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Angiotensin II-mediated expression of mitochondrial peroxiredoxin-3 in rat cardiac fibroblasts: Association with oxidative stress in myocardium

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ABSTRACT

The aim of this study was to determine whether angiotensin II (ANG II) affects the protein and mRNA expression of the mitochondrial antioxidant peroxiredoxin-3 (Prx-3) in cardiac fibroblasts through inducing the phosphorylation of the proteins Akt and FOXO3a, thereby contributing to the oxidative stress in the myocardium.

Cardiac fibroblasts (passage 2) from normal male adult rats were cultured to confluency and incubated in serum-free Dulbecco's modified Eagle's medium for 24 h. The cells were then preincubated with (out) the tested inhibitors for 1 h and further incubated with (out) ANG II (1 µmol/l) for 24 h.

ANG II increased ($p<0.001$) the intracellular and mitochondrial reactive oxygen species (ROS) production in cardiac fibroblasts. ANG II also decreased ($p<0.01$) the mRNA and protein expression of Prx-3 by 36.9 ± 3.0% and 29.7 ± 2.7%, respectively. This ANG II-induced decrease in Prx-3 expression was blocked by the angiotensin II type 1 receptor blocker losartan and not by the angiotensin II type 2 blocker PD 123,319 The ANG II-released transforming growth factor-â$_1$ did however not affect the ANG II-enhanced intracellular ROS generation. The mitochondrial complex II and IV activity were not affected by ANG II and the mitochondrial complex I and III activity were reduced ($p<0.05$) by ANG II.

The likely mechanism through which ANG II produces the effect of reducing Prx-3 expression is by reducing the extent of binding of FOXO3a to the Prx-3 promoter. In control fibroblasts inhibition of FOXO3a transcription with small-interfering RNA (siRNA) led to a reduction in Prx-3 gene expression.

Our data also shown that when Akt is phosphorylated by ANG II, P-Akt is translocated from the cytoplasm to the nucleus, subsequently nuclear phosphorylation of FOXO3a by P-Akt leads to relocalization of FOXO3a from the nucleus to the cytosol, resulting in a decrease in its transcriptional activity, and consequently in Prx-3 expression. The likehood of such a mechanism is further strengthened by the fact that inhibition of phosphoinositide 3-kinase with wortmannin or LY 294002 was shown to lead to a decrease in P-Akt and to a consequent increase in Prx-3 expression.

Our data indicate that ANG II inactivates FOXO3a by activating Akt, leading to a reduction in the expression of the antioxidant Prx-3, and thereby potentially contributing to oxidative stress in the myocard.

KEYWORDS

Peroxiredoxin-3;
Angiotensin II;
Cardiac fibroblasts;
Reactive oxygen species;
Mitochondria;
Complex I, II, III, IV activity.

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INTRODUCTION

Angiotensin II (ANG II), the primary circulating component of the renin-angiotensin system, is a multifunctional hormone responsible for many cellular processes such as fibrosis, inflammation, migration, proliferation, hypertrophy and apoptosis, resulting in the cardiovascular remodeling[1]. Unfavorable left ventricular remodeling after myocardial infarction leads to cardiac dysfunction.

ANG II is also known to contribute to oxidative stress damage by stimulating the generation of both nitric oxide and NAD (P)H oxidase-derived superoxide in the cytosol of different cell types including endothelial cells, vascular smooth muscle cells, fibroblasts and tubular epithelial cells[2].

Cardiac fibroblasts are protected from oxidative stress, triggered by inflammation after myocardial injury or induced by ANG II or growth factors, by expressing potent antioxidant defenses such as superoxide dismutases, catalases, glutathione peroxidases and peroxiredoxins[3,4].

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 reactive oxygen species (ROS)[5-8]. ROS are the key mediators of pathogenesis in cardiovascular diseases. Peroxiredoxins, a family of antioxidant and redox-signaling proteins, are plentiful within the heart[9]. They take active part in scavenging ROS, thus playing an essential role in maintaining the intracellular redox status. 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[10,11]. Prx-3 is found exclusively in the mitochondria[5] and uses mitochondrial thioredoxin (Trx-2) as the electron donor for its peroxidase activity[12].

Mitochondria are considered the main intracellular source of ROS[13]. The majority of intracellular ROS generation is derived from mitochondrial matrix and the space between the inner and outer mitochondrial membrane. Mitochondria utilize more than 90% of cellular oxygen to produce energy. While most oxygen is transformed to water, 1-2% of it forms superoxide[14]. ROS production, mitochondrial DNA damage and respiratory chain impairment are linked to one another to create a vicious cycle that leads to progressive decline in mitochondrial bioenergetics and subsequent cardiac dysfunction[15]. Prx-3 functions not only by removing H_2O_2 formed after the superoxide dismutase (SOD)-catalysed dismutation but also by detoxifying peroxynitrite[16]. Peroxiredoxins are known to possess peroxidase activity, whereby H_2O_2, peroxynitrite and a wide range of hydroperoxides are reduced into water and alcohol and detoxified[11,17].

In adult rat cardiac fibroblasts we have reported that ANG II increased superoxide anion production and intracellular formation of reactive oxygen species[18,19]. It has also been shown that the serine/threonine protein kinase Akt-regulated Forkhead transcription factor FOXO3a protected cells from oxidative stress by directly increasing Mn-SOD mRNA and protein expression[20]. Upon phosphorylation of Akt by ANG II in cardiac fibroblasts, 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, thus resulting in a decrease in its transcriptional activity and in Mn-SOD expression[21]. Activation of Akt in mitochondria also suppresses apoptosis of cardiomyocutes[22].

The aim of the present study was to investigate whether ANG II affects Prx-3 mRNA and protein expression in rat cardiac fibroblasts through induction of phosphorylation of Akt and FOXO3a and repression of the FOXO3a binding to the Prx-3 promoter and consequently Prx-3 gene expression.

METHODS

Cell cultures

All animal procedures were in accordance with the laws, regulations and administrative provisions of the Member States of the European Community (Council Directive 86/609/EEC of November 24, 1986) regarding the protection of animals for experimental and other scientific purposes. This research protocol was also approved by the Ethical Committee for Animal Experiments of the Katholieke Universiteit Leuven (K.U.Leuven), Belgium.

Cardiac ventricular fibroblasts obtained from male adult Wistar rats were grown in Dulbecco's modified Eagle's medium (DMEM) in the presence of 10% fetal

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bovine serum (FBS) and used in passage 2 as previously described[23]. When cultures reached confluence, the medium was replaced with fresh phenol red free DMEM with 0.5% FBS for 24 h. The cells, identified as proliferative myofibroblasts[24], were then preincubated with (out) the tested inhibitors for 30 min to 1 h and then further incubated with (out) ANG II (1 µmol/l) for 24 h in this medium.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated from control and ANG II-treated cardiac fibroblasts with Trizol (Gibco BRL, Life Technologies Ltd, Paisley, UK), followed by chloroform extraction and precipitation with isopropanolol[25,26]. RNA pellets were washed with 80% ethanol, air dried and dissolved in distilled water. Single-stranded cDNA was synthetised from 5 µg of total RNA, using a commercial cDNA mix (Life Technologies). The mRNA levels were quantified by the RT-PCR method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as internal standard, as previously described[24]. The PCR primers for target cDNAs were as follows: Prx-3, sense GCTGAGTCTCGAACGACTT TAAGGG and antisense CTTGATCGTAGGGGACTCTGGTGT[27]; Prx-1, sense TGTGGATTCTCACTTCTGTCATCTG and antsense TGCGCTTGGGATCTGATACC[28]; FOXO3a, sense CGGACAAACGGCTCACTT and antisense TCGGCTCTTGGTGTACTTG[29].

