Modulation of Reactive Oxygen Species and Collagen Synthesis by Angiotensin II in Cardiac Fibroblasts

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Abstract: Angiotensin II increases the NAD(P)H-dependent superoxide anion production and the intracellular generation of reactive oxygen species in cardiac fibroblasts and apocynin, a NAD(P)H oxidase inhibitor, abrogates this rise. The membrane associated NAD(P)H oxidase complex is the predominant source of superoxide anion and reactive oxygen species generation in angiotensin II-stimulated adult cardiac fibroblasts. Inhibition of this NAD(P)H oxidase complex with apocynin completely blocks the angiotensin II-stimulated collagen production, collagen I and III protein and mRNA expression.

Superoxide anion production is also increased by the Cu,Zn-superoxide dismutase (SOD) inhibitor diethyldithiocarbamic acid (DETC) and decreased by the superoxide scavenger tempol in control and ANG II-treated fibroblasts. ANG II and DETC stimulate the collagen production and the collagen I and fibronectin content in fibroblasts. The SOD mimetics tempol and EUK-8 as well as polyethyleneglycol-SOD reduce the collagen production.

ANG II also decreases the activity and mRNA and protein expression of the mitochondrial antioxidants Mn-SOD and peroxiredoxin-3. Upon phosphorylation of Akt by ANG II, P-Akt is translocated from the cytoplasm to the nucleus and nuclear phosphorylation of FOXO3a by P-Akt leads to relocalisation of FOXO3a from the nucleus to the cytosol, resulting in a decrease in its transcriptional activity and in Mn-SOD expression. These data indicate that ANG II inactivates FOXO3a by activating Akt and this leads to a reduction in the expression of the antioxidant Mn-SOD. A role of SOD and the formed reactive oxygen species in the regulation and organization of collagen in cardiac fibroblasts is suggested.

Keywords: Cardiac fibroblasts, angiotensin II, reactive oxygen species, collagen, superoxide dismutases, peroxiredoxins, FOXO3a.

1. INTRODUCTION

Increased oxidative stress has been shown in the pericardial fluid formed by the myocard of patients and animals with heart failure [1,2] and antioxidants attenuate the development of myocardial failure [3]. Hypertension is also associated with an elevation of reactive oxygen species (ROS) and frequently with an impairment of endogenous antioxidant mechanisms [4-6]. The elevation of blood pressure by oxidants and its amelioration by antioxidants strongly supports a role of ROS in hypertension [6]. During the development of hypertension, ROS are generated by endogenous sources, notably the NAD(P)H oxidase enzyme family and uncoupled nitric oxide synthase [6].

In hypertension there is a mutual reinforcement between ROS and angiotensin II (ANG II) [6]. Effects of ANG II are related to the oxidative stress. Indeed, oxidative stress is increased in mice after infusion of ANG II [7, 8]. ANG II also induces oxidative stress *in vitro*, increasing ROS in various types of cultured cells such as vascular smooth muscle cells from rat thoracic aorta [9], adult cardiac microvascular endothelial cells [10], neonatal rat cardiac myocytes [11,12] and human hepatic stellate cells [13]. It has also been extensively demonstrated that ANG II markedly increases vascular NAD(P)H oxidase activity [9,14-18].

Antioxidant enzymes such as superoxide dismutase (SOD), catalase and peroxidases regulate reactive oxygen species by maintaining superoxide anion ($^{\cdot}O_2^-$) and hydrogen peroxide (H_2O_2) at low levels. A chronic increase in ROS in the myocard, possibly due to impairment of SOD or other antioxidant pathways, could contribute to myocardial remodeling and failure [2, 19]. SODs, catalase, glutathione peroxidases and peroxiredoxins constitute the principal components of the antioxidant defense system and their deficiencies can cause oxidative stress. In mammalian tissue three isoforms of SOD have been identified and differ in their location. Cu/Zn-SOD constitutes 90% of total tissue [20] and is localized in the cytosol, Mn-SOD in the mitochondria and extracellular SOD in the interstitial fluid [21]. Inhibition of endogenous SOD by the specific inhibitor diethyldithiocarbamic acid (DETC), a copper chelator, increases basal O_2^- production in aorta rings of normal and spontaneously hypertensive rats [22] and in rings of rabbit thoracic aorta [23]. An enhancement of $^{\cdot\cdot}O_2^-$ generation by DETC is also shown in neonatal rat cardiac myocytes [12] and fibroblasts [24]. The angiotensin II- induced NADPH dependent $^{\cdot}O_2^-$ production is higher in DETC-treated vascular

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smooth muscle cells than in control cells [25]. In skin fibroblasts SOD reduces transforming growth factor-β_1 and collagen type I mRNA expression [26]. SOD also strongly colocalizes with type I collagen in the extracellular matrix of blood vessels [27]. Human aortic SOD also binds collagen type I [28]. Its association with collagen is important in protecting it from oxidative fragmentation [29].

Both, ANG II and oxidative stress induce collagen *in vivo* and *in vitro*. Oxidative stress induces remodeling of the myocard [7, 11, 12, 30] and regulates collagen metabolism in a variety of noncardiac cells, such as lung and skin fibroblasts and human venous endothelial cells [31-33]. In addition to oxidative stress infusion of ANG II induces severe cardiac fibrosis [34]. Moreover, an ANG II-induced increase in collagen production has also been demonstrated in various types of cultured cells such as rat vascular smooth muscle cells [35], murine mesangial cells [36, 37], rat glomerular cells [38] and adult rat cardiac fibroblasts [39-42].

In this review paper we will discuss if inhibition of the catalytic and regulatory subunits of NAD(P)H oxidase with apocynin or diphenyleneiodonium chloride (DPI) affects the collagen production and expression in adult rat cardiac fibroblasts and if ANG II stimulates NAD(P)H oxidase in these cells through a redox sensitive mechanism. The contribution of the activity of SOD in the $^{\cdot}O_2^-$ generation and intracellular ROS production in control and angiotensin II-stimulated cardiac fibroblasts from adult rats will also be discussed as well as its involvement in the induction of protein and mRNA expression of collagen type I and III and fibronectin. SOD is irreversibly inhibited by the copper chelator DETC and its effect is compared with tempol, a stable membrane-permeable metal-independent SOD mimetic that is specific for $^{\cdot}O_2^-$ and with 4-carboxy-tempol, a structural related and inactive compound of tempol who has minimal $^{\cdot}O_2^-$ scavenging activity.

2. ANG II-INDUCED COLLAGEN PRODUCTION AND DEGRADATION DURING INHIBITION OF CARDIAC FIBROBLAST SUPEROXIDE GENERATION

ANG II dose-dependently increases collagen production in adult rat cardiac fibroblasts in culture (Fig. **1**) and this effect is blocked by the ANG II AT_1-receptor antagonist telmisartan, but not by the AT_2-receptor antagonist, P-186 [43].

Besides the stimulation of collagen synthesis, ANG II also increases mRNA levels of collagen type I, type III, pro-α_1(III) collagen and fibronectin in cultured cardiac fibroblasts [40,41,43-45]. The ANG II-induced expression of type I collagen mRNA is also completely abolished by AT_1-receptor antagonism but is unaffected by AT_2-receptor antagonism [40,44,46,47]. *In vivo*, ANG II infusions at pressor or subpressor doses in rats also induces an increase in collagen and fibronectin [48, 49].

The presence of high numbers of ANG II AT_1-receptors on neonatal and adult rat cardiac fibroblasts has been documented [40, 41, 50-52]. AT_1-receptors exhibit saturable, reversible and high-affinity binding of ANG II, that is competed for by nonpeptide AT_1 receptor antagonists, ANG I and ANG III. Competitive binding studies employing nonpeptide antagonists for AT_2-receptors fail to detect their presence in either neonatal or adult cardiac fibroblasts [40, 41, 51] although ANG II inhibition of collagenase activity in adult rat cardiac fibroblasts is attenuated only by AT_2-receptor blockade [42].

