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The oxygen free radicals control MMP-9 and transcription factors expression in the spontaneously hypertensive rat

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Abstract

Oxygen free radical and matrix metalloproteinases-9 (MMP-9) play an important pathophysiological role in the development of chronic hypertension. MMP-9 activities are regulated at different levels. We hypothesize that as mediators of the expression of MMP-9 the transcription factors like nuclear factor kappa B (NF-κB), c-fos and retinoic acid receptors-α (RAR-α) with binding sites to the MMP-9 promoter are overexpressed in the spontaneously hypertensive rat (SHR) in a process that is regulated by oxygen free radicals. Transcription factor NF-κB, c-fos and RAR-α expression levels were determined by immunohistochemistry in renal, cardiac and mesentery micro-circulation of the SHR and its normotensive control, the Wistar Kyoto (WKY) rat. The animals were treated with a superoxide scavenger (Tempol) for eight weeks. The elevated plasma levels of thiobarbituric acid reactive substances and MMP-9 levels in the SHR were significantly decreased by Tempol treatment ($P < 0.05$). The NF-κB, c-fos and RAR-α expression levels in renal glomerular, heart and mesentery microvessels were enhanced in the SHR and could also be reduced by Tempol compared to untreated animals ($P < 0.05$). The enhanced MMP-9 levels in SHR microvessels co-express with transcription factors. These results suggest that elevated NF-κB, c-fos and RAR-α expressions and MMP-9 activity in the SHR are superoxide-dependent.

Introduction

Hypertension is a leading risk factor for morbidity and mortality (Kakar and Lip, 2006). The blood pressure elevation is accompanied by enhanced production of vascular reactive oxygen species (ROS), decreased nitric oxide bioavailability and decreased antioxidant capacity (Sedeek et al., 2009). This evidence has been regarded as a possible contribution to elevation of arterial blood pressure as well as end-organ damage in hypertension. In addition to ROS, matrix metalloproteinases (MMP) (Nagase and Woessner, 1999) may be involved in end organ damage. MMPs degrade components of the extracellular matrix (ECM) in conjunction with serine proteinases and the metalloproteinase disintegrins (Lenz et al., 2000; Mahimkar et al., 2000; Woessner and Nagase, 2000) and are involved in wall hypertrophy

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(Evan et al., in press). Our recent evidence indicates that MMPs in the SHR cleave several membrane receptors. For example cleavage of the ectodomain of the beta-2 adrenergic receptor causes arteriolar constriction and elevation of central blood pressure (Rodrigues et al., 2010). MMP inhibition attenuates the receptor cleavage, the arteriolar constriction and blood pressure elevation in the SHR. In addition, MMP inhibition attenuates other co-morbidities, e.g. insulin resistance or capillary rarefaction by attenuating cleavage of the insulin receptor and the vascular endothelial growth factor receptor 2 (VEGFR2), respectively (DeLano and Schmid-Schönbein, 2008; Tran et al., 2010).

Among the MMPs, MMP-9/gelatinase B has been shown to play an important role in wound healing, angiogenesis, inflammation, tumor invasion and metastasis (Ruhul Amin et al., 2003). But in addition it plays a key role in hypertension and diabetes. In the spontaneously hypertensive rat (SHR), elevated levels of MMPs cause direct damage to cells by cleavage of the extracellular domain of several key receptors, which results in the diverse cell dysfunctions characteristic for the SHR (DeLano et al., 1997). The question arises then by what mechanisms the MMPs may be overexpressed in the SHR. MMP-9 can be induced by cytokines, such as interleukin-1 (IL-1), growth factors (Fabunmi et al., 1996; Kusano et al., 1998; Okada et al., 2001) and by transcription factors, like activating protein-1 (AP-1) and nuclear factor kappa B (NF-κB), that bind to specific elements on MMP gene promoters (Clark et al., 2008). Inhibition of NF-κB serves to reduce MMP expression in the SHR (Wu and Schmid-Schönbein, 2011). The SHR has also elevated free radical production (DeLano et al., 2005) induced by excess nitric oxide production and oxidative stress (Kobayashi et al., 2005; Rodríguez-Iturbe et al., 2005). The free radical production has been proposed to induce NF-κB expression (Morgan and Liu, 2011; Pantano et al., 2006). Thus, the present study was designed to investigate to what degree antioxidant treatment by Tempol affects the MMP-9 expression and selected transcription factors capable to bind to its promoter (NF-κB, c-fos, RAR-α) (Clark et al., 2008) in the SHR, as compared to its normotensive Wistar Kyoto (WKY) control. Tempol (4-hydroxy-2,2,6,6-tetramethylpiperdine-N-oxyl; TEMPO) is a stable antioxidant of low molecular weight that permeates biological membranes, mimics superoxide dismutase (SOD) activity, and scavenges O_2^- anions in-vitro and in-vivo (Wilcox and Pearlman, 2008). It prevents O_2^--induced damage in different conditions, such as inflammation (Karmeli et al., 1995), radiation (Mitchell et al., 1991), and cardiac ischemia/reperfusion injury (Gelvan et al., 1991). We determine with and without Tempol treatment the expression levels of MMP-9 and transcription factors NF-κB, c-fos and RAR-α levels in selected organs of the SHR, including kidney, heart and mesentery microvessels. We make use of the whole mount mesentery preparation since it allows display of the heterogeneous MMP and transcription factors expression and co-localization within a microcirculation with complete network of arterioles, capillaries and venules.