NF-κB p105/p50 sense TTGAGCCTCTCTATGACCTGG and antisense CCCAGAGACCTCATAGTTGTCC[30] and GAPDH, sense ACCACAGTCCATGCCATCAC and antisense TCCACCACCCTGTTGCTGTA[21]. Ten microliters of PCR products were separated on 1.2-1.3% agarose gels, stained with ethidium bromide and photographed[24]. Densitometric analysis of the stained images was performed using a Sharp Scanner JX-325 and a Software Image Master (Amersham Pharmacia Biotech, Roosendaal,The Netherlands). The densities of Prx-3 and FOXO3a bands in relation to the bands obtained for GADPH were expressed as Prx-3 or FOXO3a mRNA/GADPH mRNA.

FOXO3a binding site in the rat Prx-3 promoter

FOXO3a binding to Prx-3 promoter in nuclear extracts (30 µg) of control and ANG II-treated fibroblasts was detected by a NoShift Transcription Assay kit (EMD Biosciences Inc., Merck, Darmstadt, Germany) using an anti-FOXO3a antibody (Cell Signaling, Danvers, MA, USA), a biotinylated double stranded oligonucleotide CACACAAGGTTAACAAAACAGTGGGAAAT ATGGAAACAAATACCTAAT G[31] and an anti-rabbit IgG-HRP (Clontech Labs Inc, Mountain View, CA, USA) as secondary antibody.

Silencing (si) RNA transfection

FOXO3a siRNA SMARTpool, (a mixed pool of 4 target sequences GCACGGAGCUGGAUGACGU, U G G A U G A C C U G C U A G A U A A , GAACGUUGUUGGUUUGAAC and CGUCAUGGGUCACGACAAG), synthetised by Dharmacon (Lafayette, CO, USA) according to rat LOC295 (XM_215421)FOXO3a, was delivered to cardiac fibroblasts at 100 nM final concentration for 24-48 hours through a lipid-mediated DharmaFECT transfection reagent.

Prx-3 siRNA (NM_022540) was synthetised by Qiagen (Hilden, Germany) according to rat Rn_LOC502175.

Immunoblot

Western blotting[19] has been performed in cell lysates and in nuclear and cytosolic extracts prepared by NE-PER extraction reagents (Pierce Biotechnology, Rockford, Il, USA). Antibodies for FOXO3a, phospho-FOXO3a (Ser^{253}), Akt and phospho-Akt (Ser^{473}) were from Cell Signaling, antibodies for Prx-1 from Abcam Inc (Cambridge, MA, USA), for Prx-3 from LabFrontier (Seoul, Korea) and for GAPDH from Sigma Chem. Co. (St Louis, Missouri, USA) and the corresponding secondary HRP-conjugated antibodies from Chemicon Inc. (Temecula, CA, USA).

Assay of reactive oxygen species

A fluorescent probe, 2',7' -dichlorofluorescein diacetate (DCF-DA) was used for the assessment of intracellular[32,33] and mitochondrial[34] ROS formation (such as hydrogen peroxide, hydroxyl and peroxyl radicals and hydroperoxides) in cardiac fibroblasts treated with (out) ANG II. Mitochondria, free of peroxisomes and lysosomes, were isolated from cardiac fibroblasts with the isolation kit MITOISO1 from Sigma Chem. Co.

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Assay of mitochondrial complex I, II, III and IV acitivity in cardiac fibroblasts

Complex I (NADH ubiquinone oxidoreductase) activity was assayed as rotenone-sensitive oxidation of NADH with decylubiquinone as acceptor[35].

Complex II (succinate: 2, 6-dichlorophenolindophenol (DCIP) oxidoreductase) activity was measured as thenoyltrifluoroacetone (TTFA)-sensitive reduction of DICP with succinate as substrate[36]. Complex III (ubiquinol ferricytochrome c oxidoreductase) activity was assayed as antimycin sensitive reductase activity[36]. Complex IV (cytochrome c oxidase) activity was determined with the CYTOCOX1 assay kit from Sigma Chem Co.

Statistical analysis

Values are expressed as mean ± SEM. The statistical methods used were repeated measures of variance (Tukey's) and Students 2-tailed test for (un)paired data when appropriate. A value of $p<0.05$ was considered statistically significant.

RESULTS

Effect of ANG II on Prx-3 mRNA and protein expression in cardiac fibroblasts

ANG II reduced dose-dependently the mRNA expression of the mitochondrial antioxidant Prx-3 (figure 1). Addition of ANG II (1 µmol/l) decreased ($p<0.01$) the mRNA expression of Prx-3 in cardiac fibroblasts (n=4) by 36.9 ± 3.0 % (n=4), while mRNA expression of the cytosolic homologue Prx-1 was unaffected by ANG II (figure 2).

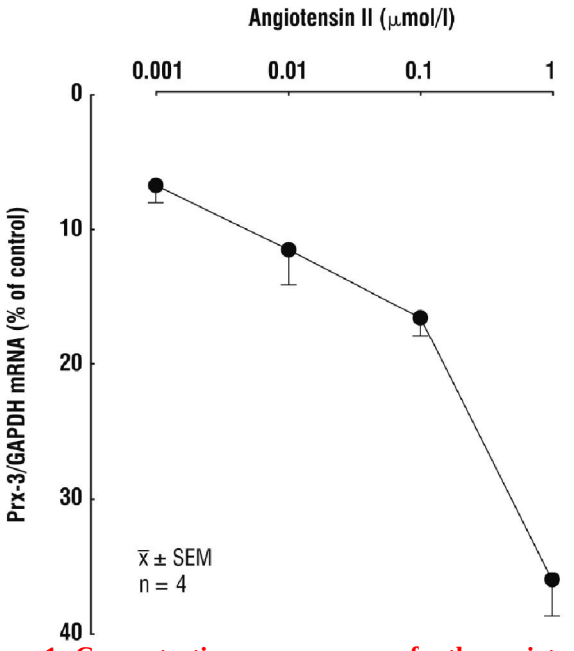
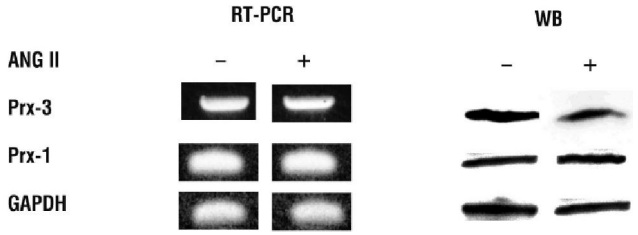


Figure 1 : Concentration-response curve for the angiotensin II (ANG II)- induced changes in peroxiredoxin-3 (Prx-3) mRNA expression. Cardiac fibroblasts were treated with(out) ANG II (0.001, 0.01, 0.1 and 1 µmol/l) for 24 h.



RT-PCR: reverse transcription polymerase chain reaction; WB: Western blotting

Figure 2 : Representative gels for mRNA and protein expression of peroxiredoxin-3 (Prx-3), peroxiredoxin-1 (Prx-1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in cardiac fibroblasts treated with(out) ANG II (1 µmol/l) for 24 h.

The Prx-3 protein level was also reduced ($p<0.01$) by 29.7 ± 2.7 % (n=4) in ANG II-treated (n=4) fibroblasts compared to controls; the protein expression of Prx-1 was however not affected by ANG II (figure 2).

The ANG II-induced decrease in mRNA expression of Prx-3 was prevented by the angiotensin type 1 receptor blocker, losartan but not by the angiotensin type 2 receptor blocker, PD 123,319 (figure 3).

The de novo DNA synthesis, assayed as incorporation of ^{3}H-thymidine, was not affected by ANG II relative to the controls (18,330 ± 336 vs. 15,940 ± 560 desintegrations/minute/10^6 cells, n=6). The DNA content (5.74 ± 0.33 pg/cell) and the number of cells (1,399,751 ± 98,221) were also not different between the control cells and the ANG II-treated cells.