Of the two AT_1-receptor subtypes expressed in the rat, cardiac fibroblasts express much higher mRNA levels for the AT_{1A} than the AT_{1B} receptor [52]. Adult rat cardiac fibroblasts express higher mRNA levels for the AT_1-receptor than do neonatal cardiac fibroblasts. The expression of ANG II

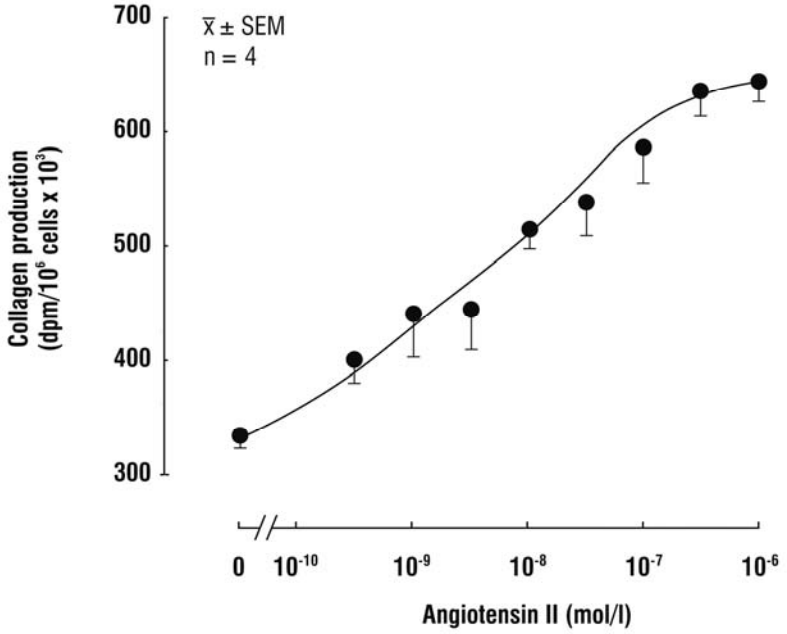

Fig. (1). Concentration-response curve for ANG II-induced changes in collagen production in adult rat cardiac fibroblasts.

receptors by left ventricular fibroblasts exhibits, however, marked species dependence. Cultured rat fibroblasts express 43,000 ± 15,000 ANG II (AT_1-specific) receptors per cell, whereas rabbit and neonatal human cardiac fibroblasts express fewer such receptors [53]. However, in isolated human cardiac fibroblasts, ANG II itself does not increase collagen I mRNA directly but through indirect pathways that may involve TGF-β_1 and osteopontin [54].

The ability of ANG II to induce collagen synthesis and expression of collagen in rat cardiac fibroblasts may be mediated by an increased TGF-β_1 production in an autocrine/ paracrine fashion. Indeed, cardiac fibroblasts are the principal cellular origin of TGF-β_1 and in cultured rat adult cardiac fibroblasts ANG II stimulates TGF-β_1 gene expression, increases total TGF-β_1 production and promotes the conversion of latent TGF-β_1 to the active form [50,55-59]. Simultaneous treatment of cardiac fibroblasts *in vitro* with ANG II and a neutralizing antibody to TGF-β_1 reduces type I and type III collagen mRNA expression [59] and collagen production (Fig. **2**).

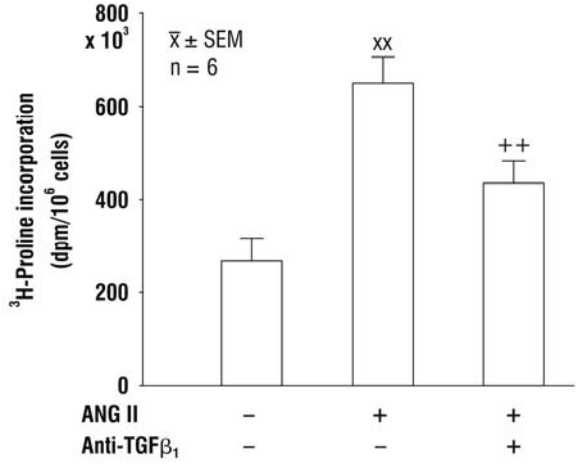

Fig. (2). Collagen production, assessed as 3H-proline incorporation, in cardiac fibroblasts treated with(out) ANG II (1 µmol/l) and a neutralizing antibody to transforming growth factor-1 (anti-TGF-1, 10 µg/ml) for 24 hours. xx $p<0.01$ compared to control; ++ $p<0.01$ compared to ANG II.

ANG II-stimulated TGF-β_1 secretion is greatly attenuated by a AT_1-receptor antagonist [60]. We [61] have also demonstrated that TGF-β_1 induces the differentiation of cardiac fibroblasts to myofibroblasts and this differentiation is accompanied by an increase in angiotensin converting enzyme (ACE) activity and protein and by a profound modification of the fibroblast phenotype, which consists of a change in cell morphology, an enlargement of cell volume and an increase in cell protein content [62]. Moreover the effects of ANG II and TGF-β_1 are also complimentary *in vivo*. Both of them induce cardiac fibrosis which, independent of the primary stimulus, is accompanied by high tissue concentrations of both ANG II and TGF-β_1 [63-65]. Both TGF-β_1 and ACE, as a means of ANG II synthesis, are thus involved in the enhancement of cardiac tissue fibrosis and these factors can create a vicious circle in the fibrotic tissue [66].

The ANG II-stimulated soluble collagen production in cultured adult rat cardiac fibroblasts is completely blocked by the NAD(P)H oxidase inhibitors apocynin and DPI (Fig. **3**) [67].

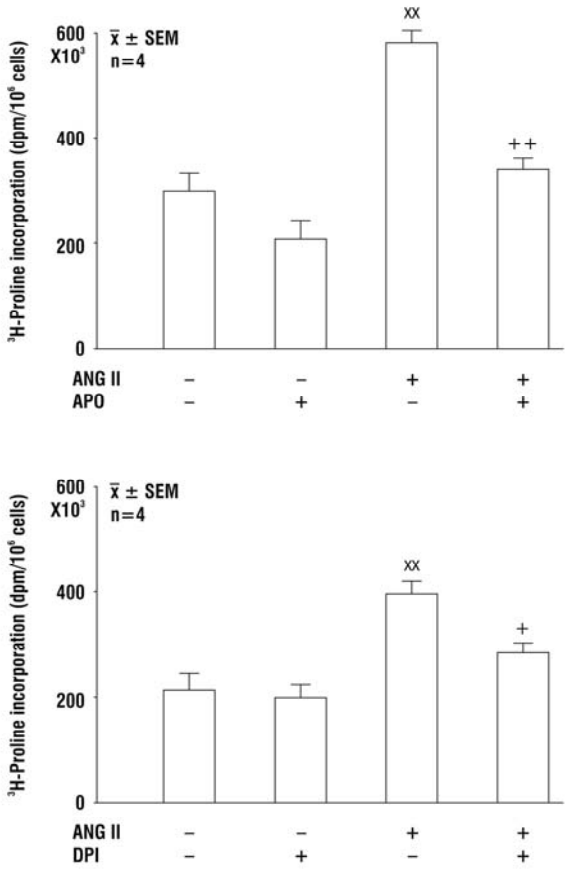

Fig. (3). Effect of apocynin (APO, 100 µmol/l) and diphenyleneiodonium chloride (DPI, 2 µmol/l) on soluble collagen production in cardiac fibroblasts treated with(out) Ang II (1 µmol/l) for 24 hours. xx $p<0.01$ compared with control without ANG II, APO and DPI; ++ $p<0.01$, + $p<0.05$ compared with samples with ANG II and without APO and DPI.

Apocynin is considered an inhibitor of the association of $p47^{phox}$ and $p67^{phox}$ with a $gp91^{phox}$ (or Nox2) homologue subunit within the membrane NAD(P)H oxidase complex [68]. DPI inhibits NAD(P)H oxidase by binding to the cellular flavoprotein moiety of the complex [69]. It should however be taken into consideration that DPI is a flavoenzyme inhibitor and not specific for NADPH oxidase, inhibiting other flavoenzymes as well. In addition, it can not be said that apocynin is specific for NADPH oxidase since apocynin has also antioxidant effects. Because the hemodynamic stress of hypertension can be excluded in these *in vitro* culture conditions, it can be assumed that the presence of ANG II and associated ROS generation is the basis of enhanced collagen synthesis by cardiac fibroblasts.

In vitro, inhibition of a membrane as well as a cytosolic component of the NAD(P)H oxidase complex suppresses the ANG II-stimulated collagen production in adult rat cardiac

fibroblasts in culture [67]. In contrast, inhibition of xanthine oxidase, cyclooxygenase, lipoxygenase, the respiratory chain, cytochrome P450 mono-oxygenase and NO synthase has no effect on ANG II-induced collagen production in cardiac fibroblasts [67]. The demonstration that apocynin and DPI block the ANG II-stimulated collagen production suggests that the predominant source of cardiac fibroblast superoxide generation is the membrane-associated NAD(P)H oxidase complex.

