ROS increase the degradation of nitric oxide (Rubanyi and Vanhoutte, 1986; Paolocci et al., 2001); (d) Peroxynitrite, a strong cytosolic oxidant generated by the reaction of the superoxide anions and nitric oxide, inactivate PGI synthase (Zou et al., 1999; Zou et al., 2002) and shifts the production of prostacyclin to that of other vasoconstrictor prostanoids (Cohen, 2002; Gluais et al., 2005). (e) In canine basilar arteries, superoxide dismutase (SOD) plus catalase abolish the A23187 induced endothelium-dependent contractions but not the production of

prostaglandins and thromboxane A_2 indicating that ROS rather than COX-derived prostanoids are the EDCF in this particular artery (Katusic and Vanhoutte, 1989); (f) In the rat pulmonary artery, ROS induce contraction involving the activity of protein kinase C in the vascular smooth muscle (Jin et al., 1991); (g) In vascular smooth muscle of the rat aorta, the ROS-induced calcium sensitization is mediated through the activation of Rho and a subsequent increase in Rho kinase activity (Jin et al., 2004), and the latter is crucial in the response to EDCF (Chan et al., 2009); and (h) ROS directly depolarize vascular smooth muscle by inhibiting ATP-sensitive potassium channel (K_{ATP}), voltage-activated potassium channel (K_v) and large conductance calcium-activated potassium channel (BK_{Ca}) (Kinoshita et al., 2004; Li et al., 2004; Tang et al., 2004).

Gap junctions

The contact between endothelial and vascular smooth muscle cells is important in the genesis of endothelium-dependent contractions. This conclusion is supported by the observation that the endothelium-dependent contractile response to acetylcholine of layered bioassay ("sandwich") preparations of SHR aortae is much smaller than that of intact aortic rings (Yang et al., 2003b). The contraction in the "sandwich preparation" is caused by prostanoids which diffuse across the intracellular gap

between the donor (containing endothelial cells) and the recipient strip (without endothelium, responsible for the contraction). Under bioassay conditions, superoxide dismutase plus catalase (both compounds with poor cell permeability) can reduce the acetylcholine-induced endothelium-dependent contractions while they have no effect in intact rings in which tiron inhibits EDCF-mediated responses (Auch-Schwelk et al., 1989; Yang et al., 2003a). These observations imply that in intact rings, ROS exert their facilitatory effect by either acting in the endothelial cells or being transported from the latter to the vascular smooth muscle cells via preferential channels not accessible to superoxide dismutase. One possible route would be the myoendothelial gap junctions (**Figure 4**), since the gap junction inhibitor carbenoxolone reduces endothelium-dependent contractions to acetylcholine and the calcium ionophore A23187 (Tang and Vanhoutte, 2008a).

Prostanoid receptors and Rho kinase

Thromboxane-prostanoid receptors (TP-receptors) are the most important prostanoid receptor subtype involved in endothelium-dependent contractions since TP receptor antagonists abolish these responses (Auch-Schwelk et al., 1990; Kato et al., 1990; Yang et al., 2003a). All prostanoids can bindto and activate TP receptors, albeit with different affinities (Dickinson and Murphy, 2002). Thromboxane A_2 is the most potent

agonist at TP receptors. Endoperoxides and prostacyclin also activate TP receptors and both of them evoke transient contractions (probably due to their short half-life) which mimic acetylcholine-induced endothelium-dependent contractions (Gluais et al., 2005). Binding of EDCF to the TP receptors in turn activates the downstream Rho kinase pathway leading to the increased contractile activity of the vascular smooth muscle (Chan et al., 2009).