ANG II-induced intracellular ROS production in cardiac fibroblasts

To determine whether ANG II affects intracellular H_2O_2, cardiac fibroblasts treated with (out) ANG II were incubated with 2',7'-dichlorofluorescein diacetate (DCF-DA), a peroxide-sensitive dye that is incorporated into the cell. ANG II caused a 1.8 -fold increase ($p<0.001$) in DCF-DA fluorescence from 389 ±36 to 695 ±66 a.u./10^6 cells (n = 10), indicating ROS generation. 5-Hydoxydecanoate and glibenclamide, specific and nonspecific inhibitors of mitochondrial ATP-sensitive potassium channels, respectively, suppressed ($p<0.01$) the intracellular ROS generation in control

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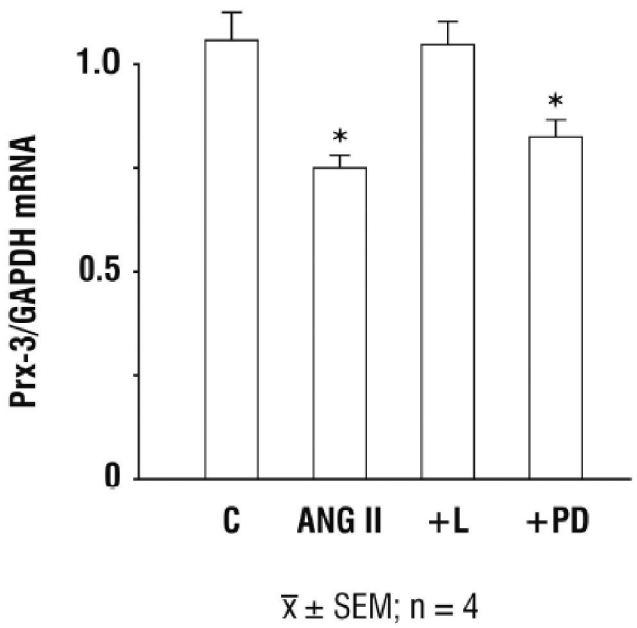
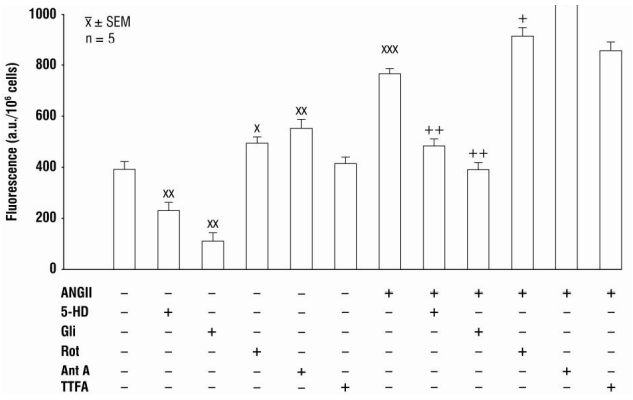
(42.0 ± 7.1% and 71.6 ± 8.7%, respectively) and ANG II-treated (32.5 ± 5.8% and 45.8 ± 4.9%, respectively) cardiac fibroblasts (figure 4).



x p<0.05 as compared to control (C).

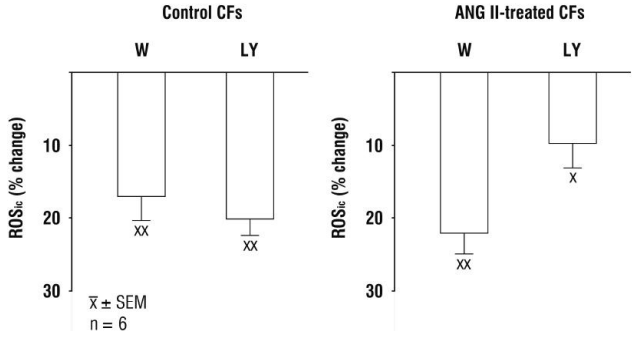
Figure 3 : Peroxiredoxin-3 (Prx-3)/ glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA ratio in cardiac fibroblasts treated with(out) angiotensin II (1 µmol/l) for 24 h after preincubation with losartan (L, 1 µmol/l) or PD 123,319 (PD, 1 µmol/l) for 1 h.



xxx p<0.001, xx p<0.01, x p<0.05 compared to samples with(out) ANG II.; ++ p<0.01, +p<0.05 compared to samples with ANG II and without 5-HD, Gli, Rot, Ant A and TTFA

Figure 4 : Intracellular reactive oxygen species (ROS_{ic}) generation assessed in cardiac fibroblast treated with(out) ANG II (1 µmol/l) for 30 min after preincubation with 5-hydroxydecanoate (5-HD, 100 µmol/l), glibenclamide (Gli, 20 µmol/l), rotenone (Rot, 10 µmol/l), antimycin A (Ant, 10 µmol/l) or thenoyltrifluoroacetone (TTFA, 10 µmol/l) for 1 h.

The complex I inhibitor rotenone increased (p<0.05) the intracellular ROS production in control and ANG II-treated fibroblasts by 27.6 ± 3.5 and 27.3 ± 5.3 %, respectively. Antimycin A, which blocks the Q_i site located at the inner membrane and facing the mitochondrial matrix of complex III, enhanced (p<0.01) the intracellular ROS production in control (41.9 ± 8.6 %) and ANG II-treated (45.6 ± 2.9%) fibroblasts. The complex II inhibitor TTFA did not affect the intracellular ROS production in cardiac fibroblasts (figure 5).



ROS_{ic} in control and ANG II-treated cardiac fibroblasts averaged, respectively, 450 ± 23 and 805 ± 50 a.u./10^6 cells.; xx p<0.01, x p<0.05 compared to samples with(out) ANG II.

Figure 5 : Intracellular reactive oxygen species (ROS_{ic}) generation in cardiac fibroblasts treated with(out) angiotensin II (ANG II, 1 µmol/l) for 30 min after preincubation with(out) LY 294002 or wortmannin (1 µmol/l) for 1 h.

Intracellular ROS production was also reduced by the phosphoinositide -3-kinase (PI3K) inhibitors wortmannin and LY 294002 in control and ANG II-treated fibroblasts (Figure 5).

In order to elucidate if the ANG II-stimulated intracellular ROS production is affected by the ANG II-induced release of TGF-β_1 [37], cardiac fibroblasts were treated with a neutralizing antibody to TGF-β_1 (10 µg/ml) and ANG II for 24 h. The ANG II-stimulated intracellular ROS production was similar in cardiac fibroblasts treated with or without an anti-TGFβ_1 antibody (figure 6), suggesting that the ANG II-released TGF-β_1 did not contribute to the ANG II-enhanced intracellular ROS production.

Mitochondrial ROS production in cardiac fibroblasts

The ROS production in mitochondria incubated with glutamate plus malate and ATP increased 1.7-fold (p<0.001) from 187.7 ± 38.6 to 313.8 ± 30.6 a.u./ mg mitochondrial protein in ANG II-treated cardiac fibroblasts (figure 7). 5-Hydroxydecanoate and glibenclamide suppressed (p<0.05) the mitochondrial ROS production by 11.5 ± 1.1% and 15.3 ± 4.2%, respec-

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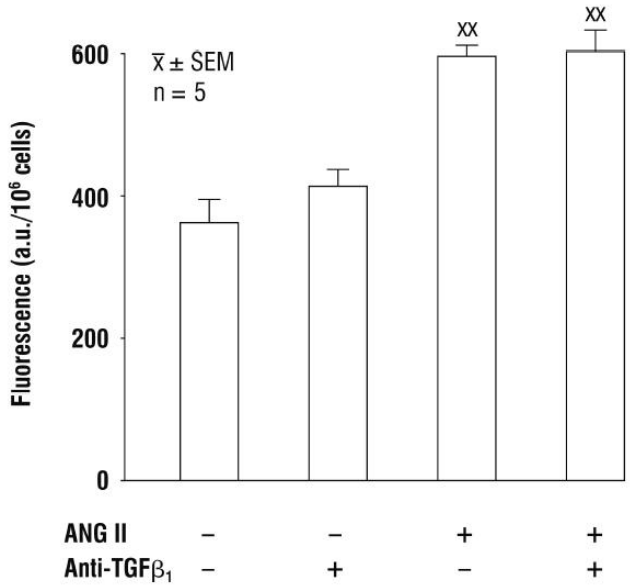
tively, in control fibroblasts and by 34.7 ± 4.8% and 44.6 ± 4.6%, respectively, in ANG II-treated fibroblasts.



xx p<0.01 compared to controls without ANG II and anti-TGFβ$_1$

Figure 6 : Intracellular reactive oxygen species (ROS) production in cardiac fibroblasts treated simultaneously with angiotensin II (ANG II, 1 µmol/l) and a neutralizing antibody to transforming growth factor-β$_1$ (anti-TGF-β$_1$, 10 µg/ml) for 24 h.