ANG II-stimulated collagen production is also associated with an increase in both the mRNA and the protein expression of collagen type I and III [67]. The synthesis of collagen in cardiac fibroblasts is thus regulated at the transcriptional and posttranslational level.

However, the extracellular matrix collagen content depends not only on its production but also on its degradation by enzymes such as MMP-1. In adult rat cardiac fibroblasts ANG II stimulates collagen production and regulates collagen degradation by attenuating MMP-1 and through enhancing TIMP-1, the tissue inhibitor of MMP-1 [67]. The decrease in MMP-1 and the increase in TIMP-1 induced by ANG II in cardiac fibroblasts would result in less collagen degradation and favors collagen synthesis, explaining at least partly the profibrotic effect of ANG II on the myocard in various pathophysiological conditions where the renin angiotensin system is activated. Brilla *et al*. [42] first showed that ANG II decreased the collagenase activity in adult rat cardiac fibroblasts. In TNFα-stimulated neonatal rat cardiac fibroblasts, ANG II increases TIMP-1 expression and decreases MMP-2 activity [70].

However, ANG II affects MMP expression/activity differently in various cell types. An enhanced MMP activity and expression is reported in endothelial cells [71], cardiac myocytes [72] and in skin fibroblasts [73]. In cardiac fibroblasts the effect of ANG II on collagen synthesis, MMP-1 and TIMP-1 content is completely blocked by apocynin suggesting that ROS generated through the membrane-associated NAD(P)H-oxidase are involved in the fibrotic process.

In vivo experiments have shown that apocynin reduces systolic blood pressure and prevents the increase in media/lumen ratio, endothelial dysfunction and collagen deposition in the media of mesenteric resistance arteries from ANG II-infused mice [74]. ANG II also increases significantly cardiac collagen content in wild type but not in $gp91^{phox-/-}$ mice [11], indicating that *in vivo* deletion of Nox2 reduces ANG II-induced cardiac fibrosis. Indeed, Nox2 is required for ANG II-induced cardiac fibrosis in rodents [11].

In salt-loaded, aldosterone-infused rats apocynin prevents collagen deposition in the perivascular regions of the left ventricle, decreases the aortic mRNA level of procollagen I, procollagen III and $p22^{phox}$, inhibits NAD(P)H oxidase activity, normalizes cardiac hypertrophy and reduces systolic blood pressure [75-77]. Inhibition of NAD(P)H oxidase activity by gp91ds-tat, a chimeric peptide that inhibits $p47^{phox}$ association with $gp91^{phox}$ in NAD(P)H oxidase, attenuates blood pressure increase, regresses cardiac and vascular remodeling and improves endothelial function in ANG II-infused rodents, as does apocynin [20,78]. The increased collagen volume fraction of the ventricles of aldosterone-treated rats is also prevented by cotreatment with the antioxidant N-acetyl cysteine or pyrolidine dithiocarbamate which also abrogates the aldosterone-induced NAD(P)H oxidase and NF-κB activation [79]. Iglarz *et al*. [80] have shown that aldosterone infusion induces a significant increase of interstitial cardiac fibrosis in terms of collagen deposition in the mid-myocard which is prevented by the superoxide dismutase mimetic tempol. The profibrotic action of aldosterone is mediated, at least in part, by ROS generation and involves an interaction with the renin-angiotensin system. Inhibition of ANG II-induced ROS generation in cardiac fibroblasts by the peroxisome-activated receptor-γ ligand, pioglitazone, is also associated with a concomitant inhibition of ANG II-stimulated collagen production [81]. In hypertensive rats with activation of the renin-angiotensin system, apocynin also attenuates hypertension and increases the antioxidant defenses glutathione peroxidases and glutathione-S-transferase [82].

Taking these *in vitro* and *in vivo* data together suggest that myocardial accumulation of collagen and/or the deposition of collagen in cardiac fibroblasts is, at least partly, mediated by activation of NAD(P)H oxidase and that blockade of ROS at the level of NAD(P)H oxidase may exert beneficial effects on the heart as well as large arteries in hypertension associated with oxidative stress.

3. ANG II-INDUCED ROS GENERATION AND MODULATION BY APOCYNIN

Quiescent rat cardiac fibroblasts treated with ANG II for 24 hours demonstrate NAD(P)H oxidase activity that was 180 ± 11% of vehicle control; this activity is inhibited by coincubation with apocynin [67]. These findings are in accordance with the stimulation (165 ± 19%) of NAD(P)H oxidase by ANG II in quiescent rat aortic fibroblasts and the reduction by gp91ds-tat [78].

In cultured adult rat cardiac fibroblasts the specificity of the oxidase is found for NADH and not for NADPH [67]. In human fetal lung fibroblasts [83] NADH is also reported to be a better substrate than NADPH for superoxide-generating oxidases. A superoxide-generating system, primarily NADPH dependent, is however demonstrated in rabbit aorta adventitial fibroblasts [23], in mouse thoracic aorta vascular smooth muscle cells [84] and in cultured rat aortic smooth muscle cells [35]. The preferred substrate for oxidases in macrophages and neutrophils is also NADPH rather than NADH [35]. A similar NADH- and NADPH-dependent activity is found in human skin fibroblasts [85], neonatal rat cardiac myocytes [86] and in human embryonic kidney cells [87], while in rat liver plasma membranes the activity with NADPH is approximately 80% that of NADH [88]. The substrate preference of the superoxide-generating oxidase is thus highly cell type dependent with specificity to NADH oxidase in adult rat cardiac fibroblasts. Since NAD(P)H does not cross plasma membranes their consumption rates measured in the extracellular medium are likely to represent oxidation by NAD(P)H oxidase on the outer aspect of the membrane.

The activation of NAD(P)H oxidase by ANG II in adult rat cardiac fibroblasts leads to a resultant increase in superoxide anion production, assessed as SOD-inhibitable cyto-

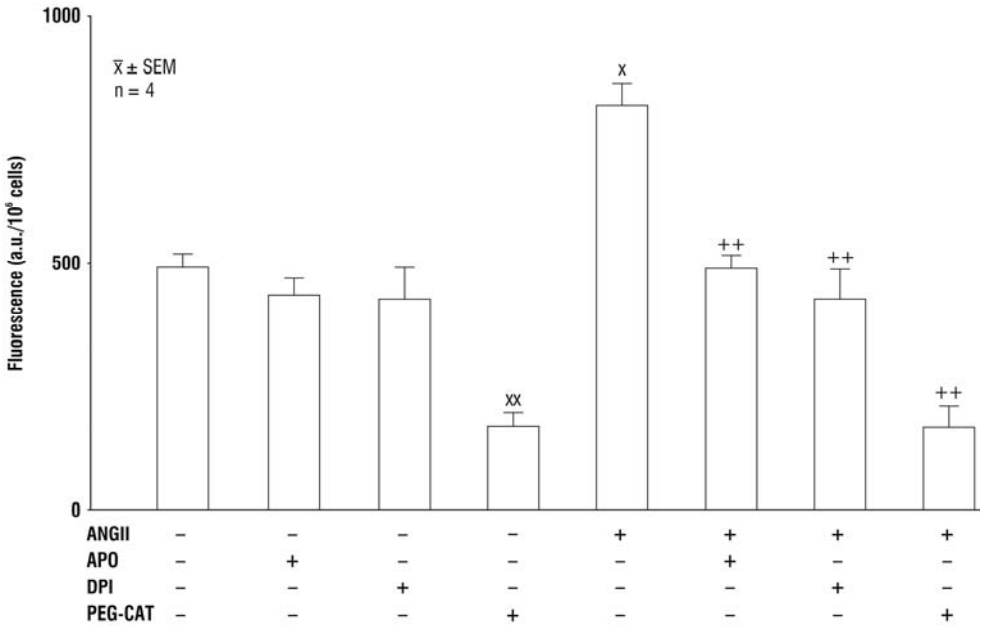

Fig. (4). Intracellular reactive oxygen species (ROS) generation, assessed in cardiac fibroblasts treated with(out) ANG II (1 µmol/l) for 24 hours after preincubation with apocynin (APO, 100 µmol/l), diphenyleneiodonium chloride (DPI, 2 µmol/l) or polyethylene glycol-catalase (PEG-CAT, 350 Units/ml) for 1 h, and incubated with 2',7'-dichlorofluorescein diacetate (20 µmol/l) for 30 min. xx $p<0.01$, x $p<0.05$ compared with control without ANG II and APO, DPI and PEGCAT; ++ $p<0.01$ compared with samples with ANG II and without APO, DPI and PEG-CAT.

chrome c reduction [67]. The intracellular generation of ROS such as hydrogen peroxide, hydroxyl radical, and hydroperoxides, assessed with the fluorescent probe 2',7'-dichlorofluorescein diacetate , is also increased by ANG II in adult [67, 79] and neonatal [89, 90] rat cardiac fibroblasts. This increase is completely blocked by DPI, apocynin and polyethyleneglycol-catalase (Fig. **4**).

