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Vascular oxidative stress: the common link in hypertensive and diabetic vascular disease

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Abstract

Vascular disease in hypertension and diabetes is associated with increased oxidants. The oxidants arise from NADPH oxidase, xanthine oxidase, and mitochondria. Superoxide anion and hydrogen peroxide are produced by both leukocytes and vascular cells. Nitric oxide is produced in excess by inducible nitric oxide synthase, and the potent oxidant, peroxynitrite, is formed from superoxide and nitric oxide. The damage to proteins caused by oxidants is selective, affecting specific oxidant-sensitive amino acid residues. With some important vascular proteins, for example endothelial nitric oxide synthase, prostacyclin synthase, and superoxide dismutase, oxidation of a single susceptible amino acid inactivates the enzyme. The beneficial effects of antioxidants, at least in animal models of hypertension and diabetes, can in part be ascribed to protection of these and other proteins. Mutant proteins lacking their reactive constituent can recapitulate some disease phenotypes suggesting a pathogenic role of the oxidation. Thus, many of the shared functional abnormalites of hypertensive and diabetic blood vessels may be caused by oxidants. Although studies using antioxidants have failed in patients, the successful treatment of vascular disease with HMG CoA reductase inhibitors, thromboxane A_2 antagonists, and polyphenols may depend upon their anti-inflammatory effects and ability to decrease production of damaging oxidants.

1. Introduction

Among all cardiovascular risk factors, diabetes mellitus (DM) and hypertension are the leading causes of cardiovascular diseases. Unfortunately, these two risk factors often co-exist, such that 60% of patients with diabetes are hypertensive, and up to 20% of subjects with hypertension are diabetic[1]. The worldwide morbidity of DM has increased rapidly even in developing countries, doubling the combined risk of cardiovascular events in patients with hypertension[2, 3]. The endothelium is the principal target of cardiovascular risk factors, including hypertension and diabetes, and is the cell most involved in the development of vascular inflammation and atherosclerosis[4]. Although low levels of reactive oxygen species (ROS) can play a physiological role in maintaining cardiac and vascular integrity, elevated levels of ROS play a pathophysiological role in cardiovascular dysfunction associated with hypertension and diabetes. Normally, ROS are produced in the vessel wall in a controlled and tightly regulated manner. Under physiological conditions, low concentrations of superoxide anion ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) are produced in cells by mitochondria and NADPH oxidases. They are controlled by endogenous antioxidants, manganese and copper/zinc superoxide dismutase (MnSOD, Cu/Zn SOD), catalase, and glutathione peroxidases. Together with nitric oxide (•NO) these ROS function as cell signaling initiators by their ability to introduce reversible post-translational protein

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modifications, such as *S*-nitroso- and *S*-glutathione adducts on cysteine thiols[5]. For example, introducing these adducts on proteins including p21ras[6, 7] and the sarcoplasmic reticulum calcium ATPase (SERCA)[8, 9] can regulate vascular smooth muscle cell (VSMC) contraction and relaxation and VSMC growth. Under pathological conditions increased ROS production leads to endothelial dysfunction, impaired vascular relaxation, increased VSMC growth and hypertrophy, as well as increased deposition of extracellular matrix proteins. Although mitochondria and xanthine oxidase are implicated as sources of damaging ROS in disease, NADPH oxidases, either in inflammatory leukocytes or vascular cells, account for the bulk of the current literature on vascular pathology. In contrast to the perceived generalized nature of the damage induced by elevated ROS, it is now being recognized that ROS can have selective effects on protein constituents, and they may selectively affect important cardiovascular proteins (Figure 1).