Rotenone and TTFA reduced (p<0.05) the mitochondrial ROS production in control fibroblasts by 39.2 ± 3.9% and 34.9± 5.1% and in ANG II-treated (n=6) fibroblasts by 27.5 ± 2.4% and 38.6 ± 3.7%, respectively (figure 7)

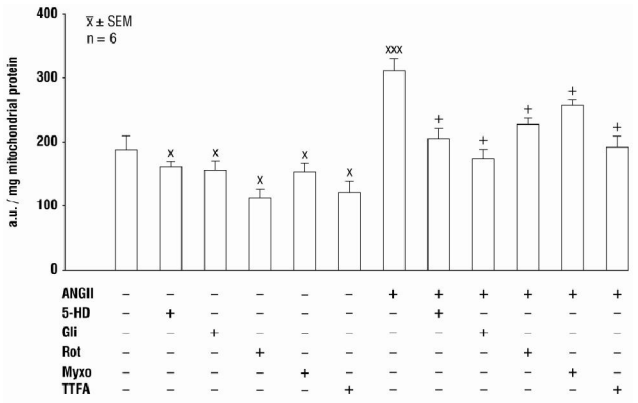
Myxothiazol which prevents semiquinone radical (UQ^-) formation at the Q_p site and ROS production at complex III[38], decreased the ROS production (p<0.05) in mitochondria from control (19.4 ±3.9%) and ANG II-treated (18.5 ± 3.0%) cardiac fibroblasts (figure 7).

Mitochondrial ROS production was also reduced (p<0.05) by LY 294002 and wortmannin in control (59.4 ± 1.8% and 52.4 ± 0.3%, respectively) and in ANG II-treated (n=6) cardiac fibroblasts (50.9 ± 13.3% and 32.2 ± 0.8%, respectively).

Effect of ANG II on FOXO3a binding to Prx-3 promoter

The FOXO3a binding activity to the Prx-3 DNA was lower in nuclear extracts of ANG II-treated fibroblasts as compared to control fibroblasts (Figure 8).

ANG II induced a time dependent reduction in FOXO3a binding activity to the Prx-3 promoter. The reduction was 21.8 ± 4.9 % at 30 min, with a further reduction of 83.1 ± 3.5% at 2 h after ANG II treatment.



xxx p<0.001, xx p<0.01, x p<0.05 compared to samples without ANG II.; ++ p<0.01, +p<0.05 compared to samples with ANG II and without 5-HD, Gli, Rot, Myxo and TTFA

Figure 7 : Mitochondrial reactive oxygen species (ROS) generation assessed in cardiac fibroblast treated with(out) ANG II (1 µmol/l) and 5-hydroxydecanoate (5-HD, 100 µmol/l), glibenclamide (Gli, 20 µmol/l), rotenone (Rot, 10 µmol/l), myxothiazol (Myxo, 1 µmol/l) or thenoyltrifluoroacetone (TTFA, 10 µmol/l) for 10 min.

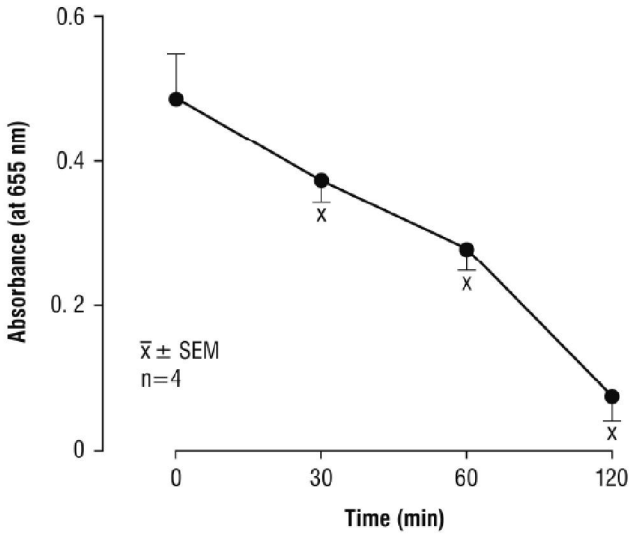


Figure 8 : Time dependency of the effect of angiotensin II on FOXO3a binding to peroxiredoxin-3 DNA in nuclear extracts of cardiac fibroblasts. x p< 0.05 compared vs. controls.

FOXO3a controls Prx-3 transcription

ANG II phosphorylated Akt on Ser^{475} and increased FOXO3a (pSer^{253}) levels in cardiac fibroblasts (figure 9 A, B).

In order to establish whether FOXO3a is causally involved in the transcriptional regulation of the Prx-3 gene, the siRNA technique was used. Cardiac fibro-

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blasts treated with FOXO3asiRNA demonstrated a ~69% reduction of FOXO3a mRNA level, a ~ 43 % reduction of nuclear FOXO3a protein and a decrease in Prx-3 mRNA and protein levels of, respectively, ~38 % and ~ 29%, but no change in Prx-1 mRNA and protein levels (figure 10).

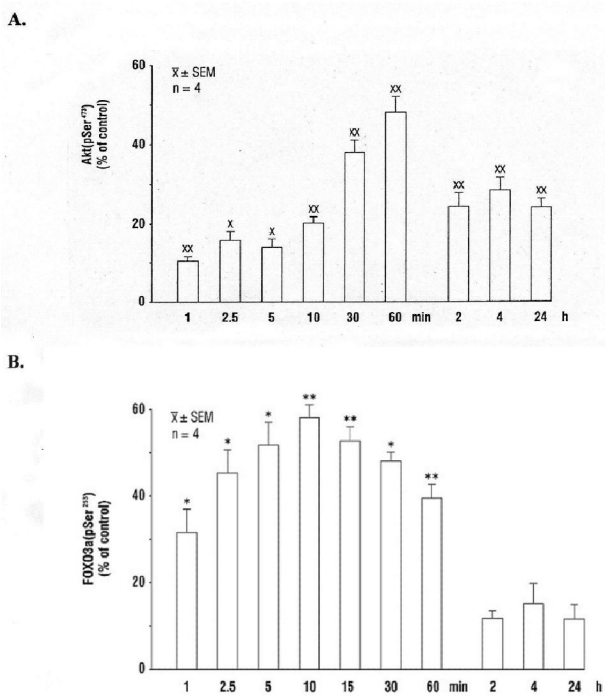
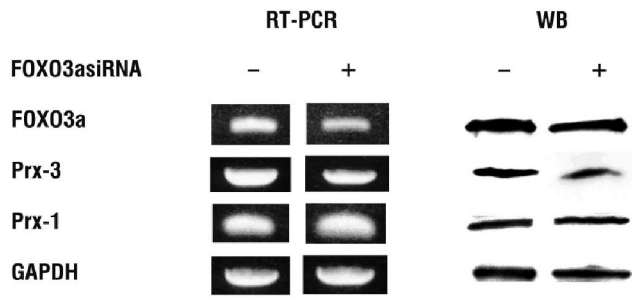


Figure 9 : Time course of Akt protein levels phosphorylated at residue Ser[473] (A) and of FOXO3a phosphorylated at residue Ser[253] (B) , by angiotensin II (1 µmol/l) in cardiac fibroblast lysates, as assessed by an enzyme-linked immunoabsorbent assay (A) and in intact cardiac fibroblasts by a fast activated cell-based enzyme-linked immunoabsorbent (B).