These data indicate that ANG II increases oxidative stress and NAD(P)H oxidase activity in adult rat cardiac fibroblasts and that the enhanced generation of ROS is blocked by inhibition of the NAD(P)H oxidase complex with apocynin or DPI.

ANG II activates NAD(P)H oxidase to generate ROS via the angiotensin subtype 1 receptor and those ROS are , at least partly, involved in the activation of ERK, JNK and p38MAPK pathways in cardiac fibroblasts [89]. p38 MAPK activated protein kinase is indeed a critical component of the redox-sensitive signaling pathway activated by ANG II [91]. However, ERK1/2 and JNKs, but not p38kinase, are involved in ROS-mediated induction of osteopontin gene expression by angiotensin II in adult rat cardiac fibroblasts [92]. Osteopontin, expressed in the myocard co-incident with heart failure, plays an important role in post myocardial infarction remodeling by promoting collagen synthesis and accumulation.

ANG II also activates the transcription factors AP-1 and NF-κB, the best characterized transcription factors to be influenced by the cellular redox state [89, 93]. In addition to influencing tyrosine kinases, protein phosphatases and MAPkinases, ROS modulate intracellular Ca^{2+} signaling in endothelial and vascular smooth muscle cells [68]. ROS increase intracellular Ca^{2+} by stimulating inositol 1,4,5-triphosphate-mediated mobilization of intracellular Ca^{2+}, by increasing cytosolic Ca^{2+}accumulation through inhibition of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase and by stimulating Ca^{2+}influx through voltage-dependent Ca^{2+}-channels in these cells [91]. ROS also influence contractile processes by stimulating Rho/Rho kinase cascades [94].

In cardiac fibroblasts the reduction in intracellular calcium levels results also in enhanced endogenous superoxide anion production, which underscores a possible link between extracellular calcium and superoxide anion production [95]. In hypomagnesemia, serum factors may stimulate cardiac fibroblast proliferation via a superoxide anion-mediated mechanism and contribute to the fibrogenic response in the heart, even when cardiac tissue levels of magnesium are well preserved [96]. The exact mechanism, either direct or indirect, by which ANG II affects ROS in rat cardiac fibroblasts and especially the NOX-subunit of the NAD(P)H oxidase complex involved in the production of ROS need , however, to be further elucidated. In human cardiac fibroblasts Cucoranu *et al.* [97] provided recently compelling evidence that Nox-4 and Nox-5 are the primary Nox subunits.

In rat aortic smooth muscle cells Nox4 is responsible for the basal production of H_2O_2, while Nox1 is required for ANG II-stimulated $^{\cdot}O_2^-$ production [98].

4. ANG II-INDUCED EFFECT ON SUPEROXIDE DISMUTASE ACTIVITY IN CARDIAC FIBROBLASTS

ANG- II decreases total SOD, CuZn-SOD and Mn-SOD activity in rat cardiac fibroblasts [99]. No EC-SOD activity

was however detectable in adult rat cardiac fibroblasts [99]. Marklund [100] reported that the CuZn-SOD content in the rat heart was much higher than the Mn-SOD content (on average 15,100 *versus* 2,125 Units/g wet weight), whereas the EC-SOD content was very low (35 U/ g wet weight). In ANG II-treated rats an increased mRNA expression of Mn-SOD, $p22^{phox}$ and Nox1 and a decreased mRNA level of EC-SOD and Nox4 is found in the kidney cortex compared to controls [101]. ANG II infusion in mice also reduces the total SOD activity in plasma, aorta and kidney cortex as well as the mRNA and protein expression for EC-SOD [102]. Compared to the normal rat heart, $gp91^{phox}$ mRNA is largely increased within the perivascular and microscopic injury in both ventricles in ANG II-treated rats and cells expressing $gp91^{phox}$ at these sites were primarily inflammatory cells; cardiac Mn-SOD and catalase protein levels, however, remain unchanged in ANG II-infused rats [103]. In ANG II-infused mice the aorta mRNA and protein expression of EC-SOD as well as the EC-SOD activity is increased, while the CuZn-SOD protein expression is unchanged [104]. ANG II also increases aortic EC-SOD protein expression in mouse organoid cultures and enhances the mRNA EC-SOD level in cultured human aortic smooth muscle cells [104]. EC-SOD activity in mouse aorta and in human aortic smooth muscle cells is also strongly induced by ANG II [105]. Incubation of mouse aortic homogenates with ANG II had no effect on total SOD activity in either wild type or CuZn-SOD deficient mice [106]. Taking these *in vitro* and *in vivo* data together indicate that the effect of ANG II on SOD is highly cell specific.

5. DOWN-REGULATION OF MN-SOD BY ANGIOTENSIN II IN CARDIAC FIBROBLASTS

In cultured rat cardiac fibroblasts ANG II decreases the mRNA and protein expression of the mitochondria-specific antioxidant Mn-SOD (Fig. **5**), but without influencing Cu, Zn-SOD, catalase or glutathioneperoxidase mRNA and protein expression [107].

Discordant findings are reported on the *in vivo* effect of ANG II on Mn-SOD activity, protein and mRNA expression in various animal tissues. In ANG II-infused rat kidney a 50% decrease in Mn-SOD activity is observed [108], while Chabrashvili *et al.* [101] reported an increased mRNA expression of Mn-SOD in the kidney cortex of ANG II-treated rats. An unchanged Mn-SOD protein expression is found in cardiac [103] and kidney [108] tissue of ANG II-infused rats, while a reduced renal Mn-SOD protein level is found in ANG II-treated rats [109]. In ANG II-infused mice, the aortic protein level Mn-SOD is unchanged, but declines in extracellular SOD deficient mice [110]. In Dahl salt-sensitive rats, characterized by an up-regulated angiotensin system [111], renal Mn-SOD protein expression is decreased and increased by the angiotensin converting enzyme inhibitor, trandolapril [112]. In spontaneously hypertensive rat (SHR) kidneys, chronic ANG II-blockade with losartan prevents the decrease in Mn-SOD activity and the increase in H_2O_2 production observed in untreated SHR [113]. Losartan also inhibits the ANG II-induced Mn-SOD mRNA down-regulation in cardiac fibroblasts [107]. A reduced Mn-SOD gene and protein expression is also observed in the infarcted myocard of rats [114, 115] and this fall is partially prevented by losartan [115]. The diminished Mn-SOD mRNA levels are not related to changes in Mn-SOD mRNA stability. The down-regulation of Mn-SOD is linked to a down-regulation of its mRNA levels, thus indicating that the alterations in Mn-SOD expression result from an altered gene expression rather than from alterations in post-transcriptional regulation. Moreover, the protein expression of α-smooth muscle actin, a marker of the differentiation of fibroblasts into myofibroblasts, is not affected by ANG II, indicating that the effects of ANG II on Mn-SOD expression do not result from changes in the phenotype of the cultured fibroblasts [107].