Materials and methods

All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, San Diego. Eight-week-old male spontaneously hypertensive rats (SHR, Harlan Laboratories, Inc., Indianapolis, IN) and their normotensive controls, the WKY rats, were used (n = 6 rats per group). Both SHR and WKY rat groups were subjected to antioxidant treatment for 8 weeks with cell permeable superoxide

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scavengers, Tempol (4-hydroxy-2,2,6,6-3,5-disulphonic acid disodium salt; TEMO; Sigma-Aldrich, St. Louis, MO), dissolved in drinking water (1 mmol/L). Age-matched controls were maintained on regular water. The systolic blood pressure of animals in each group was measured by the tail-cuff method (Fritz and Rinaldi, 2008).

Immunohistochemistry

At the end of the treatment period the rats were given general anesthesia (ketamine, 75 mg/kg, with xylazine, 4 mg/kg). Heart and kidney were immediately harvested and frozen sections prepared (5 μm thickness, −80 °C storage). To facilitate quantitative comparisons between groups, the sections were immunolabeled in a standardized procedure using anti-NF-κB p65 rabbit polyclonal antibody (sc-109, Santa Cruz Biotechnology, Santa Cruz, CA), anti-c-fos rabbit polyclonal antibody (sc-52, Santa Cruz Biotechnology, Santa Cruz, CA), and anti-RAR-α rabbit polyclonal antibody (sc-551, Santa Cruz Biotechnology, Santa Cruz, CA). In addition, the matrix matalloproteinase-9 (MMP-9) protein density was determined by immunolabeling with anti-MMP-9 goat polyclonal antibody (sc-6840, Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C overnight. The primary antibodies were visualized by binding of secondary antibody conjugated to peroxidase activity with diaminobenzidine (DAB) substrate (VECTOR *NovaRED*). Buffer alone or nonspecific purified rabbit immunoglobulin G (IgG) served as controls.

Whole mount mesentery

A sector of the mesentery was carefully exteriorized to expose a microvascular network with arterioles, capillaries, and venules. Mesentery sectors were selected in which the microvascular networks were located in connective tissue regions devoid of adipose tissue. To achieve full penetration of the antibody into the entire mesentery, the tissue was pretreated with a permeabilizing agent as follows. Following fixation in formalin (10%) and two rinses of the fixed tissue in tap water (10 min each), the tissue was placed into a digest-all 4 proteinase K solution (Invitrogen, Gaithersburg, MD) for 20 min. After a rinse in tap water (10 min, in hydrogen peroxide (0.5%, 30 min), and in phosphate-buffered saline (PBS) buffer (pH 7.4, 10 min), the tissue was incubated in normal serum (20 min) and rinsed again in buffer (10 min). To achieve optimal primary antibody infiltration, the mesentery sectors were incubated (1 h at room temperature) with diluted primary antibody (1:300), or in control specimen with nonbinding IgG antibody (Vector Laboratories, 0.5–5 mg/mL). Specimen without use of primary antibody served as controls.

After a buffer rinse (10 min), the biotinylated secondary antibody was added (for 45 min), the specimens were rinsed in buffer (10 min), and labeling of the secondary antibody was enhanced using an avidin/biotinylated enzyme complex (Vectastain *Elite* ABC Kit, rabbit IgG, Vector) for 30 min. The tissue was rinsed in buffer (10 min), incubated in VECTOR *NovaRED* enzyme substrate (no. SK-4800; Vector Laboratories; 2 min), washed in tap water (5 min), dehydrated (in 90%, 100% ethyl alcohol, followed by 100% xylene for 1 min each), and mounted (Vecta Mount, Vector Laboratories) on a glass slide. The specimens were viewed under high-resolution, bright-field microscopy (Leitz Wetzlar SM-LUX) using standardized light intensities to facilitate measurements for comparison of groups. The selected microvessels were limited to arterioles of 20 to 35 μm, venules of 25 to 40 μm and

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capillary of 5 to 10 μm and approximately 100 μm in length. Arterioles and venules were identified based on their network position within the microcirculation of the mesentery.

Plasma thiobarbituric acid species (TBARS)

The plasma samples were stored at −80 °C and thawed at room temperature before use. All plasma samples were measured using a TBARS commercial kit (ZeptoMetrix Corporation, Buffalo, NY) following the manufacturer's instructions. TBARS measurements are expressed as nmol/L of malondialdehyde (MDA).