In the SHR aorta, the gene expression levels and protein presence of TP receptors are not altered, but the responsiveness to endoperoxides is augmented compared to WKY preparations (Ge et al., 1995; Tang and Vanhoutte, 2008b). This hyperresponsiveness contributes to the prominence of EDCF-mediated responses in the aorta of the SHR. Another crucial aspect in this prominence is that the vascular smooth muscle of aging WKY and of the SHR have lost the ability to respond with relaxation to prostacyclin, despite an unchanged expression of IP receptors and the large production of prostacyclin by endothelial cells exposed to acetycholine or A23187 (Levy, 1980; Rapoport and Williams, 1996; Gluais et al., 2005; Tang and Vanhoutte, 2008b)

Interactions between NO, EDHF and EDCF

In the SHR aorta, the concomitant release of NO inhibits endothelium-dependent contractions to acetylcholine (Auch-Schwelk et al., 1992; Yang et al., 2004b), an

observation that has lead to the systematic use of inhibitors of NO synthases when studying EDCF-mediated responses. In addition, previous exposure to endothelium-derived NO or exogenous NO-donors causes a long-term inhibition of EDCF-mediated responses (Tang et al., 2005a; Feletou et al., 2008). Likewise, in the renal artery of the rat, the absence of EDHF favours the occurrence of endothelium-dependent contractions (Michel et al., 2008).

Alternatively, EDCF may also counteract the action of endothelium-derived relaxing factors. Thus, in WKY mesenteric artery, EDHF-mediated relaxations are attenuated by the release of EDCF (Sekiguchi et al., 2002). This attenuation is explained best by the EDCF-induced activation of TP-receptors which depolarizes the vascular smooth muscle cells by inhibiting K_v and BK_{Ca} (Scornik and Toro, 1992; Cogolludo et al., 2003).

Physiological importance

In the early nineteenth century, Bayliss showed that an increase in the internal pressure in the carotid artery of the dog caused its constriction, a seminal observation leading to the concept of autoregulation (Bayliss, 1902). In isolated basilar arteries of the same species, stretch induces a contraction which disappears after the removal of the endothelium, demonstrating an endothelium-dependent process (Katusic et al.,

1987). This contraction is sensitive to both the COX inhibitor indomethacin and the calcium-influx blocker diltiazem, suggesting that the activity of COX (presumably in the endothelial cells) and the influx of extracellular calcium (presumably in the vascular smooth muscle cells) are required for the active response to stretch (Katusic et al., 1987). Likewise, in bovine coronary arteries, stretch elicits an endothelium-independent contraction which requires the activation of NAD(P)H oxidase (Oeckler et al., 2003). Stretch also directly activates various cation channels on the smooth muscle cells of small arteries facilitating their contraction (Setoguchi et al., 1997; Hill et al., 2001; Wu and Davis, 2001). Oxygen-derived free radicals play a key role in endothelium-dependent contractions of the canine basilar artery (Katusic and Vanhoutte, 1989). Thus, it is tempting to speculate that the endothelium-dependent contraction evoked by stretch (resulting from activation of endothelial COX, the production of ROS and the hypersensitivity of the vascular smooth muscle) may initiate the autoregulatory response, at least in cerebral arteries.

Pathophysiological relevance

As mentioned already, endothelium-dependent contractions are exacerbated by aging, diabetes, hypertension and atherosclerosis (Taddei et al., 2006; Vanhoutte and Tang, 2008; Vanhoutte et al., 2009). Foe example, the blunted endothelium-dependent

relaxations in response to acetylcholine in diabetic animals is partly due to the augmented production of EDCF, resulting from the over-expression and activation of COX and increased ROS production after the chronic exposure of the endothelial cells to high glucose levels (De Vriese et al., 2000). In essential hypertensive patients, the blunted vasodilatation induced by acetylcholine can almost be normalized by the COX inhibitor indomethacin indicating that COX-derived vasoconstrictors are key players responsible for the abnormal endothelial response (Taddei et al., 1998). This indomethacin-sensitive impairment of the response to acetylcholine is accentuated by aging (Taddei et al., 2006). However, in secondary hypertension, inhibition of COX does not restore the acetylcholine-induced vasodilatation suggesting that EDCFs are not equally important in all cases of hypertension. It is likely that the prominence of endothelium-dependent contractions observed in arteries of aging and diseased (essential hypertension, diabetes) animals and human reflects the progressive inability of the endothelial cells to generate enough NO to curtail the production of EDCF (Tang et al., 2005a; Feletou et al., 2008; Vanhoutte et al., 2009). Shifting from the normal release of NO (and EDHF) to that of EDCF likely plays an important role in the development of vascular disease (Vanhoutte, 1997; Vanhoutte et al., 2009).