6. MITOCHONDRIAL ROS PRODUCTION AND ANGIOTENSIN II IN CARDIAC FIBROBLASTS

In quiescent cells most of ROS are produced through an univalent reduction of molecular oxygen to $\cdot O_2^-$ by electrons

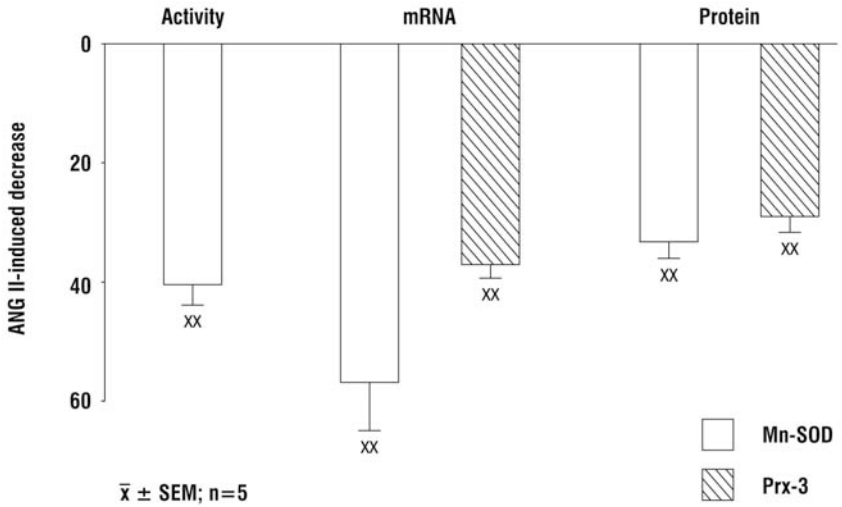

Fig. (5). ANG II-induced decrease in activity, mRNA and protein expression of manganese superoxide dismutase (Mn-SOD) and peroxiredoxin-3 (Prx-3) in cardiac fibroblasts treated with(out) ANG II (1μmol/l) for 24 hours. xx $p<0.01$, x $p<0.05$ compared to control.

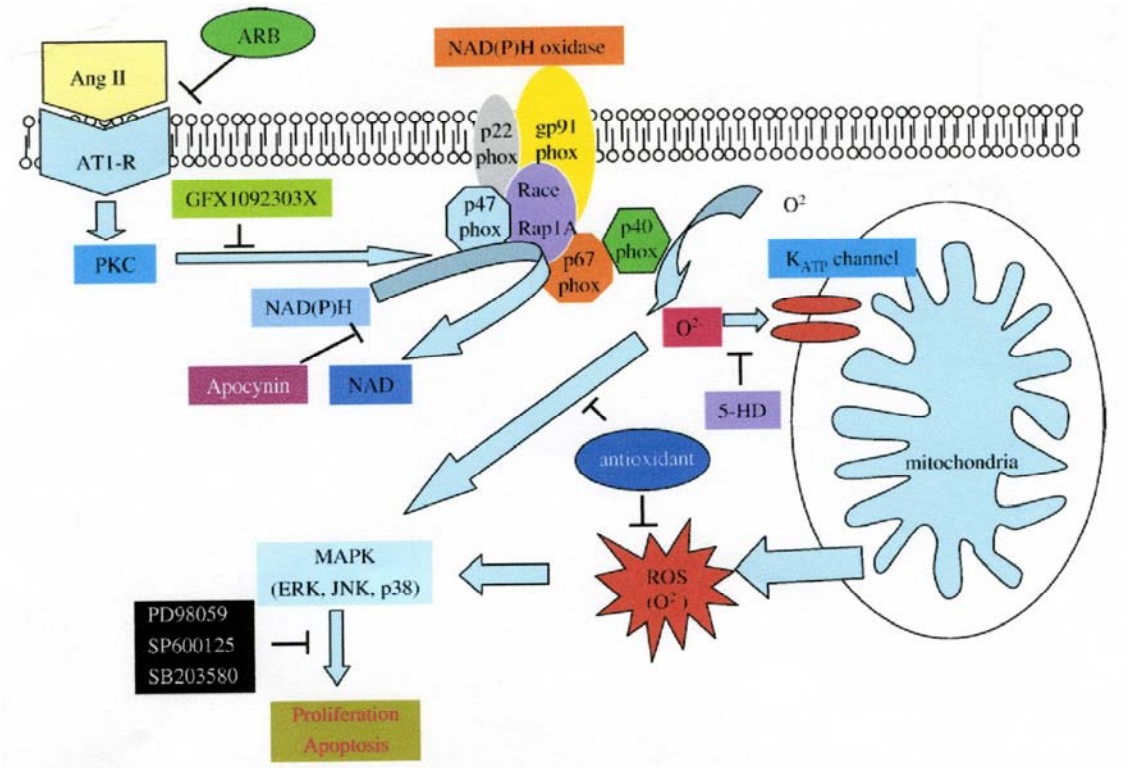

Fig. (6). Potential signaling pathways in ANG II-stimulation. ARB Angiotensin II receptor blocker, AT1-R angiotensin II subtype 1 receptor; PKC protein kinase C; PKC inhibitor (GFX1092303X), NADPH oxidase inhibitor (apocynin), Mitochondrial K_{ATP} channel inhibitor (5-hydroxydecanoate, 5-HD), ERK inhibitor (PD98059), JNK inhibitor (SP600125), p38 inhibitor (SB203580). *Reprinted from [120] with permission from Oxford University Press, Oxford, U.K.*

that leak from complex I and III of the mitochondrial electron transport chain. The mitochondrial electron transport system consumes approximately 85% of the oxygen utilized by cells, and about 5% of that oxygen is converted to ROS [116, 117]. The burden of $^{\cdot}O_2^-$ is largely countered by the mitochondrial enzyme Mn-SOD. Although Mn-SOD relieves oxidative stress in mitochondria caused by $^{\cdot}O_2^-$, it generates H_2O_2 a mild oxidant which is readily converted to the more powerful oxidant $^{\cdot}OH$.

Chronic increases in mitochondrial ROS production can lead to a catastrophic cycle of further oxidative stress and ultimate cellular injury [118]. This deleterious process may play an important role in the development and progression of myocardial remodeling and failure [119]. Indeed, recent findings [120] have demonstrated that ANG II stimulation induces opening of mitochondrial K_{ATP} channels and further amplifies ROS formation from mitochondria. ROS produced initially in the mitochondria have been shown to act in a positive feedback, where mitochondria can respond to elevated ROS by increasing their own ROS production in a process known as ROS-induced ROS release [121]. ANG II stimulation activates NADPH oxidase to generate ROS which activates mito K_{ATP} channels to induce a ROS burst in the mitochondria to activate downstream signaling pathways, such as activation of p38 and JNK MAPK involved in cell apoptosis, hypertrophy and differentiation [120]. ANG II binds to ANG type 1 receptors (AT_1-R), activates protein kinase C (PKC) which activates NADPH oxidase to induce $^{\cdot}O_2^-$ generation (Fig. **6**). Mitochondrial K_{ATP} channels can be activated by $^{\cdot}O_2^-$ to produce more generation of ROS to induce activation of MAPK, which mediates ANG II-induced cell proliferation, apoptosis and differentiation [120].

Exposure of cardiac fibroblasts to ANG II can thus lead to increased oxidative stress because of down-regulation of antioxidant enzymes such as manganese superoxide dismutase. The Mn-SOD mimetic tempol [122] on the contrary completely abolishes the ANG II-induced increase in $^{\cdot}O_2^-$ production in cardiac fibroblasts [67].

It has indeed been shown that lack of Mn-SOD in mice results in dilated cardiomyopathy [123], induces progressive heart failure with excess formation of superoxide and transcriptional alterations of genes associated with heart failure [124] and leads to severe impaired vasorelaxation, high levels of mitochondrial ROS formation and mitochondrial DNA damage [125]. Decreased Mn-SOD activity promotes also atherosclerotic lesion development and increases aortic mtDNA damage in $apoE^{-/-}$ mice [126, 127]. Miller *et al.* [128] reported that chronic increases in oxidative stress, produced by mitochondrial Mn-SOD deficiency in mice, impair vascular function via a H_2O_2-dependent, cyclooxygenase 1-dependent endothelium-derived contracting factor.

On the contrary, mice over-expressing Mn-SOD has reduced infarct size compared to wild type [129] and diminished severity of diabetic cardiomyopathy [130]. In rats, over-expression of Mn-SOD in carotid arteries results in reduced $^{\cdot}O_2^-$ production in response to endothelin-1 [131] and in the rostral ventrolateral medulla it attenuates the ANG II-induced pressor response and suppressed ANG II-induced

ROS production [132]. Transgenic mice with increased Mn-SOD expression in the liver have 23% fewer bromodeoxy-uridine-positive cells and a marked attenuation of proliferative cell nuclear antigen expression, leading to delayed entry into S phase [133]. The increase in Mn-SOD activity also leads to an increase in the mitochondrial thioredoxin-2, but not in other peroxidases, suggesting the importance of thioredoxin-2 in maintaining redox balance in mitochondria with elevated levels of Mn-SOD [133]. Studies with fibroblasts have shown that increased Mn-SOD expression prolongs cell type transition time in G_1/S and favors entrance into the quiescent state [134, 135].