Data analysis

Images of the immunolabel intensities were digitally recorded (with 10× and 40× objectives). At least 10 randomly selected windows (2 × 5 μm) per section along endothelial cells in the case of microvessels, 10 glomeruli and 10 selected windows per section of myocardium were used to determine immunolabel density by light intensity measurements. All measurements were carried out under standardized light microscopy settings. The light intensities for each pixel were determined in digital units (such that 0 = white and 255 = black; ImageJ). Pixel intensity divided by tissue area was computed and presented as mean ± standard deviation. Unpaired comparisons of mean values between groups were carried out by 2-way ANOVA and Student *t* test. A value of $P < 0.05$ was considered statistically significant.

Results

Blood pressures

The systolic blood pressures in the WKY rats and SHRs were 110 ± 12 mm Hg and 200 ± 10 mm Hg, respectively ($P < 0.05$). After Tempol treatment the blood pressure marginally decreased (107 ± 17 mm Hg in WKY, 188 ± 8 mm Hg in SHR).

Body and heart weights

The heart/body ratios were 0.0042 ± 0.001 in WKY rats, 0.0054 ± 0.002 in SHRs, 0.0036 ± 0.0002 in WKY rats treated with Tempol, and 0.0047 ± 0.003 in SHRs treated with Tempol. In both strains there was a significant reduction in the heart weight/body weight ratio after Tempol treatment ($P < 0.05$) (Table 1).

Plasma TBARS

Plasma concentrations of thiobarbituric acid were significantly decreased by Tempol treatment (Fig. 1, SHR Tempol vs. non-treated SHR; $P < 0.05$), whereas plasma concentrations of thiobarbituric acid of the Tempol-treated and non-treated WKY rats remained unchanged.

Effect of Tempol on MMP-9 and transcription factors in whole mount mesentery

The general pattern of the MMP-9 protein labeling in arterioles, capillaries, and venules was similar for both WKY rats and SHRs mesentery (Fig. 2). The average antibody label density as measured by light intensity over randomly selected arterioles, venules and capillaries in

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mesentery was significantly higher in the SHRs (by 10%, 17% and 15% respectively) ($P < 0.05$). The MMP-9 expression levels in mesentery microcirculation were significantly decreased ($P < 0.05$) in arterioles, venules and capillaries (18%, 16% and 17%) of Tempol-treated.

The average NF-κB antibody label density in randomly selected capillaries in mesentery was significantly higher in the SHRs ($P < 0.05$). In arteriole and venules the NF-κB expression levels were not significantly elevated (Fig. 3A). Tempol administration served to reduce NF-κB expression in all classes of mesentery microvessels in the SHR and in part also in the WKY rats. The c-fos expression levels were significantly increased by 5% and 7% in mesentery venules and capillary, respectively, but not significantly in arterioles (Fig. 3B). c-Fos expression was reduced by the Tempol treatment in arterioles ($P < 0.05$), capillaries and venules (18%, 23% and 20% respectively). In arterioles, the RAR-α expression levels of SHR were significantly elevated by 14% as compared with WKY rat, whereas the RAR-α expression levels were not significantly elevated in mesentery capillaries and venules (Fig. 3C). Tempol reduced RAR-α expression in mesentery arterioles and venules in the SHR but not in WKY rats, whereas in capillaries no significant differences between treated and untreated rats were detected.

Renal and myocardial MMP-9 expression level

Compared with WKY, the SHR had on average 19% higher MMP-9 expression in renal glomeruli ($P < 0.05$) and 20% in myocardium ($P < 0.05$) (Fig. 4A and B). The MMP-9 expression levels after Tempol treatment were significantly decreased on average by 12% compared with non-treated SHR in renal glomeruli and by 4% in the myocardium, whereas no significant differences were detected between Tempol-treated WKY and non-treated WKY.

Renal and myocardial transcription factor expression levels

Untreated SHR had higher NF-κB expression ($P < 0.05$) in renal glomerular areas on average by 18% and by 6% in myocardium compared with WKY. The NF-κB expression level in renal glomeruli after Tempol treatment was significantly decreased by 12% compared with untreated SHR, whereas in myocardium no significant differences were detected (Fig. 5A and B).

The SHR has elevated c-fos expression compared with WKY in renal glomeruli and myocardium (Fig. 6A and B). The c-fos expression level was not significantly different in renal glomeruli after Tempol treatment, whereas in myocardium it significantly decreased by 10% compared with untreated SHR (Fig. 6A and B).

Untreated SHR showed higher RAR-α label density compared with age-matched WKY rats in renal glomeruli and myocardium (Fig. 7A and B). The elevation of RAR-α expression in the SHR was partially prevented by systemic antioxidant treatment with Tempol in renal glomeruli and myocardium.

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Discussion

The present results indicate that in the SHR an antioxidant, such as the SOD mimetic Tempol, reduces MMP-9 expression and expression levels of NF-κB, c-fos and RAR-α that can bind to the promoter of the MMP-9 gene. Oxidative stress is increased in the SHR in form of increased production of superoxide anion, hydrogen peroxide and the hydroxyl radical, and/or can be decreased by antioxidant enzymes such as SOD (Manning et al., 2003). Tempol (e.g. 1 mmol/L in drinking water) reduces oxidative stress (Kobayashi et al., 2005), decreases in a mild fashion the blood pressure in several models of hypertension, including the SHR (Schnackenberg and Wilcox, 1999) and salt-dependent forms of hypertension (Hoagland et al., 2003), and also reduces endothelial apoptosis, capillary rarefaction (Kobayashi et al., 2005) and lymphocyte apoptosis (Kobayashi and Schmid-Schönbein, 2006).