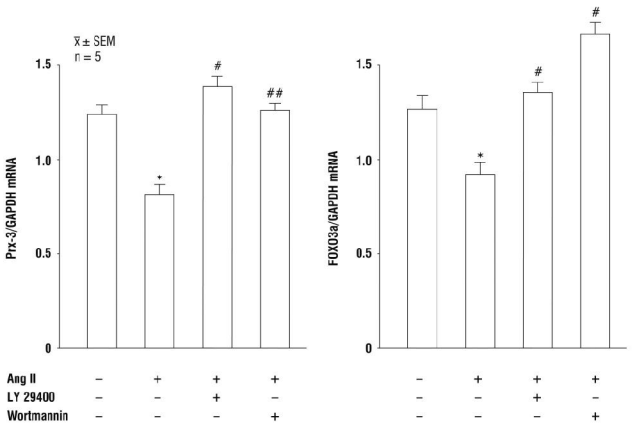


RT-PCR: reverse transcription-polymerase chain reaction.; WB: Western blots show FOXO3a in the nucleus and Prx-3, Prx-1 and GAPDH in the cytosol.

Figure 10 : Representative gels of mRNA and protein expression of FOXO3a, peroxiredoxin-3 (Prx-3), peroxiredoxin-1 (Prx-1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in cardiac fibroblasts transfected with(out) FOXO3asiRNA.

These data indicate that FOXO3a controls transcription and show consistent changes between Prx-3 mRNA and protein expression levels using the same approach.

In order to elucidate whether the ANG II-mediated regulation of Prx-3 mRNA expression is under control of PI3K-Akt signaling, ANG II-treated fibroblasts were preincubated with the PI3K-inhibitors wortmannin and LY 294002. The Prx-3 mRNA expression was increased (P<0.05) in ANG II-treated cells relative to the values before incubation with the inhibitors (figure 11).



x p<0.05 as compared to controls without ANG II , LY and wortmannin.; ## p<0.01, # p<0.05 as compared to samples with ANG II and without LY and wortmannin.

Figure 11 : mRNA expression of peroxiredoxin-3 (PRX-3) and FOXO3a in cardiac fibroblasts treated with(out) angiotensin II (ANG II, 1 µmol/l) for 24 h after preincubation with LY 294002 or wortmannin (1 µmol/l) for 1 h.

The ANG II- reduced levels of FOXO3a transcripts in cardiac fibroblasts was abolished in cells pretreated with wortmannin and LY (figure 11), indicating a role of PI3K in the control of FOXO3a at the transcriptional level.

PI3K is an upstream mediator of Akt activation in cardiac fibroblasts[21]. Western blot analyses were carried out in nuclear and cytosolic extracts of control fibroblasts to demonstrate differences in the localization of P-FOXO3a and P-Akt. Western blot analysis (figure 11) indicated that the phosphorylation of Akt at Ser [472] in the nucleus was increased 2.7 fold after exposure to ANG II for 30 min. The same blot, reprobed with an antibody to P-FOXO3a at Ser [253], showed minimal changes in P-FOXO3a in the nucleus, but a 4.3 fold rise in the cytosol, indicating that P-FOXO3a was transported to the cytosol after ANG II treatment (figure 12).

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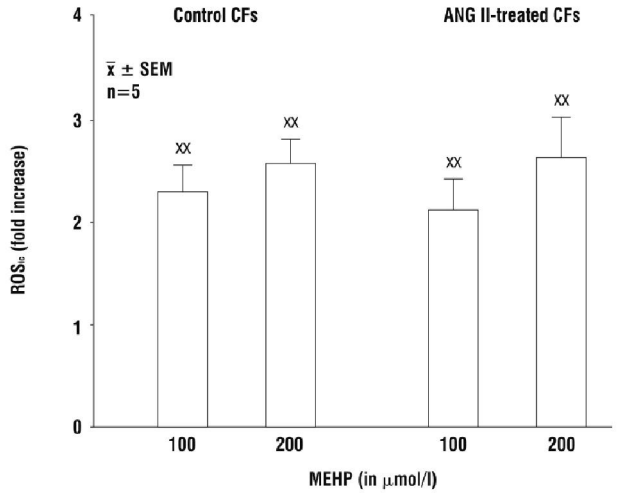
Figure 12 : Western blots of phosphorylated Akt at Ser 473 and FOXO 3a at Ser253 in nuclear and cytosolic extracts of cardiac fibroblasts treated with(out) ANG II (1 µmol/l) for 30 min.

Intracellular ROS production in Prx-3- and FOXO3a-depleted fibroblasts

Prx-3 depletion was obtained by Prx-3siRNa and administration of mono-(2-ethylhexyl)-phthalate (MEHP) since Prx-3 gene expression is reduced by phtalates[27]. MEHP (at 100 and 200 µmol/l) increased the generation of intracellular ROS in control and ANG II-treated cardiac fibroblasts more than 2-fold (figure 13).

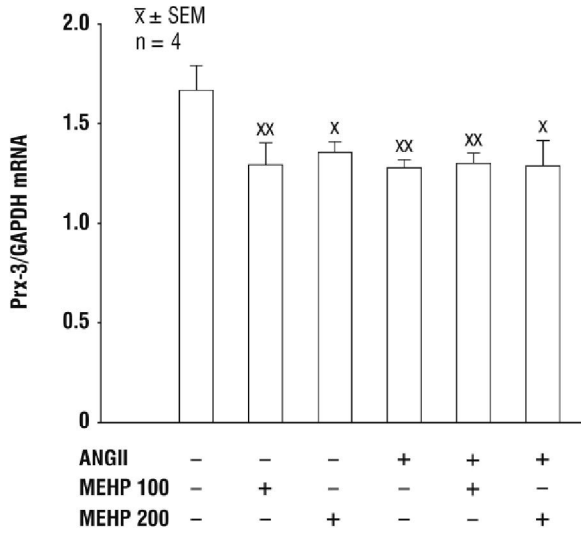


CFs: cardiac fibroblasts

Figure 13 : Intracellular reactive oxygen (ROS) production in cardiac fibroblasts treated with(out) angiotensin II (ANG II, 1 µmol/l) for 30 min after preincubation with(out) mono-(2-ethylhexyl)phthalate (MEHP, 100 and 200 µmol/l) for 1 h.

MEHP reduced the mRNA expression of Prx-3 (figure 14). The ANG II-induced decrease in Prx-3 mRNA was however not further affected by MEHP.

In order to test whether the upregulation of Prx-3 by FOXO3a is required for protection against cellular ROS protection, we depleted fibroblasts from Prx-3 or FOXO3a by performing transfections with siRNA against Prx-3 or FOXO3a and measured ROS production. Depletion of FOXO3a increased ($p<0.01$) the ROS production in cardiac fibroblasts by 70 ± 8% (n=6). Depletion of FOXO3a had a more potent effect on ROS production than Prx-3 depletion (17.4 ± 1.1%), indicating that the FOXO3a-Prx-3 axis is required to protect against oxidative stress induced by Ang II.



x p<0.05, xx p<0.01 compared to control

Figure 14 : Peroxiredoxin-3 (Prx-3)/ glyceraldehyde-3-phosphate dehydrogenase (GADPH) mRNA ratio in cardiac fibroblasts treated with(out) angiotensin II (1 µmol/l) and mono-(2-ehylhexyl)phthalate (MEHP, 100 and 200 µmol/l) for 24 h.

Effect of ANG II on mitochondrial complex I, II, III and IV activity

As shown in TABLE 1, ANG II did not affect the mitochondrial complex II and IV activity in cardiac fibroblasts and reduced ($p<0.05$) the mitochondrial complex I and III activity by 66.2 ± 13.1% and 48.8 ± 14.1 % (n=4), respectively.

Complex I, II and III activity are expressed as nmol /min/mg mitochondrial protein and complex IV activity as mUnits/ml.

TABLE 1 : Mitochondrial complex I, II, III and IV activity in control and ANG II-treated cardiac fibroblasts (CFs) .