Mitochondrial oxidative stress and damage seems also to play a major role in the development and progression of left ventricular remodeling and failure after myocardial infarction [136, 137]. In the failing ventricular myocard from patients with end-stage heart failure a marked decline in mitochondrial Mn-SOD protein and activity is detected [138]. Mitochondrial biogenesis is also severely impaired as evidenced by reduced mtDNA replication and depletion of mtDNA in the human failing heart [139].

Both acute (24 hours) and chronic (14 days) ANG II treatment in mice results also in decreased expression of mitochondrial metabolic genes, notably those for the electron transport chain and Krebs-TCA cycle [140]. Chronic ANG II treatment also results in decreased expression of genes involved in fatty acid metabolism [140]. In contrast, genes involved in protein translation and ribosomal activity increase expression following both acute and chronic ANG II administration [140]. It has also been demonstrated by Kienhöfer *et al.* [141] that mtDNA is associated with an antioxidant system in mammalian cells with Mn-SOD binding directly to mtDNA. Mn-SOD is thus essential to protect mitochondrial genome from oxidative damage.

7. DOWN-REGULATION OF PEROXIREDOXIN-3 EXPRESSION BY ANGIOTENSIN II IN CARDIAC FIBROBLASTS

Cardiac fibroblasts are protected from oxidative stress, triggered by inflammation after myocardial injury or induced by ANG II or growth factors, by expressing potent anti-oxidant defenses such as superoxide dismutases, catalases, glutathione peroxidases and peroxiredoxins [142, 143].

Multiple peroxiredoxins or thioredoxin peroxidases, (Prx-1 through -6) are identified in mammalian cells in different intracellular locations and protect cells and tissues from damage caused by ROS [144-147]. All six peroxiredoxins are present in cardiac fibroblasts. Prx-1, -2 and -6 are localized in the cytosol, Prx-3 in mitochondria, Prx-4 in the extracellular space and Prx-5 is localized intracellularly to cytosol, mitochondria and peroxisomes [148, 149]. Prx-3 is found exclusively in the mitochondria [143] and uses mitochondrial thioredoxin (Trx-2) as the electron donor for its peroxidase activity [150]. Prx-3 functions not only by removing H_2O_2 formed after the SOD-catalysed dismutation but also by detoxifying peroxynitrite [151].

ANG II decreases the mRNA and protein expression of the mitochondrial specific antioxidant Prx-3 (Fig. **5**), while the cytosolic Prx-1 fraction is unaffected [152].

A down-regulation of Prx-3 has been described in various experimental models that are characterized by an increased cellular oxidative stress [153-155]. In human heart failure Brixius *et al.* [156] have also reported a selective down-regulation of the mitochondrial Prx-3 and Prx-5 as well as that of the extracellular Prx-4 isoform and that of the cytosolic Prx-6, while the cytosolic Prx-1 and Prx-2 isoforms are unaffected by the enhanced ROS production.

Thus reducing Prx-3 sensitizes cells to oxidative stress [157]. Furthermore, Prx-3 knockdown by siRNA increases mitochondrial ROS [158] and Prx-3 knock-out mice are more susceptible to lipopolysaccharide-induced oxidative stress than their wild-type littermates [159]. Higher levels of ROS are detectable in macrophages derived from these mice and they release increased amounts of TNFα [160]. Taken all data together suggest that the loss of Prx-3 results in increased susceptibility to oxidative stress. Thus, it may be concluded that an increase in cellular oxidative stress seems also to be paralleled by a down-regulation of mitochondrial Prx-3.

Chronic increases in mitochondrial ROS production can lead to a catastrophic cycle of further oxidative stress and ultimate cellular injury [161]. This deleterious process may play an important role in the development and progression of myocardial remodeling and failure [162].

Given that mitochondria contain Prx-3 30 times more abundant than glutathione peroxidase and that they lack catalase [157], Prx-3 is thought to be a primary line of defense against H_2O_2 produced by the mitochondrial respiratory chain, as Mn-SOD does against $^{\cdot}O_2^-$.

The specific localization of Prx-3 in the mitochondria suggests that mitochondrial oxidative stress plays an important role in the development and progression of heart failure and the antioxidant localized specifically within the mitochondria provides a primary line of defense against the disease process [143].

Indeed, over-expression of Prx-3 protects the heart against post-MI remodeling and failure in mice [161]. It reduces LV cavity dilatation and dysfunction as well as myocyte hypertrophy, interstitial fibrosis and apoptosis of the non-infarcted myocard. These beneficial effects of Prx-3 gene over-expression are associated with the attenuation in oxidative stress, mtDNA decline and dysfunction [163]. Prx-3 over-expression has also been shown to improve glucose homeostasis, with transgenic mice displaying resistance to diet-induced elevations in blood glucose and increased glucose clearance [164].

Prx-3 is thus an important candidate for therapy against LV failure after MI, in which ROS production has been found to be increased within the mitochondria [161]. Recently it has been shown that an elevated cytosolic Na^+ increases mitochondrial formation of ROS in failing cardiac myocytes of guinea pigs [165].

8. REGULATION OF MN-SOD EXPRESSION BY FOXO3A IN CARDIAC FIBROBLASTS

In human cardiac fibroblasts the Forkhead box class o transcription factor FOXO3a mediates the expression of

peroxiredoxin-3, which functions to protect mitochondria against oxidative stress by scavenging H_2O_2 [166].

FOXO transcription factors may be important in the regulation of the antioxidant defense in many species [163]. It has indeed been shown that FOXO3a interacted with the promoter of the rat Mn-SOD gene at a specific binding site, located 1272 bp upstream of the coding region of the rat Mn-SOD promoter [167]. In rat cardiac fibroblasts ANG II also reduces the binding of FOXO3a to the Mn-SOD promoter [107]. Inhibition of FOXO3a transcription with small interfering RNA leads to a reduced FOXO3a binding to the Mn-SOD promoter and a concomitant reduction in Mn-SOD gene expression in control cardiac fibroblast [107], implying that FOXO3a does up-regulate Mn-SOD. Specific activation of FOXO3a by 4-hydroxy-tamoxifen, a modified ligand of the estrogen receptor, increases Mn-SOD mRNA expression in control fibroblasts and reverses the ANG II-induced reduction in Mn-SOD gene expression [107]. In FOXO3a depleted fibroblasts the reduced Mn-SOD expression is also associated with an increased $^{\cdot}O_2^-$ production [105]. Our data suggest thus that FOXO3a may be the transcription factor responsible for the ANG II-induced down-regulation of Mn-SOD in cardiac fibroblasts. ANG II-stimulated Akt (protein kinase B) activity might thus responsible for the phosphorrylation and inactivation of FOXO3a which in turn down-regulates Mn-SOD transcription in adult rat cardiac fibroblasts. In DL23 cells FOXO3a also increases the expression of Mn-SOD [167] and a lower Mn-SOD protein and activity in vascular smooth muscle cells from old rats is also paralleled by a reduction in FOXO3a transcriptional activity [168]. Although FOXO3a is able to regulate mRNA and protein levels of catalase in neonatal rat cardiomyocytes [168], no changes in activity, mRNA and protein expression of catalase are found in adult rat cardiac fibroblasts [107]. This has to be further elucidated in various cardiac cell types of neonatal and adult rat hearts. It should however taken into account that catalase has been detected in mitochondria from rat heart [169,170], but this is at low nanomolar concentrations (0.025% of rat heart mitochondrial protein) and therefore probably not regarded to play a significant role in mitochondrial ROS detoxification in rat heart mitochondria [171].

ANG II is thus linked with increased Akt phosphorrylation in cardiac fibroblasts and consequent reduction in FOXO3a transcriptional activity leading to downregulation of Mn-SOD gene expression. Upon phosphorylation of Akt by ANG II P-Akt is indeed translocated from the cytoplasm to the nucleus and nuclear phosphorylation of FOXO3a by P-Akt leads to relocalization of FOXO3a from the nucleus to the cytosol [107], thus resulting in a decrease in its transcriptional activity and in Mn-SOD expression. Inhibition of PI3K with wortmannin and LY294002 and Akt inhibition leads to a decrease in P-Akt, to an increased Mn-SOD mRNA expression and a reduced $^{\cdot}O_2^-$ production [107]. This shuttling mechanism contributes to the down-regulation of Mn-SOD gene expression. In adult rat cardiac fibroblasts ANG II also provides protection against NO-stimulated apoptosis by activating a PI3kinase-Akt-mediated survival signaling pathway [172]. ANG II inactivates thus FOXO3a by activating Akt and this leads to a reduction in the expression of the antioxidant Mn-SOD contributing to the ANG II-induced ROS production. ANG II may thus cause an increased oxidative stress by inhibiting the expression and activity of the mitochondrial enzyme Mn-SOD engaged in ROS breakdown.