Kobayashi et al. (2005) showed that Tempol does not significantly reduce systolic blood pressure when treatment was started at young age (four weeks old) and continued for four weeks but there was a mild reduction of blood pressure when treatment was continued for 12 weeks. In the current study treatment was initiated at eight weeks of age and while we found that it tended to reduce on average the blood pressure, this trend was not statistically significant. Since the treatment in this and the Kobayashi studies was otherwise the same, these results suggest that the ability of Tempol to reduce the blood pressure is relative modest and depends on age at the time of treatment and its duration.

MMPs are a family of zinc-dependent endopeptidases which we recently recognized for their ability to cleave the extracellular domain of several cell receptors causing co-morbidities such as insulin resistance (Schmid-Schönbein, 2012; Tran et al., 2011). Elevated MMP-9 activities are found in plasma of hypertensive patients (Ahmed et al., 2006; Yasmin et al., 2005) and in plasma, arteries and microvessels of hypertensive animals (both in vascular smooth muscle and endothelial cells) (Castro et al., 2008, 2010; Flamant et al., 2007; Rizzi et al., 2009). Chronic inhibition of MMPs strongly reduces blood pressures in the SHR (Rodrigues et al., 2010). The expression levels of MMPs can be regulated at multiple levels including gene transcription, post-translational modification and by interaction with their endogenous tissue inhibitors (TIMPs). Among several other MMPs, MMP-2 and MMP-9 are involved in cardiac remodeling (Polyakova et al., 2004) and may be related to progression of cardiac hypertrophy (Ahmed et al., 2006). Our results are consistent with the idea that increased MMP-9 activity may enhance matrix turnover, thus contributing to adverse myocardial remodeling associated with hypertensive heart disease (Campbell et al., 2005; Heymans et al., 2005).

Several studies have shown a link between ROS and MMPs (Hao et al., 2006; Schulz, 2007; Viappiani et al., 2009). MMP-9 was confirmed by immunohistochemistry and activity levels, and the oxidative stress marker by MDA concentration and superoxide formation with nitroblue tetrazolium reduction. Using antioxidants, such as Tempol, recent in-vivo evidence indicates that MMP-9 activity may be an important downstream mechanism of ROS production and ROS-induced vascular endothelial damage in hypertension (Castro et al., 2009). Conversely, it was also suggested that MMPs may be upstream mediators of ROS

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generation, as chronic MMP inhibition serves to reduce superoxide production in the SHR (DeLano and Schmid-Schönbein, 2008). In the present study, however, Tempol also inhibited both oxidative stress and MMP activity, and thus it remains uncertain whether increased ROS levels are responsible to trigger MMP activation or whether MMPs mediate the generation of oxidative stress, thus leading to hypertension. The levels of MMP-9 expression parallel levels of MDA activity. These findings support the concept that induction and activation of MMPs are upregulated by oxidative stress, including superoxide (Jian Liu and Rosenberg, 2005).

Furthermore, the MMPs have the ability to cleave the extracellular domain of a variety of membrane receptors in the SHR, such as the beta-2 adrenergic receptor in arteries and arterioles, that contribute to the elevated arteriolar tone and elevated blood pressure in the SHR (Rodrigues et al., 2010). The MMPs can cleave VEGFR2, which causes endothelial cell apoptosis and consequently capillary rarefaction (Castro et al., 2008). The MMPs also cleave the ectodomain of the insulin receptor, contributing to the insulin resistance in the SHR (DeLano and Schmid-Schönbein, 2008), as well as several other receptors whose function is compromised in the SHR and contributes to its co-morbidities (Tran et al., 2011). The MMP involvement can lead to ECM remodeling as well as specific co-morbidities via a receptor cleavage mechanism, while ROS generation may contribute to arteriolar vasoconstriction and central blood pressure elevation and systemic organ damage via lipid peroxidation and other forms of oxidation reactions in the SHR.

The involvement of transcriptional regulation of MMP-9, e.g. in the heart, suggests that MMP-9 activation is part of the cardiac cellular response in form of ventricular remodeling (Peterson et al., 2000). c-Fos, NF-κB and RAR-α have binding sites to the promoter of MMP-9 (Clark et al., 2008). In this study, up-regulation of transcription factors c-fos, NF-κB and RAR-α in kidney, heart, and microvessels may contribute to the MMP-9 over-expression in the SHR and thus contribute to the tissue damage found in these organs. Evidence to date indicates that oxidative stress increases NF-κB expression and therefore after translocation into the nucleus the MMP-9 expression activity (Konishi et al., 2001), thereby playing a critical role in determining cellular responses to extracellular stimuli, especially oxidative stress (Karin and Lin, 2002; Surh et al., 2005).