2. Reactive oxygen and nitrogen species

The principal ROS made by cells is $O_2^{-\bullet}$, an anion radical produced by reduction of oxygen. H_2O_2 is produced from $O_2^{-\bullet}$ either by spontaneous dismutation or enzymatic dismutation by the three isoforms of SOD - Cu/Zn, Mn, and extracellular Cu/Zn SOD. In normal tissues, most H_2O_2 is converted to water by catalase. •NO produced in vascular cells from constitutive endothelial nitric oxide synthase (eNOS) or inducible NOS, induced by inflammatory cytokines, reacts rapidly with $O_2^{-\bullet}$ to form the short-lived peroxynitrite ($OONO^-$). $OONO^-$ is termed a reactive nitrogen species (RNS) because of its high reactivity with protein, DNA, and lipids. Leukocyte myeloperoxidase (MPO), H_2O_2, and chloride anion can also produce hypochlorous acid (HOCl). When $O_2^{-\bullet}$ is kept low, •NO remains functionally active. When $O_2^{-\bullet}$ is elevated it not only destroys •NO, but the ROS and RNS mentioned here also oxidize protein targets of •NO (Figure 1).

3. NADPH oxidase as a source for increased ROS production

NADPH oxidase is the primary source of ROS in the vasculature and is functionally active in all cells within the vessel wall, including endothelial cells, VSMCs, fibroblasts and monocytes/macrophages[10, 11]. NADPH oxidase is a multi-component enzyme that is comprised of membrane components p22phox and gp91phox (termed Nox2, or its homologues Nox1, 3–5), and cytoplasmic components p47phox, p67phox and the small G protein, rac1, which plays a role in activating NADPH oxidase. Unlike leukocytes which express Nox2, rat VSMCs express mainly the Nox4 isoform, which together with p22phox are the major components of the active Nox4-based NADPH oxidase complex[12, 13]. Unlike the leukocyte oxidase which produces $O_2^{-\bullet}$ in a burst during activation, there is a continuous low-level of Nox4-derived ROS production in vascular cells, the activation of which does not require rac1, p67phox or p47phox[14–16]. Also, unlike the leukocytic NADPH oxidase whose NADPH oxidase releases $O_2^{-\bullet}$ into the extracellular space, the vascular NADPH oxidases release O_2^- intracellularly, where it and the ROS and RNS produced from it can act as intracellular signaling molecules or, when produced in excess, can cause damage.

4. Angiotensin II-induced Hypertension and ROS

Oxidative stress caused by increased ROS and RNS plays an important pathophysiological role in hypertension. Treatment with antioxidants or agents to inhibit NADPH oxidase decrease ROS production, prevent target-organ cellular damage, and decrease blood pressure in animal models and in human hypertension (reviewed in [ref. 17]). The renin-angiotensin-aldosterone system is a major activator of NADPH oxidase and ROS production in hypertension[18–20]. Angiotensin II (Ang II) stimulates NADPH oxidase both by increasing expression of NADPH oxidase subunits as well as by increasing ROS production in VSMCs, endothelial cells, adventitial fibroblasts[21, 22], and in intact arteries[23–26]. Many of

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these effects are mediated by Ang II type-1 (AT_1) receptors and are blocked by losartan[27, 28], except apparently in cultured adventitial fibroblasts[21]. Some of the therapeutic blood pressure-lowering effects of AT_1 receptor blockers in human patients may be attributed to reduction of oxidative stress and increase of plasma antioxidant capacity[29].