9. ANG II-INDUCED OXIDATIVE STRESS AND MODULATORY EFFECT OF DETC

The irreversible CuZn-SOD inhibitor DETC increases, while the $^{\cdot}O_2^-$ scavenging agent tempol inhibits completely,

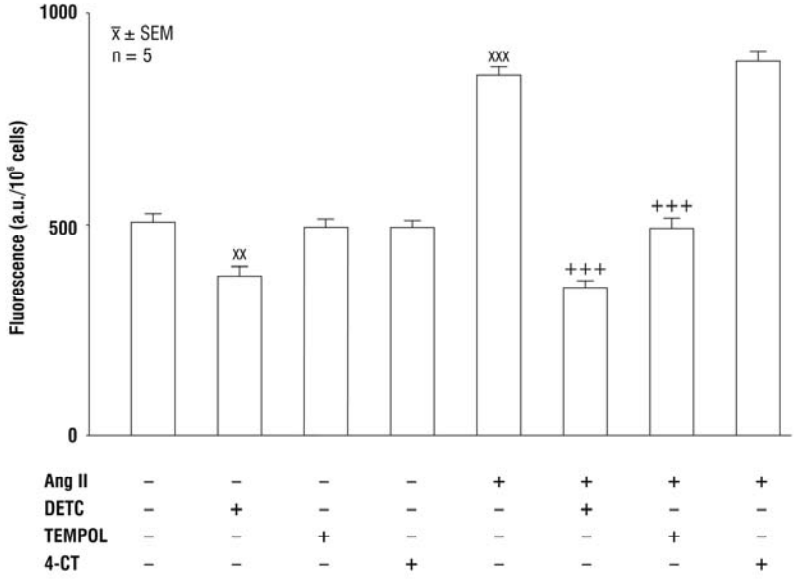

Fig. (7). Intracellular reactive oxygen species (ROS) generation, assessed in cardiac fibroblasts treated with(out) ANG II (1 μmol/l) for 24 hours after preincubation with diethyldithiocarbamic acid (DETC, 100 μmol/l), tempol or 4-carboxy-tempol (4-CT, 1 μmol/l) for 1 h, and incubated with 2',7'-dichlorofluorescein diacetate (20 μmol/l) for 30 min. xxx $p<0.001$, xx $p<0.01$ compared with control without ANG II and DETC, tempol and 4-CT; +++ $p<0.001$ compared with samples with ANG Ii and without DETC, tempol and 4-CT.

the $^{\cdot}O_2^-$ production in control and ANG II-treated adult rat cardiac fibroblasts (Fig. **7**) [97].

An enhanced $^{\cdot}O_2^-$ generation by DETC in rats has been reported in various cell types such as neonatal cardiac fibroblasts [24] and myocytes [12], aortic rings [22,173], renal medullary tissue [174], vascular smooth muscle cells [26], aortic adventitial fibroblasts [175] and intact middle cerebral and basilar arteries [176]. DETC also causes a dose-dependent increase in levels of $^{\cdot}O_2^-$ in rings of rabbit aorta [23]. Besides an increase in $^{\cdot}O_2^-$ production in cardiac fibroblasts DETC also reduces the intracellular ROS production in control and ANG II-treated cardiac fibroblasts [97]. Colston *et al.* [177] also reported a decreased ROS production by DETC in adult rat cardiac fibroblasts, while in adult rat cardiac myocytes an increased ROS generation is found [178]. These data indicate that in adult rat cardiac fibroblasts oxidative stress is increased by ANG II, partially through down-regulation of antioxidant enzymes as SOD, and that DETC enhances $^{\cdot}O_2^-$ production and decreased intracellular ROS formation as a consequence of inhibition of CuZn-SOD.

10. ANG II-INDUCED COLLAGEN PRODUCTION AND DEGRADATION DURING INHIBITION OF CARDIAC FIBROBLAST SUPEROXIDE DISMUTASE

Inhibition of SOD by DETC and the subsequently increased $^{\cdot}O_2^-$ production induces an increase in collagen production and fibronectin level in adult cardiac fibroblasts, while inhibition of $^{\cdot}O_2^-$ generation by the SOD mimetics tempol and EUK-8 lowers the collagen production and fibronectin content (Fig. **8**) [95].

The antioxidant tempol is a cell permeant scavenger of superoxide anion. In diabetic rats tempol administration also inhibits the mesangial expression of fibronectin and matrix expansion [179]. The increased cardiac collagen content as well as the collagen type I mRNA expression in chronic isoproterenol infused rats is reversed by tempol treatment [180]. In mice subjected to pressure overload, EUK-8 also atenuates cardiac hypertrophy and fibrosis, prevents myocardial oxidant stress and improves LV end-systolic dimensions and fractional shortening [181].

In neonatal rat cardiac fibroblasts however DETC inhibits collagen synthesis and increased total MMP activity [24], while in neonatal cardiac myocytes DETC reduces cardiac hypertrophy through a ROS-dependent mechanism [12].

Administration of PEG-SOD to cardiac fibroblasts induces however a decrease in collagen deposition [95]. In pig skin fibroblasts in a three-dimensional model, SOD administered as liposod CuZn-SOD also significantly lowers the level of the extracellular matrix components α_1(I) collagen and tenascin-C and of the myofibroblast marker α-smooth muscle actin [26]. In ANG II-induced hypertension in rats, both hypertension and the impairment of vasodilator responses to acetylcholine and calcium ionophores are improved by treatment with liposome-encapsulated SOD [15], In ANG II-treated rats liposome-entrapped SOD increases total SOD activity by 30 % in vascular homogenates, normalizes vascular $^{\cdot}O_2^-$ production and reduces blood

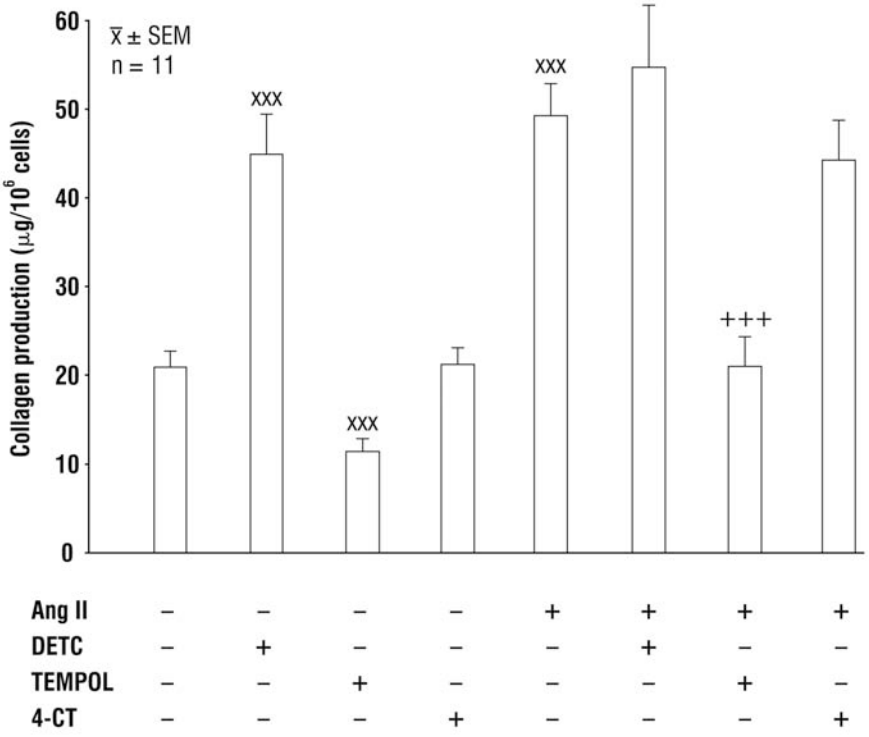

Fig. (8). Soluble collagen production in cardiac fibroblasts treated with(out) ANG II (1 µmol/l) for 24 hours after preincubation with diethyldithiocarbamic acid (DETC, 100 µmol/l), tempol or 4-carboxy-tempol (4-CT, 1 µmol/l) for 1 h. xxx $p<0.001$ compared with control without ANG II and DETC, tempol and 4-CT; +++ $p<0.001$ compared with samples with ANG II and without DETC, tempol and 4-CT.