The high expression of MMP-9, NF-κB, c-fos and RAR-α in SHRs is prominent not only in arterioles but also in venules and capillaries. Direct immunolabeling of whole-mount specimens and of circulating cells that pass on a periodic basis through the higher-pressure regions of the SHR circulation (arteries and arterioles) and regions with the same blood pressure as the normotensive controls (capillaries and venules) exhibits elevated MMP levels (Fries et al., 2004). The evidence then supports the notion that elevated MMP activity and its consequences may be not entirely caused by blood pressure elevation but may be the consequence of a genetically derived systemic mechanism. Capillary rarefaction has been observed in patients with borderline hypertension (Sullivan et al., 1983) and in normotensive young adults with a genetic predisposition to high blood pressure (Noon et al., 1997). In the SHR model of hypertension capillary rarefaction may be produced by endothelial apoptosis, which in turn is caused by extracellular VEGFR-2 cleavage via enhanced and unchecked MMP activity (Tran et al., 2010).

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The elevated NF-κB expression and activation found in glomerular areas of the SHR are in agreement with previous studies in a renal model of hypertension (Quiroz et al., 2003). The activation of NF-κB in myocardium in SHR also supports the association between myocardial infarction, hypertrophy, and hypertension (Madhavan et al., 1994). Several AP-1 sites are present throughout the MMP promoters, and may contribute to gene expression. c-Fos controls the transactivation of inflammatory cytokines and MMPs (Hess et al., 2001; Sun et al., 2002) leading to hypertension. The interaction of c-fos with other transcription factors may also regulate tissue-specific expression of MMPs. Although initial studies demonstrated the pivotal role of the AP-1 site in MMP transcription in many cells, later studies have clearly shown that it must cooperate with a variety of cis-acting sequences found in the upstream regions of the MMP promoters (Matthew et al., 2002).

An in-vitro approach shows that the production of MMP-9 in J3B1A mammary epithelial cells is induced, in a dose- and time-dependent manner, by the addition of retinoic acid (RA) via the RAR-α receptor. RA is essential for the maintenance of the ECM in renal basement membranes (Marı'n et al., 2005) pointing towards a potential role of RA in hypertension in the SHR. Despite many studies that have shown that oxidative stress is elevated in hypertensive individuals compared with normotensive (Kobayashi and Schmid-Schönbein, 2006; Lacy et al., 1998), few studies have provided evidence that a reduction in oxidative stress is associated with a reduction in blood pressure and cardiovascular disease risk in hypertensive humans. This issue remains a challenge in hypertension research and points towards the fact that ROS may not be a causative event for the elevated arterial blood pressure. While in humans unexplored, the majority of the ROS production in the SHR vasculature occurs in venules and thus indicates that elevated blood pressure and ROS production may be parallel events, i.e. co-morbidities. Tempol treatment serves to reduce ROS production in the SHR with less effect on the blood pressure. Thus Tempol may reduce ROS production and consequently have an effect on apoptosis and other organ damage mechanisms, even though its effect on blood pressure may be less pronounced.

In conclusion, the current results suggest that elevated NF-κB, c-fos and RAR-α expressions and MMP-9 activity in the SHR are superoxide-dependent.

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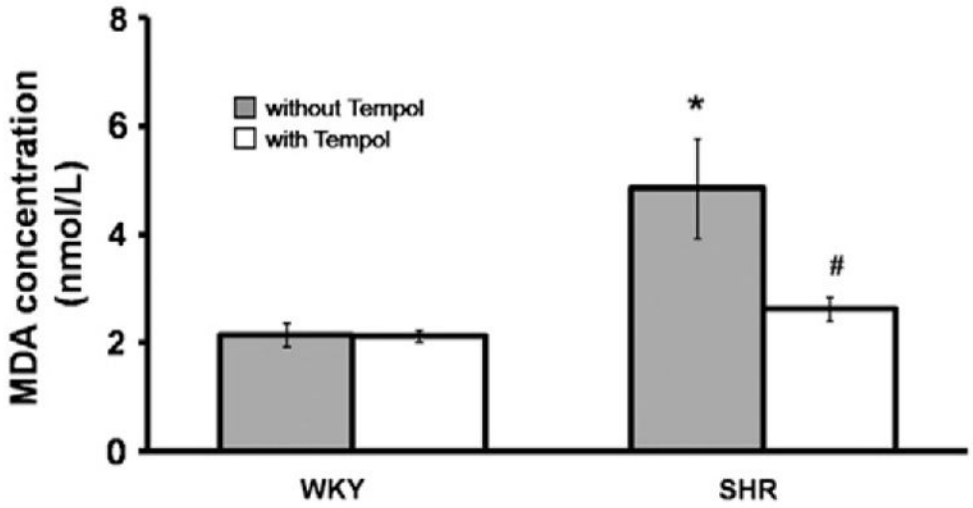

Fig. 1.