Several NADPH oxidases may play a role in blood pressure control. Overexpression on Nox1 in mouse VSMC causes a marked increase in systolic blood pressure and hypertrophy in response to Ang II[30]. In addition, in Nox1 knockout mice, the basal blood pressure is lower, and there was complete protection against Ang II-induced increase in blood pressure and medial hypertrophy. This was attributed to the elimination of $O_2^{-\bullet}$ production and improved •NO function[31, 32]. Nox2 appears to be equally important for the sequelae of Ang II-induced hypertension. Nox2 knockout mice had lower basal blood pressure compared to their controls[33], but blood pressure increased similarly in the two strains when Ang II was infused. Despite this, Ang II-induced vascular hypertrophy was entirely prevented in the Nox2 knockout mice and the elevated oxidants were eliminated. This indicates that while the pressor response to Ang II itself does not depend on Nox2 or $O_2^{-\bullet}$, the vascular oxidants and smooth muscle hypertrophy do[33]. In a model of renovascular hypertension Nox2-derived $O_2^{-\bullet}$ decreased •NO function, and there was marked protection from hypertension in the Nox2 knockout mice[34]. In low renin salt-sensitive hypertension, a tat-peptide inhibitor of Nox2 normalized ROS generation and endothelium-dependent vascular relaxation[35]. The dual roles of Nox1 and Nox2 in Ang II-induced hypertension in the mouse may be due to the fact that Nox1 is localized in VSMC, but Nox2 is primarily located in endothelial cells and adventitial fibroblasts in normal mice or those infused with Ang II[33]. Interpreting the roles of the different oxidases is also made more difficult by the potential paracrine roles played by diffusible •NO, $O_2^{-\bullet}$, and H_2O_2[25, 36]. Ang II increases leukocyte infiltration into the adventitia and intima, accounting for additional contribution to ROS production by leukocyte Nox2. The importance in Ang II-induced hypertension of p47phox, another component of NADPH oxidase involved in its activation, was demonstrated in p47phox knockout mice which failed to develop hypertension in response to Ang II infusion[37].

5. NADPH oxidase and Diabetes

Both acute and chronic hyperglycemia is associated with endothelial dysfunction[38, 39]. The deleterious effects of hyperglycemia in type 2 diabetes are often amplified by coexisting conditions associated with insulin resistance, including hyperlipidemia and hypertension. Activation of NAD(P)H oxidase is implicated in oxidative stress associated with hyperglycemia. Treatment of human umbilical vein endothelial cells with high glucose increases NADPH oxidase expression, levels of oxidative stress markers, and apoptosis[40]. Moreover, ROS production and expression of p22phox and p47phox are increased in mouse microvascular ECs treated with high glucose[41]. p47phox siRNA decreases glucose-stimulated O_2^- production in SMC, implicating involvement of p47phox upregulation and/or phosphorylation in ROS generation in response to hyperglycemia[42].

Accumulating evidence suggests that sustained NAD(P)H oxidase ROS generation contributes to endothelial dysfunction in diabetes[43, 44]. Antioxidants protect against the deleterious effects of high glucose on vascular endothelial cells[45]. The rac1-regulated NADPH oxidase subunits, Nox1 and Nox2, have been implicated in the abnormal endothelial vasodilatorfunction in diabetes[46]. Consistent with a role for these Nox isoforms, adenoviral vectors expressing DN rac-1 decrease $O_2^{-\bullet}$ production and significantly improve vascular relaxation[47]. The renin-angiotensin system is activated in diabetes, so maybe an important activator of NADPH oxidase. Ang II acts through the AT_1 receptor to inhibit many of the actions of insulin in the vasculature, including vasodilation. The increased AT1

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receptor/NAD(P)H oxidase activation appears to contribute to vascular insulin resistance, endothelial dysfunction, apoptosis, and inflammation[48].

6. Other Sources of Oxidants in Hypertension and Diabetes

Endothelial nitric oxide synthase (eNOS) consists of both oxidase and reductase domains. When the two enzymatic activities are uncoupled by lack of arginine substrate or tetrahydrobiopterin (BH_4) cofactor, eNOS produces $O_2^{-\bullet}$ in addition to •NO, making the enzyme a generator of $OONO^-$. $OONO^-$ resulting from uncoupling of eNOS has been implicated in Ang II-induced hypertension and diabetes[49, 50]. Xanthine oxidase is also a source of ROS in atherosclerosis and has been implicated in hypertension and diabetes[51, 52]. Some of the therapeutic effects of allopurinol used to control uric acid levels in many patients with hypertension and diabetes may result from inhibiting this enzyme. Excess mitochondrial production of ROS is implicated in the setting of hyperglycemia and hyperlipidemia in diabetes[53], but in part because mitochondrial inhibitors are so toxic and no deficient mouse models are available, the role of excess ROS from mitochondria during *in vivo* pathologies is poorly understood.