Conclusion

Endothelial cells release COX-derived vasoconstrictor prostanoids and reactive oxygen species, which have been termed EDCF. In the SHR, prostacyclin becomes a prominent EDCF acting on TP-receptors, even more so that IP receptor signaling is impaired (Rapoport and Williams, 1996; Gluais et al., 2005; Feletou et al., 2009). EDCF-mediated responses are amplified in aging normotensive animals (Koga et al., 1989; Wong et al., 2009), hypertensive (Luescher and Vanhoutte, 1986) and diabetic (Tesfamariam et al., 1989; Shi et al., 2007b; Shi et al., 2008; Shi and Vanhoutte, 2009) animals. In humans, EDCF plays a role in the endothelial dysfunction that accompanies aging, atherosclerosis, myocardial infarction and essential hypertension (Boulanger, 1999; Vita and Keaney, 2002; Taddei et al., 2006).

Author contribution

Sze-Ka Wong and Paul M. Vanhoutte wrote this review article together.

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Figure 1 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 $iPLA_2$. Activated $iPLA_2$ 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 $cPLA_2$ which catalyze the production of arachidonic acids (AA). The later is then metabolized by cyclooxygenase-1 (COX-1) to prostanoids. 1,25-Dihydroxyvitamin D_3 (Vit D) acutely reduces endothelium-dependent contraction by inhibiting the calcium surge. $cPLA_2$ = calcium dependent phospholipase A_2; EC = endothelial cells; $iPLA_2$ = calcium independent phospholipase A_2; PGD_2= prostaglandin D_2; PGE_2 = prostaglandin E_2; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; PGH_2 = endoperoxides; PGI_2 = prostacyclin; PL = phospholipids; SERCA = sarco/endoplasmic reticulum Ca^{2+}-ATPase; SR = sarcoplasmic reticulum; TXA_2 = thromoboxane A_2.

Figure 2 Metabolism of arachidonic acid and its downstream metabolites. Arachidonic acid is converted to endoperoxides by the activity of cyclooxygenases (COX). Endoperoxides are then converted to various prostaglandins by their respective synthase. **Michael: I added Cox-1 & 2; can you fit that into the usual endothelial cells?**

Figure 3 Formation of oxygen-derived free radicals of relevance for endothelium-dependent responses, and pharmacological agents commonly used to determine their importance. Superoxide anions (O_2^-) can be generated from molecular oxygen by the actions of various enzymes. O_2^- can react with NO to form peroxynitrite ($ONOO^-$). It can also be converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). H_2O_2 can be transformed to hydroxyl radicals by ferrous ions or converted to H_2O by catalase and glutathione. Tiron scavenges O_2^- inside cells. DETCA inhibits SOD. Deferoxamine is an iron chelator that scavenges hydroxyl radicals. L-NAME inhibits NO synthase. MnTMPyP mimics the combined effect of SOD and catalase. DETCA = diethyldithiocarbamic acid; GSH = glutathione; GSSG = glutathione disulphide; L-NAME = N^{ω}-nitro-L-arginine methyl ester hydrochloride; MnTMPyP = Mn(III)tetrakis(1-methyl-4-pyridyl)porphyrin pentachloride; NO = nitric oxide; tiron = 4,5-dihydroxy-1,3-benzenedisulphonic acid. (Aapted from Shi et al. 2007, by permission).

Figure 4 Endothelium-dependent contraction comprises of two components: endoperoxides (and their downstream metabolites) and ROS. Both components require an increase in endothelial calcium concentration, the activity of endothelial COX and the stimulation of the TP receptors to elicit contraction. Prostanoids diffuse from the endothelial cell to the vascular smooth muscle cell while ROS utilize both diffusion and possibly myoendothelial gap junctions. AA = arachidonic acid; ACh = acetylcholine; COX-1 = cyclooxygenase-1; H_2O_2 = hydrogen peroxide; 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; PGIS = prostacyclin synthase; PLA_2 = phospholipase A_2; ROS = reactive oxygen species; TP = thromboxane-prostanoid receptors; TXA2 = thromboxane A2; TXAS = thromboxane synthase. X = xanthine; XO = xanthine oxidase. **Michael: please ask Eva for the exact reference!**