Malondialdehyde (MDA in nmol/L) analyzed by thiobarbituric acid reactive substances in plasma treated without and with Tempol (1 mmol/L). Results are expressed as mean ± SD ($N = 6$ for each group). *$P < 0.05$ compared to WKY rats. $^{\#}P < 0.05$ compared to untreated SHR.

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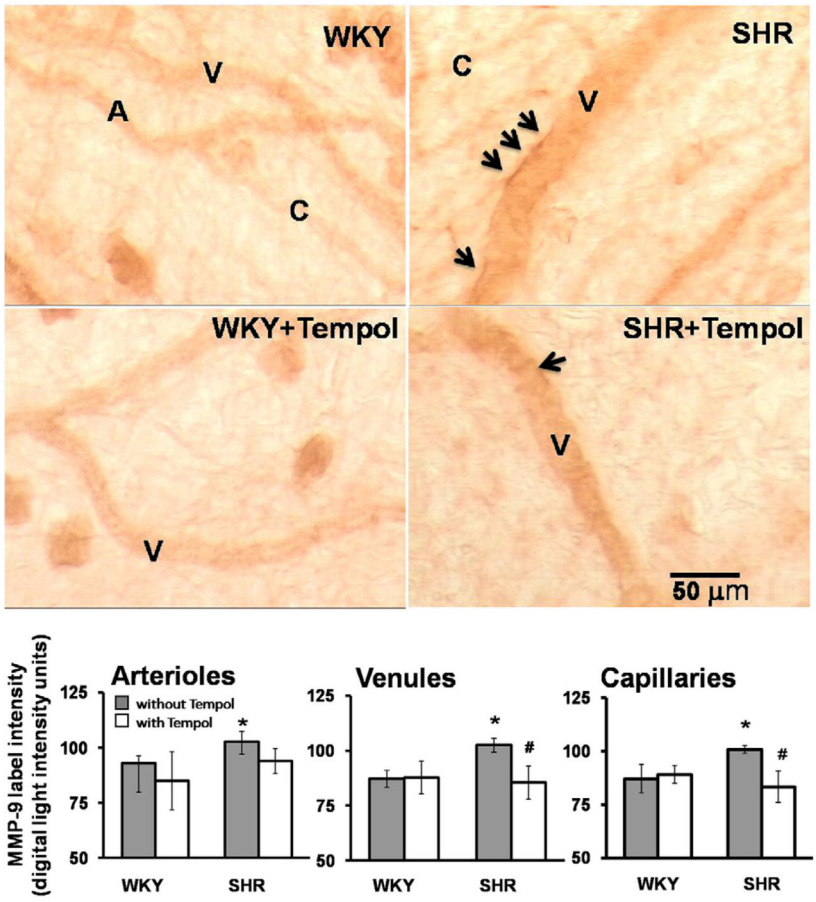

Fig. 2.

Low-magnification overviews of MMP-9 levels immunolabeled with VECTOR *NovaRED* substrate in whole-mount rat mesentery microcirculation of WKY (left) and SHR (right). Selected arterioles (A), venules (V) and capillaries (C) are shown (top panels). Mean ± standard deviation of digital light intensity of MMP-9 substrate label of control normotensive and hypertensive rat (bottom panels).*$P < 0.05$ compared to WKY rats. $^{\#}P < 0.05$ compared to untreated groups.

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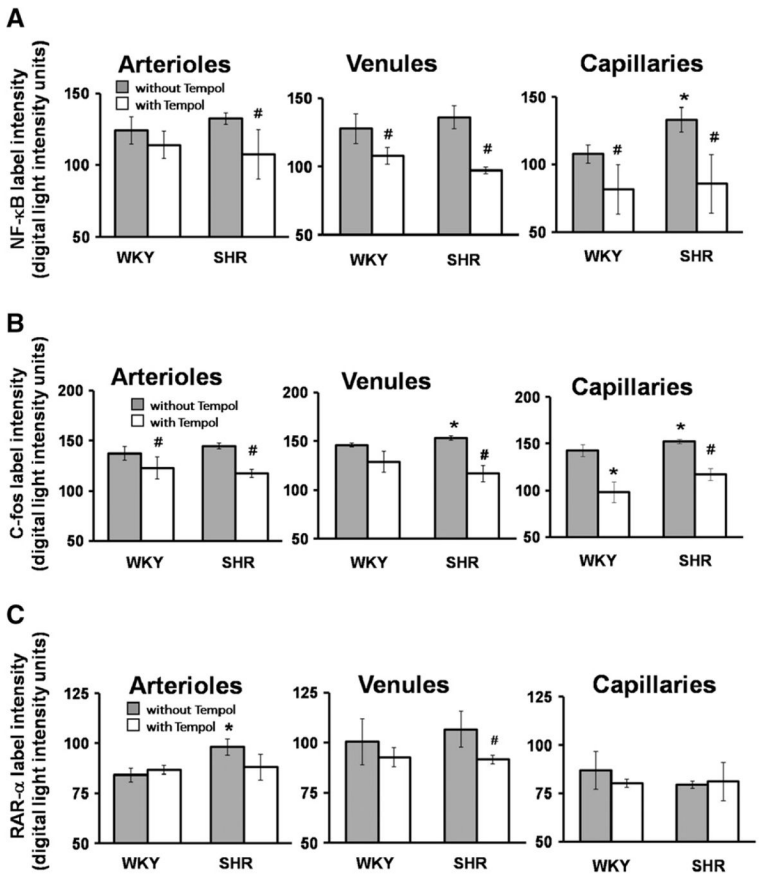

Fig. 3.