7. Oxidants and Vascular Function in Hypertension and Diabetes

Both in animal models and patients with diabetes and hypertension endothelium-dependent vasodilator responses to acetylcholine may be attenuated. Studies of diabetic and hypertensive rodent arteries have shown that resting arteries can contract in response to acetylcholine, suggesting that an endothelium-derived contractile agent is produced. In isolated aortic rings from diabetic rabbits, or rings from normal rabbits incubated in high glucose, both the impaired relaxations and endothelium-dependent contractions are prevented by cyclooxygenase inhibitors and thromboxane receptor antagonists, but not by thromboxane synthase inhibitors, suggesting that an eicosanoid, such as prostaglandin endoperoxide (PGH_2) or hydroxyeicosatetraenoic acids (HETE's) are produced (Figure 2)[54]. Antioxidant enzymes, SOD and catalase, and antioxidant compounds, including allopurinol, prevent or restore normal function, indicating a role of ROS in the responses. Although the eicosanoids and the ROS involved have yet to be precisely defined, observations in other rodent models of diabetes and hypertension show similar findings[55–61]. ROS can both disrupt eicosanoid metabolism as well as produce isoprostanes by direct oxidation of arachidonic acid to account for thromboxane A_2 receptor (TP) activation. Indeed, many of the beneficial therapeutic effects of TP antagonists in preventing vascular dysfunction, atherosclerosis, hypertension, and nephropathy in rodent models of hypertension and diabetes are mimicked by antioxidants. Furthermore, TP receptor activation markedly enhances inflammatory signaling in vascular cells[62], and a TP antagonist markedly decreased vascular inflammation and tissue oxidants in atherosclerotic diabetic mice[63, 64], suggesting that TP receptor activation can be implicated in oxidant generation. S 18886, a TP receptor antagonist improves endothelium-dependent vasodilation in patients with coronary artery disease, suggesting that TP receptor agonists contribute to human vascular dysfunction.

8. Antioxidant defenses in hypertension and diabetes

A number of antioxidants are involved in maintaining defenses against oxidative stress. These mechanisms vary in different intracellular and extracellular compartments and comprise enzymatic and non-enzymatic types. The major vascular enzymatic antioxidants are SOD, catalase, and glutathione peroxidase. Non-enzymatic antioxidants include endogenous ascorbic acid (Vitamin C), α-tocopherol (vitamin E), glutathione, and exogenous carotenoids and flavonoids. Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. Overproduction of ROS

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and RNS depletes both enzymatic and non-enzymatic antioxidants leading to additional ROS/RNS accumulation and cellular damage.

Low antioxidant bioavailability promotes cellular oxidative stress and oxidative damage associated with hypertension[65]. In hypertensive patients, the ratio of oxidized to reduced glutathione was significantly higher, and the activities of SOD, catalase, and glutathione peroxidase were significantly lower in whole blood and peripheral mononuclear cells when compared with normal subjects[66]. In spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP), the levels of 8-hydroxy-2'-deoxyguanosine, a marker for oxidative stress-induced DNA damage, and protein carbonylation, a marker for protein oxidation, were enhanced in aorta, heart, and kidney, while the expression of thioredoxin was markedly suppressed in those tissues compared with Wistar-Kyoto rats (WKY)[67].

Increased oxidative stress and impaired antioxidant defense mechanisms are believed to be the important factors contributing to the pathogenesis and progression of diabetes mellitus. In alloxan-induced diabetic rabbits, the concentration of glutathione and the activities of Cu,Zn-SOD, catalase, and glutathione peroxidase in aortic endothelial cells were significantly decreased compared with controls[68]. The levels of endogenous antioxidants metallothionein I and II were significantly decreased in skeletal muscle and plasma of type 2 diabetic patients compared with control subjects[69].