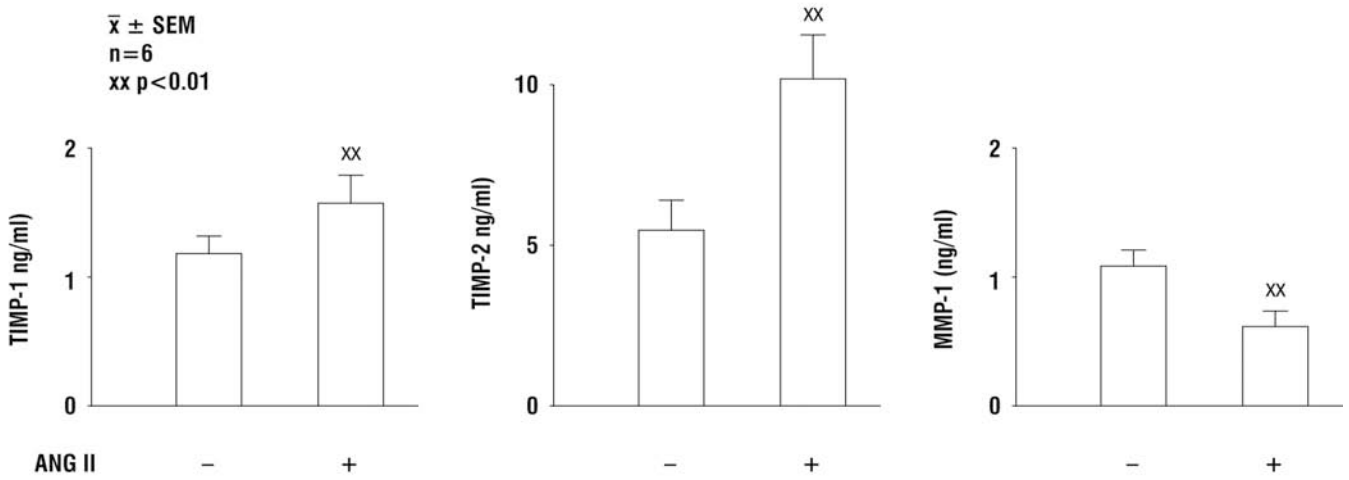

Fig. (9). TIMP-1, TIMP-2 and MMP-1 level in the medium of cardiac fibroblasts treated with(out) ANG II (1 μmol/l) for 24 hours. xx $p<0.01$ compared to control (-).

pressure [182]. *In vivo* administration of lecithinized SOD in rats inhibits oxidative stress and improves outcomes after focal cerebral ischemia [183]. An inhibitory effect of phosphatidylcholine-SOD on bleomycin-induced pulmonary fibrosis in mice is also reported [184]. SOD is indeed already successfully used as an antifibrotic agent in the treatment of cutaneous fibrosis both in clinical trials [185, 186] as in animal experimental studies [187].

The stimulation of the collagen production by SOD inhibition with DETC and the inhibition of the collagen production by PEG-SOD and by the SOD mimetics tempol and EUK-8 [95] suggest a vital role of SOD and the formed ROS in the regulation and organization of collagen in cardiac fibroblasts. Indeed, over-expression of SOD in mouse aortic adventitial fibroblasts inhibits the ANG II-induced type I procollagen α-I protein expression and $\cdot O_2^-$ generation [188].

In adult rat cardiac fibroblasts DETC and ANG II stimulate collagen production and TIMP-1 and TIMP-2 synthesis [97] which inhibits collagen degradation by MMPs and leads to collagen accumulation. Indeed, ANG II decreases MMP-1 activity in adult rat cardiac fibroblasts [42,67,79] and increases TIMP-1 and TIMP-2 levels (Fig. **9**) [97], with a more pronounced effect on TIMP-2 than on TIMP-1, but no effect on MMP-2 and MMP-9 level.

It has indeed been shown that adenovirus-mediated over-expression of TIMP-2 but not of TIMP-1, TIMP-3 or TIMP-4 in cardiac fibroblasts increases collagen synthesis [189]. In TNFα-stimulated neonatal rat cardiac fibroblasts ANG II also increases TIMP-1 expression and decreases MMP-2 activity [70]. Bergman *et al.* [190] on the contrary reported that ANG II moderately increased MMP-2 secretion in neonatal rat cardiac fibroblasts.

Compared with left ventricle hypertrophy, MMP-2 but not MMP-9 activity level in LV myocardium is increased with congestive heart failure (CHF) and inhibited by the ANG II-receptor blocker telmisartan, while the mRNA expression of MMP-2, MMP-9, MMP-13, TIMP-1 and TIMP-2 was increased with CHF and blocked by telmisartan [191].

At first glance MMP-2 expression should result in a decreased level of cardiac interstitial collagen. However, significant increases in interstitial collagen volume fraction are noted in MMP-2 transgenic mice compared with age-matched littermate controls [192]. Analysis of cardiac tissue from patients with idiopathic dilated cardiomyopathy also shows a large increase in gelatinase activity associated with increased total collagen content [193]. Similarly, Matsusaka *et al.* [194] reported increased cardiac collagen content in mice with intact levels of MMP-2 as opposed to MMP-2 knockout mice. In MMP-1 transgenic mice Kim *et al.* [195] showed that cardiac expression of MMP-1 produced a net loss of cardiac interstitial collagen coincidence with a marked deterioration of both systolic and diastolic function. However, it has also to be taken into account that the more collagen is degraded the more is synthesized [196]. Consequently, synthesis exceeds degradation and leads also to accumulation of collagen [196]. It has also demonstrated that under physiological relevant conditions interstitial collagen is not efficiently cleaved by MMP-2 [197, 198].

11. CONCLUSIONS

The ANG II-stimulated collagen production, collagen type I and III protein and mRNA expression as well as the ANG II-enhanced superoxide anion and intracellular ROS production in adult rat cardiac fibroblasts are completely blocked by the membrane-associated NAD(P)H oxidase inhibitor apocynin. The ANG II-decreased MMP-1 activity and increased TIMP- activity are also reversed by apocynin. The collagen production in cardiac fibroblasts is however stimulated by SOD inhibition with DETC and inhibited by the SOD mimetics tempol and EUK-8 and by PEG-SOD administration. These data suggest a role of SOD and the generated ROS in the myocardial accumulation of collagen.

12. PERSPECTIVES

Superoxide anion generated by NAD(P)H-oxidase has an important role in the pathogenesis of cardiovascular diseases and scavenging superoxide anion can be considered as a reasonable therapeutic strategy [199,200]. In this review we

demonstrate that scavenging superoxide anion by tempol or EUK-8 or administration of PEG-superoxide dismutase inhibits collagen production in cardiac fibroblasts. On the contrary increasing superoxide anion formation by inhibition of superoxide dismutase stimulates collagen production. A vital role of superoxide dismutase and the generated reactive oxygen species can be suggested in the myocardial collagen accumulation.

Specific pharmacological intervention with superoxide dismutase mimetics can probably be an alternative approach for reducing myocardial fibrosis.

A growing body of evidence suggests that oxidative stress, a chronic increase in reactive oxygen species (ROS), in the myocard can contribute to myocardial remodeling and failure. Furthermore, antioxidants have been shown to exert protective and beneficial effects against this process. Mitochondria are the predominant source of ROS and miotochondria antioxidants are expected to be the first line of defense against mitochondrial oxidative stress-mediated myocardial injury. Angiotensin II increases mitochondrial ROS production in cardiac fibroblasts and decreased the protein and mRNA expression of the mitochondrial antioxidant Mn-SOD by induction of the phosphorylation of Akt and FOXO3a and repression of the FOXO3a binding to the Mn-SOD promoter gene. Exposure to angiotensin II could thus lead to increased oxidative stress because of down-regulation of antioxidant enzymes such as Mn-SOD and Prx-3. These antioxidants are specifically localized in the mitochondria. It could thus provide a primary line of defense against angiotensin II-induced oxidative stress and myocardial injury. Therapies designed to interfere with mitochondrial oxidative stress by using antioxidants such as SOD-mimetics might be beneficial in hypertensive heart diseases and in preventing heart failure.

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DISCLOSURE

Part of the information included in this article has been previously been published in Cardiovascular Therapeutics. This article is however an extended and updated version with nine additional figures on the stimulation of reactive oxygen species and collagen synthesis by angiotensin II in cardiac fibroblasts.

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