Intensity of NF-κB (A), c-fos (B) and RAR-α (C) levels immunolabeled with VECTOR *NovaRED* substrate in whole-mount rat mesentery microcirculation of WKY (left) and SHR (right). $^{*}P < 0.05$ compared to WKY rat. $^{\#}P < 0.05$ compared to untreated groups.

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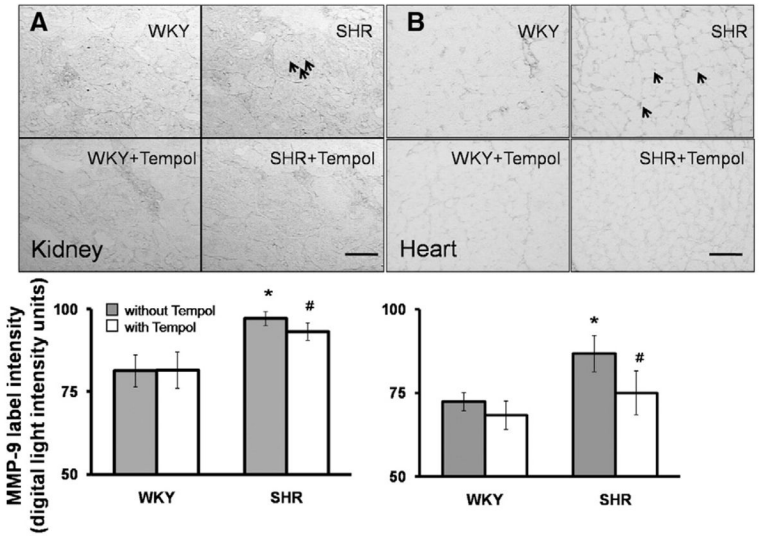

Fig. 4.

Micrographs of frozen immunohistochemical sections with primary antibody against MMP-9 expression in renal glomerular tissue (A) and myocardium (B). The light absorption measurements expressed as digital units (bottom panels). Images are shown without counterstain to facilitate digital light absorption measurements. $^{*}P < 0.05$ compared to WKY. $^{\#}P < 0.05$ compared to untreated SHR. Scale bar = 50 μm.

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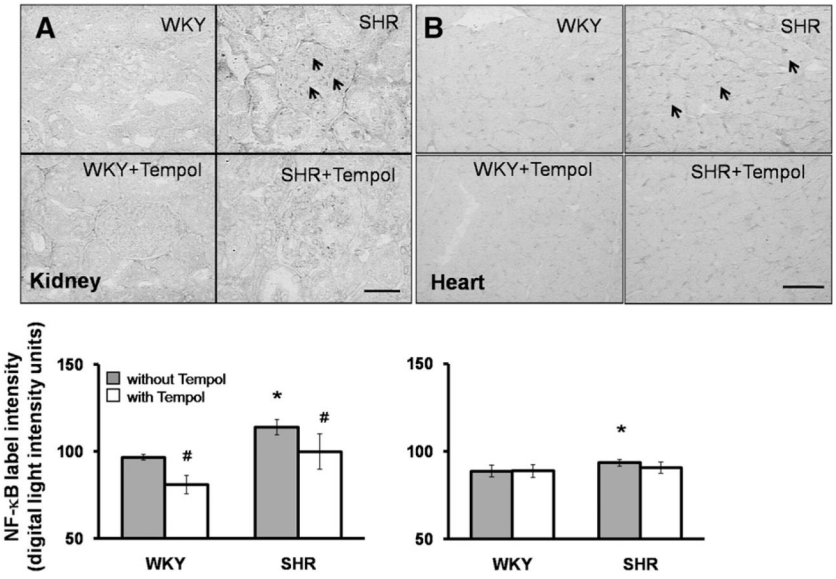

Fig. 5.

Micrographs of frozen immunohistochemical sections with primary antibody against-NF-κB expression in renal glomerular tissue (A) and myocardium (B). Light absorption measurements expressed in digital units (bottom panels). Images are shown without counterstain to facilitate digital light absorption measurements. **P* < 0.05 compared to WKY. $^{\#}P$ < 0.05 compared to untreated SHR. Scale bar = 50 μm.