9. Vascular Oxidant Targets in Hypertension and Diabetes

Rather than indiscriminately affecting cellular constituents, ROS and RNS may affect proteins and their amino acid residues selectively. Sites within proteins that participate in enzyme catalysis and regulation may be particularly susceptible. $OONO^-$ in particular is highly reactive with tyrosine – particularly tyrosyl radicals, with reactive cysteine thiols, and with methionine and tryptophan amino acids. Because some of these residues are essential for enzyme function, “one hit” by an oxidant can inactivate a protein, requiring its resynthesis. Oxidants may therefore produce targeted disruption of vascular function.

9.1. Prostacyclin Synthase

Prostacyclin (PGI_2) is a potent vasodilator which has anti-platelet, anti-inflammatory, and antioxidant actions within the vasculature. A tyrosyl radical on tyrosine-430 of PGI_2 synthase is essential for its catalytic activity. The enzyme is extremely sensitive to $OONO^-$ which causes tyrosine nitration at the site and enzymatic inactivation at concentrations as low as 50 nanomolar[70, 71]. To the extent that PGI_2 is a major metabolite of arachidonic acid, the degree to which PGI_2 synthase is inactivated, eicosanoid metabolism can be redirected towards vasoconstrictor products including PGH_2, $PGF_{2\alpha}$, TxA_2, and HETE's, all of which can be implicated in causing vascular contraction, inflammation, and thrombosis by stimulating TP receptors (Figure 2).

9.2. eNOS

As mentioned above, eNOS can generate $OONO^-$ when it is uncoupled. eNOS normally exists as a dimer joined by a zinc atom coordinated by four sulfur atoms, termed a zinc thiolate center (ZnS_4). As the sulfur atoms in this center are highly reactive to $OONO^-$, and when oxidized lead to uncoupling of enzymatic activity[49]. Thus, $OONO^-$ produced by eNOS can decrease •NO production and further increase $OONO^-$. The $OONO^-$ thus formed can also affect nearby proteins as demonstrated by the fact that in endothelium exposed to high glucose, not only is the eNOS ZnS_4 oxidized and the enzyme uncoupled, but PGI_2 synthase is tyrosine-nitrated and inactivated. Inhibiting eNOS activity prevents the PGI_2 synthase inactivation[72], indicating that eNOS uncoupling accounts for the decrease in PGI_2.

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9.3. MnSOD

MnSOD, the SOD2 isoform, is located in mitochondria where it is thought to be needed to protect cellular constituents from $O_2^{-\bullet}$ derived from the electron transport chain. Like PGI_2 synthase, MnSOD has a tyrosyl radical which is essential for its enzymatic activity on tyrosine-34. As much as 20% of all tyrosine nitration in kidneys of Ang II-mediated hypertensive rat and mouse kidney is accounted for by MnSOD tyrosine nitration[64, 73]. An antibody that specifically recognizes the nitrotyrosine in MnSOD stains a variety of diseased tissues including blood vessels and heart from hypertensive and diabetic animals and patients indicating that the enzyme is attacked by oxidants in disease[74] (Figure 3). As nitration of MnSOD inactivates the enzyme, loss of its scavenging activity can be implicated also in further increases in $O_2^{-\bullet}$ levels. Realization that this positive reinforcing of oxidant stress could contribute to pathology has led to development of several MnSOD mimetics for therapeutic use[75]. Also, treatment with a TP antagonist protects against tyrosine nitration of MnSOD, loss of its enzymatic activity, and proteinuria in diabetic atherosclerotic mice[64].