	Control CFs	ANG II-treated CFs
Complex I	103.0 ± 20.9	34.8 ± 13.5^{x}
Complex II	43.1 ± 11.1	40.8 ± 3.1
Complex III	72.7 ± 15.1	38.6 ± 14.9^{x}
Complex IV	9.05 ± 1.57	9.23 ± 1.85

Mean ± SEM is given; n=4; x p<0.05 compared to control CFs

Effect of ANG II on nuclear factor-κB (NF-κB) expression

ANG II increased the mRNA expression of the p50

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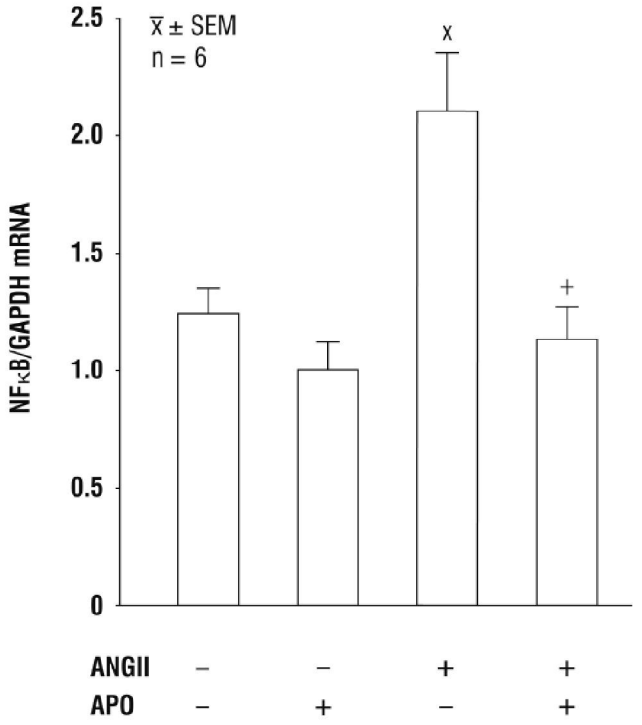
subunit of NF-κB in cardiac fibroblasts (figure 15). Apocynin, a NADPH oxidase inhibitor, abolished the ANG II-stimulated gene expression of NF-κB p50.



x p<0.05 compared to samples without ANG II; + p<0.05 compared to samples with ANG II and without apocynin

Figure 15 : Nuclear factor-κB (NF-κB) / glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA ratio in cardiac fibroblasts treated with(out) angiotensin II (ANG II, 1 µmol/l) for 24 h after preincubation with apocynin (APO, 100 µmol/l) for 1 h

The ANG II-induced ROS production, through the ROS-sensitive transcription factor NF-κB, increases the expression of collagen in cardiac fibroblasts and reduces the ANG II-stimulated ROS production in cardiac fibroblasts[39].

DISCUSSION

The present in-vitro data have shown, in cultured adult rat cardiac fibroblasts, that ANG II decreased the mRNA and protein expression of the mitochondrial specific antioxidant Prx-3, while the cytosolic Prx-1 fraction was unaffected (figures 1 and 2). This downregulation of Prx-3 is linked to a downregulation of its mRNA levels, indicating that the alterations in Prx-3 expression result from an altered gene expression rather than from alterations in post-transcriptional regulation. This study also shows that losartan inhibits the ANG II-induced downregulation of Prx-3 in cardiac fibroblasts (figure 3). Previously we[21] have shown that the protein expression of α-smooth muscle actin, a marker of the differentiation of fibroblasts into myofibroblasts, was not significantly increased by ANG II, indicating that the effects of ANG II on Prx-3 expression do not result from changes in the phenotype of the cultured fibroblasts. Myofibroblasts are not part of normal cardiac tissue and appear only after cardiac injury[40]. These myofibroblasts differ from normal resident cardiac fibroblasts by de novo expression of α-smooth muscle actin containing stress fibers[41,42].

A downregulation of Prx-3 has been described in various experimental models that are characterized by an increased cellular oxidative stress[43-45]. In human heart failure Brixius et al[46] also reported a selective downregulation of the mitochondrial Prx-3, while the cytosolic Prx-1 and Prx-2 isoforms were unaffected by the enhanced ROS production. Thus reducing Prx-3 sensitizes cells to oxidative stress[27].

It is also known that ANG II decreased the protein expression of cardiac thioredoxin in female mice[47]. Cardiac inhibition of thioredoxin increased oxidative stress and cardic hypertrophy[47-49]. Increased oxidative stress in ANG II-induced hypertension is also associated with the downregulation of the antioxidant thioredoxin system[47]. An impaired induction of thioredoxin expression in spontaneously hypertensive rats (SHR) and stroke-prone SHR tissues, despite the increased oxidative stress, is reported by Tanito et al.[50]. Expression of the mitochondrial antioxidant enzymes Prx-3, MnSOD and Trx2 was greatly reduced in familial hypercholesterolemic pigs, while mitochondrial oxidative stress was increased[51]. In obese mice and human subjects adipose Prx-3 levels were also significantly decreased, indicating its association with obesity[52].

ANG II also induces oxidative stress by increasing intracellular and mitochondrial ROS production in cardiac fibroblasts (figures 4 and 7). In quiescent cells most of ROS are produced through an univalent reduction of molecular oxygen to $\cdot O_2^-$ by electrons that leak from complex I and III of the mitochondrial electron transport chain[14,53-55]. $\cdot O_2^-$ does not readily cross membranes due to its charged nature. It inhibits mitochondrial function by inactivating the Fe-S centers in complex I and III and the tricarboxylic acid cycle (aconitase)[56]. The

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capacity of mitochondria to produce ROS is significantly increased in aged rats[15]. The mitochondrial content of cardiolipin, a phospholipid required for optimal activity of complex I, significantly decreased as a function of aging[15].

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 readly converted to the more powerful oxidant ·OH.

Indeed, recent findings have demonstrated that ANG II stimulation induces the opening of mitochondrial K_{ATP} channels and further amplifies ROS formation from mitochondria[57]. ROS produced initially in the mitochondria have been shown to provoke a positive feedback with mitochondria responding to elevated levels of ROS by increasing their own ROS production in a process known as ROS-induced ROS release[58]. Recently, local dynamic mitochondrial ROS production in the form of superoxide flashes, described in cardiomyocytes and fibroblasts, distinguishes itself from the well-established constitutive basal mitochondrial ROS production[59].

Blockade of the mitochondrial K_{ATP} channels by glibenclamide or 5-hydroxydecanoate suppresses the intracellular ROS production in control and ANG II-treated cardiac fibroblasts (figure 4). On the contrary inhibitors of complex I and III, rotenone and antimycin A, stimulated intracellular ROS production at these sites, while the complex II inhibitor TTFA had no effect on intracellular ROS production in control or ANG II-treated cardiac fibroblasts (figure 4). Rotenone also induced $\cdot O_2^-$ release in both primary microglia and peritoneal macrophages from wild-type mice, but failed to do so in cells from $gp91^{phlox}$–deficient mice[60].

Antimycin A blocks the electron transfer from the Q_i to Q_o sites of complex III and increases ROS production in the innermembrane space of mitochondria[61,62]. Rotenone blocks complex I near the binding site for ubiquinol, the electron acceptor of complex I[62]. Blockade of complex I at this distal site in the complex increases the reduction of the NADH dehydrogenase of complex I, increasing the electron leak to ROS. Thus rotenone blockade enhances oxyradical production by complex I. Indeed, antimycin A and rotenone increased the intracellular ROS production also in various cell types, such as rat and dog heart mitochondria[62-64], bovine aortic endothelial cells[65], rat aortic vascular smooth muscle cells[66], bovine coronary arterial smooth muscle cells[67,68], cremaster muscle arterioles[69], Hek-293 and U87-cells[70] and AS4.1 cells[71].