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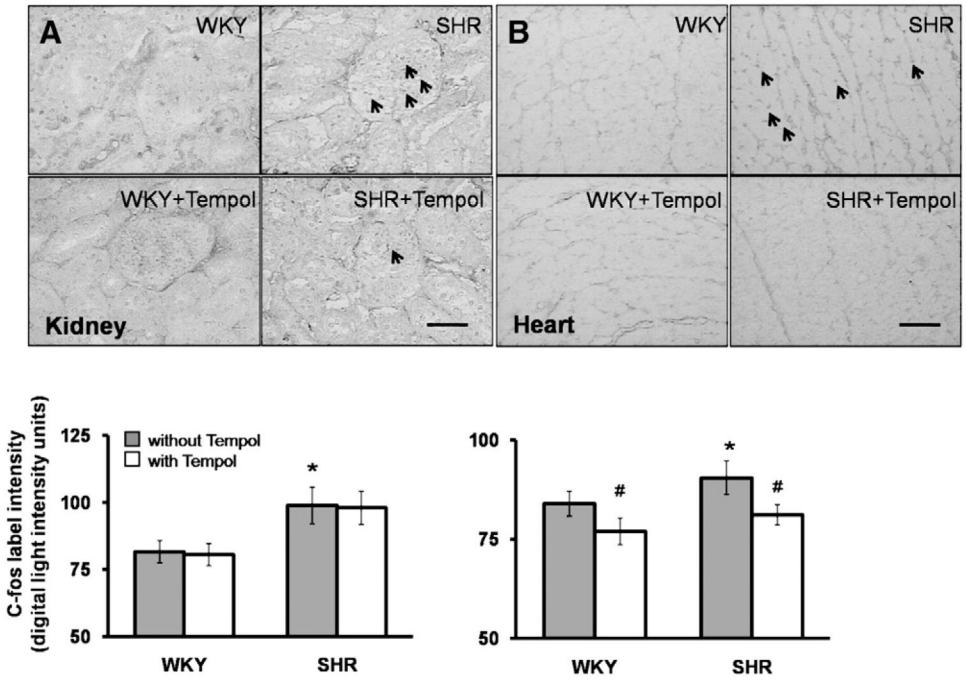

Fig. 6.

Micrographs of frozen immunohistochemical sections with primary antibody against-c-fos expression in renal glomerular tissue (A) and myocardium (B). Light absorption measurements are expressed in digital units (bottom panels). Images are shown without counterstain to facilitate digital light absorption measurements. *$P < 0.05$ compared to WKY. $^{\#}P < 0.05$ compared to untreated SHR. Scale bar = 50 μm.

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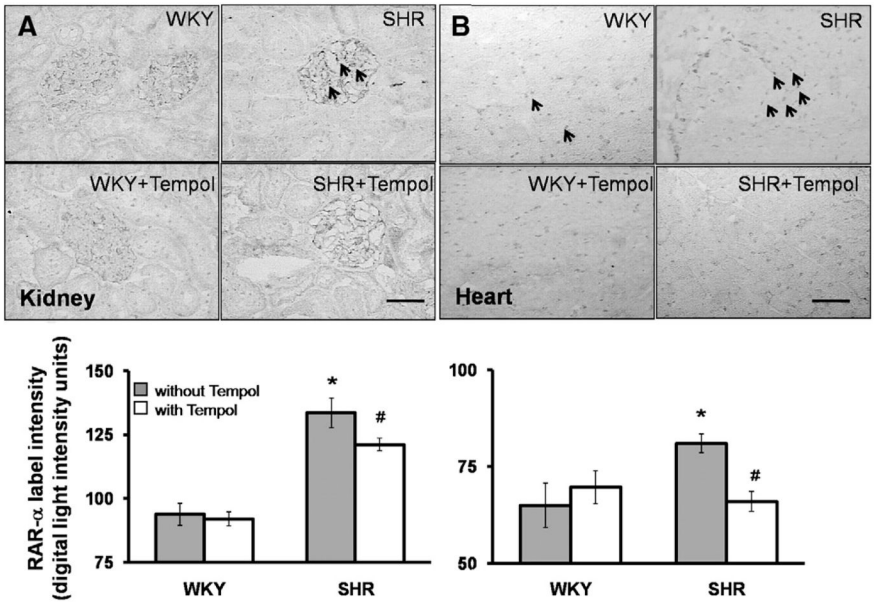

Fig. 7.

Micrographs of frozen immunohistochemical sections with primary antibody against RAR-α expression in renal glomerular tissue (A) and myocardium (B). The light absorption measurements are expressed in digital units (bottom panels). Images are shown without counterstain to facilitate digital light absorption measurements. **P* < 0.05 compared to WKY. $^{\#}P < 0.05$ compared to untreated SHR. Scale bar = 50 μm.

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Table 1

Final body and heart weights.

Parameter	WKY	SHR	WKY with Tempol	SHR with Tempol
Body weight (g)	267.3 ± 1.8	330.7 ± 9.1	284.5 ± 6.3*	330.2 ± 10.5
Heart weight (g)	1.14 ± 0.02	1.67 ± 0.09	1.05 ± 0.04*	1.46 ± 0.12
Heart weight/body weight ratio	0.0042 ± 0.001	0.0054 ± 0.002	0.0036 ± 0.002*	0.0047 ± 0.003**

* $P < 0.05$ compared to WKY rats.

** $P < 0.05$ compared to untreated SHR.