9.4. SERCA

SERCA is a 110 kDa protein which accumulates Ca^{2+} into the sarcoplasmic/endoplasmic reticulum of all cells. As more than 99% of total cell Ca^{2+} resides in the stores and the levels there inversely regulate Ca^{2+} influx from the extracellular space into the cell, SERCA is a major regulator of intracellular free Ca^{2+} which is responsible for regulating many cell functions. SERCA is also responsible for rapid uptake of Ca^{2+} into cardiac myocytes, accounting for diastolic relaxation of the heart. SERCA has a reactive thiolate anion on cysteine-674 which when adducted with glutathione adducts increases Ca^{2+} uptake activity (Figure 4). The same reactive thiol is quantitatively and irreversibly oxidized by concentrations of $OONO^-$ in the 100–400 micromolar range. Despite the requirement for this high concentration *in vitro*, chronically elevated oxidants oxidize SERCA in diseased tissues. Iodoacetamide labeling of the free reactive cysteine thiol on cysteine-674 indicates that more than 50% of the thiol is oxidized in atherosclerotic rabbit aorta, accounting for the impaired ability of •NO to relax the blood vessel[8]. An antibody recognizing irreversible sulfonic acid oxidation of cysteine-674 reveals widespread oxidation of this cysteine in blood vessels and heart from hypertensive, diabetic, and atherosclerotic, blood vessels in rodents and patients[76] (Figure 5). To the extent that redox regulation of SERCA is important for vascular and cardiac relaxation, irreversible oxidation of cysteine-674 is an indicator of physiological impairment. In the aorta of chronically diabetic pigs, SERCA with oxidized cysteine-674 was found in a 70 kDa form, consistent with irreversible oxidation of the protein resulting in its degradation[76].

9.5. Antioxidant Therapy

In rodents, antioxidant agents such as Tempol, apocynin, butylated hydroxytoluene, and vitamin E and C show remarkable effects in preventing both oxidation of proteins and hypertensive and diabetic cardiovascular disease[77–83]. The same can be said of transgenic mouse models in which NADPH oxidase components are genetically eliminated[34, 84]. Despite the ability to prevent disease, the evidence that antioxidants can reverse the effects of disease either in rodents and human patients is limited. Acute administration of antioxidants may restore vascular function, suggesting that elevated oxidants do play a role[85–88]. However, treatment of patients with vitamin C and E have not revealed any significant long term benefit[29, 78, 89–91]. The lack of efficiency may be attributed to the low doses used, to the failure to demonstrate antioxidant efficacy *in vivo*, or potentially to interference with physiological roles of oxidants in cell function.

Because inflammation plays such an important role in the pathophysiology of vascular disease, and oxidant generation is an inherent participant in that process, anti-inflammatory

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therapies might achieve antioxidant effects. However, there is no evidence that anti-inflammatory therapy *per se* with aspirin, nonsteroidal, or steroid anti-inflammatory agents are able to prevent vascular disease progression or excess oxidants in tissues. On the other hand, therapeutic agents that have proven effective in decreasing atherosclerotic plaque in human patients, including HMG CoA reductase inhibitors (statins)[89] and metformin[92–94], do have both anti-inflammatory and anti-oxidant effects[95–99]. For example, statins ameliorate endothelial dysfunction mainly by an attenuation of $O_2^{-\bullet}$ production by NADPH oxidase. A recent study showed that atorvastatin can decrease COX2-dependent 8-isoprostane generation which causes endothelial dysfunction in SHR[100]. Atorvastatin also restored NO bioavailability by increasing phosphorylation of extracellular signal-regulated kinase 1/2, Akt, and eNOS, as well as increasing expression of inducible NO synthase levels and decreasing vascular NADPH oxidase-driven $O_2^{-\bullet}$ production[101]. TP antagonists[63, 102] and polyphenols[103, 104] are two additional examples of therapeutic agents that provide anti-inflammatory effects and vascular protection accompanied by significant improvement in protein oxidation, at least in rodent models.

9.6. Conclusions

ROS are regulators of normal cellular function, but when produced in excess they contribute to the disease process. High levels of •NO produced by iNOS can create the more reactive $OONO^-$, and leukocyte myeloperoxidase produces HOCl, making the accumulation of inflammatory leukocytes possessing these two enzymes within vascular lesions particularly injurious. Oxidants attack specific amino acids within proteins, and these residues are often those that are more reactive because they have evolved to mediate normal enzymatic catalysis or regulation, as in PGI2 synthase, eNOS, MnSOD, and SERCA. Despite the ability of antioxidants to prevent vascular disease in rodents, little evidence exists for their efficacy in patients. Rather, agents that have pleotropic anti-inflammatory effects including statins, metformin, TP antagonists, and polyphenols appear to limit oxidant production because of the integral role of oxidant production in inflammation.