The ROS production in mitochondria incubated with glutamate plus malate and ATP[34] is also 1.7-fold increased by ANG II in cardiac fibroblasts and inhibited by the K_{ATP} channel inhibitors 5-hydroxydecanoate and glibenclamide (figure 7). ANG II stimulates thus both cytosolic and mitochondrial ROS generation in cardiac fibroblasts.

Two segments of the respiratory chain are primarly responsible for ROS generation in the mitochondria: the NADH-ubiquinone reductase in complex I and the ubiquinol-cytochrome c reductase in complex III[64]. In the present study a decreased mitochondrial ROS production was observed with rotenone and myxothiazol in control and ANG II-treated fibroblasts, while the intracellular ROS generation was enhanced by rotenone and antimycin A (figures 4 and 7). It should be taken into consideration that myxothiazol prevents ROS generation at complex III, although it may increase their release at complex I[38]. The divergent effects of complex I, II and III inhibitors on mitochondrial and intracellaular ROS production has to be further elucidated and is partially related to the content of the used substrates in the media[62] and to the various sources of the mitochondria[72]. In the present study the ROS production was assayed in mitochondria, isolated from rat adult cardiac fibroblasts and incubated with the complex I substrates glutamate/malate and ATP[33]. In rabbit heart mitochondria the H_2O_2 production was reduced by rotenone when incubated with succinate, but increased when incubated with glutamate/malate as substrate[72]. However, in rat liver mitochondria rotenone did not affect the H_2O_2 production when incubated with glutamate/malate, but decreased it when incubated with succinate[72].

A direct interaction between ANG II and mitochondrial components can be suggested by the presence of ANG II in mitochondria of brain, heart and smooth muscle cells in rodents; moreover, renin, angiotensinogen and angiotensin converting enzyme were also described within intramitochondrial dense bodies[2,73,74]. A functional mitochondrial ANG system has recently be described by Abadir et al[75].

In the present study, ANG II did not affect the mi-

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tochondrial cmplex II and IV activity in cardiac fibrolasts; the mitochondrial complex I and III were however reduced by ANG II. Discordant findings are reported on the effect of ANG II on mitochondrial complex I, II, III and IV activity in various tissues and cell types.

In the vascular wall Dai et al[76] showed that ANG II induces mitochondrial ROS formation indirectly through activation of NOX2 located in the plasma membrane and directly through activation of the mitochondrial NOX4. ANG II administration for 4 weeks also increased mitochondrial DNA deletion frequency as well as protein carbonyls, both of which are related to oxidative damage to mitochondria[76]. ANG II thus negatively influences mitochondrial number and function by promoting oxidative stress.

In cultured 2C12 myotubular cells from mice, ANG II reduced the mitochondrial content in association with increased mitochondrial ROS production; ANG II further reduced mitochondrial mass and membrane potential, whereas cellular ATP content was not significantly changed[77]. In skeletal muscle of ANG II-infused mice, the copy number of mitochondrial DNA was also reduced; furthermore the cytochrome c oxidase (complex IV) activity and mitochondrial protein level in the muscle was decreased in the ANG II-group[77]. However exposure of neonatal rat cardiac myocytes to ANG II did not induce any decline in mitochondrial DNA copy number despite increased ROS generation[78]. In adult rat ventricular myocytes the complex IV activity of the respiratory chain tended to be lower with ANG II[79]. In neonatal rat cardiomyocytes ANG II downregulated the level of NADH dehydrogenase subunit 5, a component of complex I of the electron transport chain[80]. In transgenic Ren2 rats with elevated endogenous ANG II levels the hepatic expression of the mitochondrial cytochrome c oxidase subunit 1 was markedly reduced[81]. The deleterious effects of ANG II on hepatic mitochondria are mediated by increased ROS generation[81]. In skeletal muscle from ANG II-infused mice the mitochondrial complex I and III, but not II and IV, activities were decreased compared with vehicle, while the superoxide anion production and NAD (P)H oxidase activity were increased[82]. After intracerebroventricular infusion of ANG II, the enzyme activity of complex I, II and III, but not of IV, in the ventrolateral medulla of rats is reduced[83]. ANG II impaired mitochondrial biogenesis and mitochondrial respiratory chain activity and deletion of the ANG II receptor type 1 in mice was associated with increased number of mitochondria, decreased ROS-induced oxidative damage and improved cardiac function[84,85]. In human vascular smooth muscle cells the angiotensin II subtype 1 receptor blocker telmisartan increased ATP levels and activation of mitochondrial complex II[86]. The absence of the angiotensin II type 1 receptor strongly attenuated the functional and structural changes that occur in kidney mitochondria following oxidative stress.

Exposure of cardiac fibroblasts to ANG II can thus lead to increased oxidative stress because of downregulation of the mitochondrial antioxidant Prx-3.

Indeed, Prx-3 knockdown by siRNA increased mitochondrial ROS[55] and Prx-3 knock-out mice were more susceptible to lipopolysaccharide-induced oxidative stress than their wild-type and progression of litternates[87]. Higher levels of ROS were also detectable in macrophages derived from these mice and they released increased amounts of TNFα[88]. In Prx-3 depleted cells, obtained by silencing RNA transfection against Prx-3 or by MEHP treatment, we also observed an increased intracellular ROS production in cardiac fibroblasts (figure 13).

Overexpression of Prx-3 protected the heart against post-MI remodeling and failure in mice[89]. It reduced LV cavity dilatation and dysfunction as well as myocyte hypertrophy, interstitial fibrosis and apoptosis of the non-infarcted myocardium. These beneficial effects of Prx-3 gene overexpression were associated with the attenuation in oxidative stress, mitochondrial DNA decline and dysfunction[89]. Prx-3 overexpression has also been shown to improve glucose homeostasis, with transgenic mice displaying resistance to diet-induced elevations in blood glucose and increased glucose clearance[90].

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 to be paralleled by a downregulation of mitochondrial Prx-3. The local antioxidant activity of Prx-3 may have a role in maintaining cardiac function[91]. ANG II-induced cardiac damage is thus associated with oxidative stress-dependent mitochondrial dysfunction[92].

Chronic increases in ROS production are thus associated with mitochondrial damage and dysfunction which lead to a catastrophic cycle of mitochondrial func-

tional decline, additional ROS generation and cellular injury. Therefore, these cellular events might be involved in myocardial remodeling and failure[78,93] Oxidative stress is indeed increased in heart failure, hypertension, cardiac fibrosis and hypertrophy[94-97]. Increased production of ROS in the failing heart is indeed associated with mitochondrial damage and dysfunction, characterized by an increased lipid peroxidation in the mitochondria, a decreased mtDNA copy number, a decrease in the number of mRNA transcripts and a reduced oxidative capacity due to low complex enzyme activities[93]. In vitro exposure of cardiac fibroblasts to superoxide anion stimulates their proliferation by increasing the production of a potent fibrogenic cytokine transforming growth factor-β_1 [3].

Ventricular fibroblasts activation and cardiac fibrosis are primary events in ventricular remodeling rather than secondary to cardiomyocyte injury[99]. In normal cardiac tissue collagen synthesis and deposition are exclusely carried out by cardiac fi!broblasts with relatively low turnover of extracellular matrix proteins[100] Contractile myofibroblasts are the relevant phenotypic variants in wound healing or in hypertrophied and failing hearts[99-101].

Given that mitochondria contain Prx-3 30 times more abundant than glutathione peroxidase[55], 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 $\cdot O_2^-$.

Approx. 90 % of mitochondrial hydrogen peroxide reacts with Prx-3. Although glutathione peroxidase has a higher rate constant than Prx-3, its lower abundance limits its ability to compete directly with Prx-3[102].

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 oxidative stress-mediated myocardial injury[95]. Antioxidant strategies specifically targeting Prx-3 or Mn-SOD could thus have therapeutic benefit in preventing a wide spectrum of adverse cardiovascular outcomes[103]. Prx-3 is indeed an important candidate for therapy against LV failure after MI, in which ROS production has been found to be increased within the mitochondria[92]. Higher levels of Prx-3 detected in the post-myocardial infarction left ventricle of matrix metalloproteinase -7 null mice may also be associated with attenuated mitochondrial oxidative stress[104].