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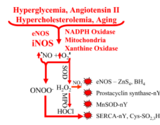

Figure 1.

Cardiovascular risk factors increase oxidants and protein oxidation. Major cardiovascular risk factors increase vascular production of nitric oxide (•NO) and superoxide anion ($O_2^{-\bullet}$) by increasing the expression and/or activity of endothelial and inducible •NO synthase (eNOS, iNOS), NADPH oxidase, xanthine oxidase, as well as increasing production of mitochondrial $O_2^{-\bullet}$. •NO and $O_2^{-\bullet}$ react rapidly to form peroxynitrite anion ($OONO^-$) which can increase tyrosine nitration (nY), cysteine (Cys) and zinc thiolate (ZnS_4) oxidation ($SO_{2,3}H$). Superoxide dismutases (SOD) form H_2O_2 which can also oxidize proteins, or together with leukocyte myeloperoxidase (MPO) can form hypochlorous acid (HOCl) and with nitrite (NO_2^-) can form nY on proteins. Important cardiovascular proteins are affected including eNOS, prostacyclin synthase, MnSOD, the sarco-endoplasmic reticulum calcium ATPase (SERCA).

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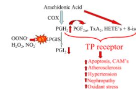

Figure 2.

Tyrosine nitration (nY) of prostacyclin synthase (PGIS) increases stimulation of thromboxane A_2 (TP) receptors. Nitration of PGIS at tyrosine-430 inactivates the enzyme resulting in shunting of arachidonic acid metabolites to products that stimulate the TP receptor. Cyclooxygenase produces prostaglandin endoperoxide (PGH_2) which produces more prostaglandin ($F_{2\alpha}$) and thromboxane (Tx) A_2. More arachidonic acid derived hydroxyeicosatetraenoic acids (HETE's) also are produced. Furthermore, oxidants generate more 8-isoprostanes (isoP) directly from arachidonic acid which also stimulates TP receptors. These products can all be implicated in apoptotic and inflammatory cell responses, increased atherosclerosis, hypertension, and nephropathy. TP receptor stimulation also further augments the generation of reactive oxygen species.

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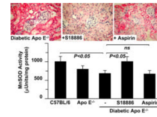

Figure 3.
Tyrosine nitration and inactivation of MnSOD in diabetic apoliprotein E deficient mice is prevented by TP antagonist. Upper panels show examples of immunohistochemical staining of kidneys of atherosclerotic, hyperlipidemic apolipoprotein E deficient mice that were given type-1 diabetes by administration of streptozotocin. The red staining was achieved with a sequence-specific antibody that detects nitration of tyrosine-34 of MnSOD[74]. Treatment of the mice with the TP antagonist, S18886 restored staining to a level indistinguishable from that in non-diabetic control mice, whereas aspirin had no significant effect. The bar graph shows that the renal MnSOD enzymatic activity was significantly decreased from control in the diabetic mice, and that treatment with S18886, but not aspirin prevented the decrease. Data from [reference 64].

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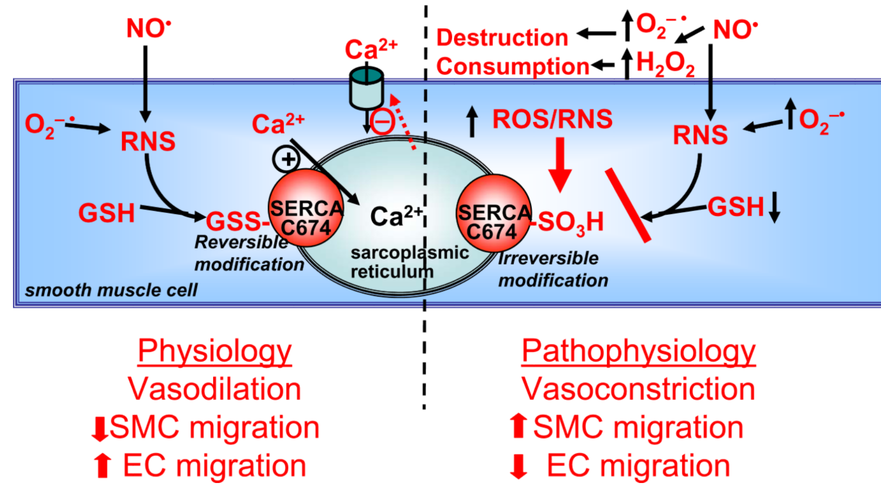

Figure 4.

Redox regulation of SERCA by reactive oxygen/nitrogen species. RNS produced by •NO and $O_2^{-\bullet}$ increase glutathione adducts (GSS-) of cysteine(C)-674 of SERCA. This increases Ca^{2+} uptake into sarcoplasmic reticulum stores, inhibiting store-dependent Ca^{2+} influx, and decreasing cytosolic Ca^{2+} which causes vasodilation, inhibits smooth muscle cell (SMC) migration, and increases endothelial cell (EC) migration. Under pathophysiological conditions higher levels of ROS increase the destruction and consumption of •NO, producing RNS which can oxidize the SERCA C674 thiol ($-SO_3H$), preventing its reversible *S*-glutathiolation and blocking the stimulation of SERCA by •NO. Thus, the redox status of C674 can determine physiological and pathophysiological changes in vascular tone and cell migration. From [reference 8].

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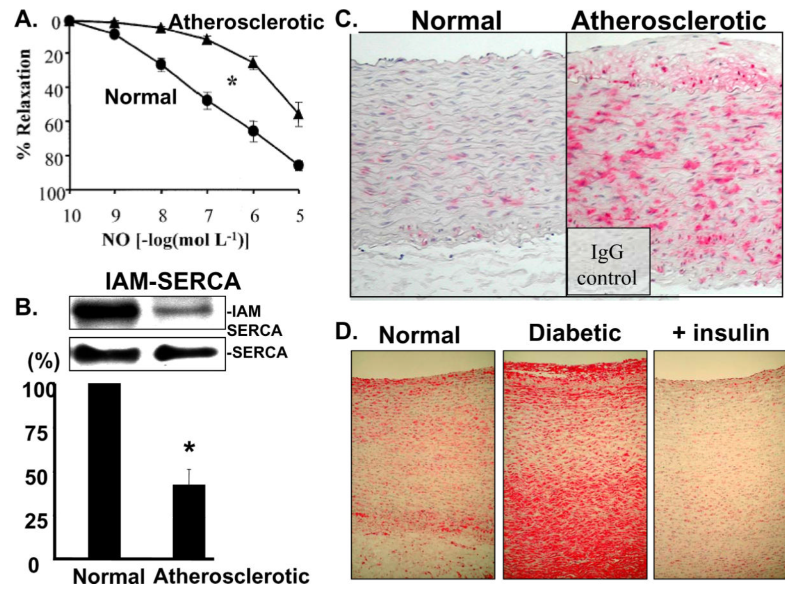

Figure 5.

Oxidation of SERCA in atherosclerotic and diabetic aorta. A. Impaired aortic relaxations to •NO in rabbits made atherosclerotic by feeding a high cholesterol diet for 10 weeks. B. The decreased response can be explained in part by decreased labeling of the free thiol on cysteine-674 with biotin-tagged IAM (upper blot) summarized in bar graph. There is no change in total SERCA expression (lower blot). C and D. Immunohistochemical staining of oxidized SERCA cysteine-674 by a sequence specific antibody that recognizes the sulfonic acid thiol. Increased staining is seen in atherosclerotic rabbit aorta (C) obtained from the same study as data in panels A and B, as well as in the aorta of a pig maintained diabetic and hypercholesterolemic for 1 year, but not in a diabetic pig treated with insulin for 1 year. Data from [references 8] and[76].