Accumulating evidence suggest thus that mitochondrial ROS contribute to the deleterious effects of ANG II mediated by activation of angiotensin type 1 receptors or by direct interaction of ANG II with mitochondrial or nuclear components.

FOXO (Forkhead box class o) transcription factors may be important in the regulation of the antioxidant defense in many species[20].

In human cardiac fibroblasts FOXO3a mediated the expression of peroxiredoxin-3, which functions to protect mitochondria against oxidative stress by scavenging H_2O_2 [30]. In rat cardiac fibroblasts ANG II was shown to reduce the binding of FOXO3a to the Prx-3 promoter (figure 8). Inhibition of FOXO3a transcription with siRNA led to a reduction in FOXO3a binding to the Prx-3 promoter, and a concomitant reduction in Prx-3 gene expression in control cardiac fibroblasts (figure 10), thereby suggesting that FOXO3a does upregulate Prx-3.

In FOXO3a-depleted fibroblasts the reduced Prx-3 expression was also associated with an increase in ROS production, which was more pronounced than in Prx-3-depleted cells. In human cardiac fibroblasts as well as in HEK293 cells[29] deletion of FOXO3a increased total cellular level of H_2O_2 more pronounced (~45%) than Prx-3 depletion (~15%). Our data suggest, therefore, that FOXO3a may be the transcription factor responsible for the ANG II-induced downregulation of Prx-3 in cardiac fibroblasts[105].

ANG II –stimulated Akt activity may be responsible for the phosphorylation and inactivation of FOXO3a (figure 9A, B)) which, in turn, downregulates Prx-3 transcription in rat cardiac fibroblasts. When Akt is phosphorylated 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 (figure 12), thereby resulting in a decrease in its transcriptional activity and consequently in Prx-3 expression.

Inhibition of PI3K with wortmannin and LY 294002 led to a decrease in P-Akt[21], an increase in Prx-3 mRNA expression (figure 11) and a reduction in ROS production (figure 5). These data indicate that this translocation-relocalisation mechanism contributed to the downregulation of Prx-3 gene expression.

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In conclusion, our data show that ANG II inactivated FOXO3a by activating Akt, leading to a reduction in the expression of the antioxidant Prx-3 and thereby contributing to ROS production. ANG II may cause, through this mechanism, an increase in oxidative stress by inhibiting the expression of the mitochondrial enzyme Prx-3 that is involved in ROS breakdown.

CLINICAL PERSPECTIVES

A growing body of evidence suggests that oxidative stress, a chronic increase in ROS in the myocardium can contribute to myocardial remodeling and failure in hypertensive heart diseases[106]. Further, antioxidants have been shown to exert protective and beneficial effects to counter this process. However, downregulation of ROS below certain levels disrupts normal cellular functions, causing adverse effects[105]. For example, the salutary effect of ischemic preconditioning against ischemia/reperfusion injury is blocked by treatment with antioxidants[107-109].

In hypertension, there is a mutual reinforcement between ROS and ANG II [110]. The effects of ANG II are related to oxidative stress. Indeed, oxidative stress was found to increase in mice after infusion of ANG II[111,112]. In vitro, ANG II also induces oxidative stress in various types of cultured cells[113-117]. Experimental hypertension is also ameliorated by SOD mimetics[118,119] and mice lacking the NADPH oxidase subunit $p47^{phlox}$ have reduced hypertensive responses to ANG II[120,121]. Mice lacking the extracellular SOD are also predisposed to excessive hypertension in response to ANG II[122,123]. An increase in vascular $\cdot O_2^-$ might promote hypertension by several mechanisms. Superoxide anion inactivates endothelium-derived NO and therefore, could indirectly enhance vasoconstriction[124]. In addition, $\cdot O_2^-$ has been implicated as an endothelium-derived vasoconstrictor agent and could enhance formation of vasoconstrictor isoprostanes[122]. Superoxide can also alter vascular smooth muscle calcium handling, predisposing to vasoconstriction[125].

Both ANG II and oxidative stress also induce collagen production, in vivo as well as in vitro (TABLE 2)[18,19,21,39,126,127]. ANG II is involved in extracellular matrix accumulation contributing to heart failure. Oxidative stress, in turn, induces remodeling of the myocardium and regulates collagen metabolism in various cell types. In addition, infusion of ANG II induces severe cardiac fibrosis. ANG II infusion in mice enhances cardiac mRNA levels of collagen I, collagen III, fibronectin, prolyl 4-hydroxylase component P4ha3, matrix metaaloproteinase-12 and the tissue inhibitor of matrix metalloproteinase TIMP-1[128]. Excess collagen deposition with a loss of collagen degradation by matrix metalloproteinases leads to fibrosis, which has a long-term impact on the function of the heart[40]. Chronic administration of ANG II via subcutaneous osmotic pump in mice results in left ventricular hypertrophy, diastolic dysfunction and cardiac fibrosis. The transcription factor NF-κB is also implicated in the ANG II-mediated collagen synthesis in cardiac fibroblasts[129-131].

TABLE 2 : Manganese superoxide dismutase (Mn-SOD), collagen type I and III level, mRNA and protein expression in control and angiotensin II (ANG II) –treated cardiac fibroblasts (CFs).

	Control CFs	ANG II-treated CFs
Mn-SOD activity (U/mg protein)	2.18 ± 0.19	1.23 ± 0.20^{xx}
Mn-SOD/GAPDH mRNA	1.37 ± 0.14	0.56 ± 0.10^{xxx}
Mn-SOD/GAPDH protein	1.04 ± 0.08	0.68 ± 0.06^{xx}
Collagen type I (μg/ml)	1.4 ± 0.1	3.4 ± 0.5^{x}
Collagen type III (μg/ml)	0.75 ± 0.18	1.52 ± 0.16^{x}
Type I collagen/GAPDH mRNA	1.03 ± 0.06	1.62 ± 0.18^{x}
Type III collagen/GAPDH mRNA	0.97 ± 0.2	1.71 ± 0.20^{x}
Collagen production ($\mu g/10^6$ cells)	20.8 ± 2.0	49.3 ± 3.3^{xxx}

Mean ± SEM; n=10; GAPDH: glyceraldehyde -3-phosphate dehydrogenase; CFs: cardiac fibroblasts; xxx p<0.001; xx p<0.01; x p<0.05 compared to control

In the present study, cardiac fibroblasts were chosen because these are the cells that are primarily responsible for the deposition of extracellular matrix proteins.

Mitochondria are the predominant source of ROS, and miotochondrial antioxidants are expected to be the first line of defense against mitochondrial oxidative stress–mediated myocardial injury. This study demonstrated that ANG II increased mitochondrial ROS production in cardiac fibroblasts and decreased the protein and mRNA expression of the mitochondrial antioxidant Prx-3 by inducing the phosphorylation of Akt and FOXO3a and repressing the binding of FOXO3a to the Prx-3 promoter gene. Exposure to ANG II is therefore likely to lead to increased oxidative stress because of downregulation of antioxidant enzymes such as Prx-3 (Figures 1-3) and Mn-SOD (TABLE 2)

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This antioxidant is specifically localized in the mitochondria.

It could therefore potentially provide a primary line of defense against ANG II–induced oxidative stress and myocardial injury. Therapies designed to interfere with mitochondrial oxidative stress by using antioxidants might be beneficial in hypertensive heart diseases and in preventing clinical heart failure. It may represent a potential target for cardiac protection from oxidative stress-induced injury. Alterations in the expression levels of thioredoxin family members also constitute effective biomarkers in various diseases, including cardiovascular complications that involve oxidative stress[132]. Recently it has been demonstrated that Mn-SOD is a negative modulator of vascular lesion formation after injury[130]. Therefore, Mn-SOD augmentation may also be a promising therapeutic strategy for the prevention of lesion formation in proliferative vascular diseases such as restenosis[133].

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DISCLOSURE

The authors declared no conflict of interest.

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