Effects of Melatonin and Pycnogenol on Small Artery Structure and Function in Spontaneously Hypertensive Rats

Rita Rezzani, Enzo Porteri, Carolina De Ciuceis, Francesca Bonomini, Luigi F. Rodella, Silvia Paiardi, Gianluca E.M. Boari, Caterina Platto, Annamaria Pilu, Daniele Avanzi, Damiano Rizzoni, Enrico Agabiti Rosei

Abstract—It was suggested that oxidative stress has a key role in the development of endothelial dysfunction, as well as microvascular structural alterations. Therefore, we have investigated 2 substances with antioxidant properties: melatonin and Pycnogenol. We treated 7 spontaneously hypertensive rats (SHRs) with melatonin and 7 with Pycnogenol for 6 weeks. We compared results obtained with those observed in 7 SHRs and 7 Wistar-Kyoto normotensive control rats kept untreated. Mesenteric small resistance arteries were dissected and mounted on a wire myograph, and a concentration-response curve to acetylcholine was performed. Aortic contents of metalloproteinase 2, Bax, inducible NO synthase, and cyclooxygenase 2 were evaluated, together with the aortic content of total collagen and collagen subtypes and apoptosis rate. A small reduction in systolic blood pressure was observed. A significant improvement in mesenteric small resistance artery structure and endothelial function was observed in rats treated with Pycnogenol and melatonin. Total aortic collagen content was significantly greater in untreated SHRs compared with Wistar-Kyoto control rats, whereas a full normalization was observed in treated rats. Apoptosis rate was increased in the aortas of untreated SHRs compared with Wistar-Kyoto control rats; an even more pronounced increase was observed in treated rats. Bax and metalloproteinase 2 expressions changed accordingly. Cyclooxygenase 2 and inducible NO synthase were more expressed in the aortas of untreated SHRs compared with Wistar-Kyoto control rats; this pattern was normalized by both treatments. In conclusion, our data suggest that treatment with Pycnogenol and melatonin may protect the vasculature, partly independent of blood pressure reduction, probably through their antioxidant effects. (Hypertension. 2010;55:1373-1380.)

Key Words: endothelial function ■ melatonin ■ Pycnogenol oxidative stress

Reactive oxygen species (ROS) are generated as by-products of cellular respiration and other metabolic processes and may contribute to oxidative damage. The ROS family includes several molecules that have divergent effects on cellular function, namely, regulation of cell growth and differentiation, modulation of extracellular matrix production and breakdown, inactivation of NO, and stimulation of protein kinases and proinflammatory genes. Importantly, some of these actions are associated with pathological changes in cardiovascular tissues. In cardiovascular disease, increased ROS production leads to endothelial dysfunction, increased vascular contractility, vascular smooth muscle cell growth and apoptosis, monocyte migration, lipid peroxidation, inflammation, and increased deposition of extracellular matrix proteins, all processes contributing to vascular damage. ROS may be important in the development and maintenance of hypertension, in terms of excess production of oxidants, decreased NO bioavailability, and decreased antioxidant capacity in the vasculature and kidneys. Hypertension may contribute to structural and functional alterations in small resistance arteries by inducing extracellular matrix reorganization, remodeling and growth (hypertrophy/hyperplasia) of vascular smooth muscle cells, and endothelial dysfunction. Alteration of NO synthesis may be related to the pathogenesis of hypertension. In different types of hypertension, the expression of inducible NO synthase (iNOS) is upregulated, and the iNOS-dependent overproduction of NO may contribute to the pathology associated with hypertension. Increased ROS can react with NO and form a very unstable and reactive oxidizing species, thus inactivating vasodilator NO. The impaired endothelium-dependent vasodilatation has been linked to decreased NO bioavailability. Previous studies hypothesize that oxidative stress promotes endothelial cell apoptosis and a reduction in microvessel density in tissues such as skeletal muscle, skin, and myocardium in spontaneously hypertensive rats (SHR). This phenomenon, designated as structural or anatomic rarefaction, leads to an
increase in peripheral vascular resistance and localized reduction in oxygen delivery to the tissue.\textsuperscript{12} ROS, as a mediator of an apoptosis/proliferation imbalance in hypertension, may modify intracellular elements, such as protein kinase, nuclear factor-κB, mitochondria, and Bcl2 family proteins.\textsuperscript{13} Moreover, ROS can induce cyclooxygenase (COX) 2 expression\textsuperscript{14} probably through an inflammatory response.\textsuperscript{15} Oxidative stress seems to induce an enhancement of COX-2 expression in hypertensive but not in normotensive rats.\textsuperscript{16} ROS can also regulate matrix metalloproteinase (MMP) activity, suggesting that the generation of such species by inflammatory cells controls MMP activation and inactivation.\textsuperscript{17} A consequence of a reduced activity of MMPs is an enhanced collagen deposition in the microvessels and in several organs.\textsuperscript{9,18} Increased production of superoxide anion and hydrogen peroxide, reduction of NO synthesis, and decreased bioavailability of antioxidants have been demonstrated in experimental and human hypertension.\textsuperscript{19} Several studies demonstrated that hypertension may be beneficially affected by antioxidants treatment\textsuperscript{20,21} and that these substances may improve vascular function and structure, prevent target-organ damage, and reduce blood pressure in animal models and in human hypertension.\textsuperscript{20–22} Antioxidants are, therefore, expected to decrease the vulnerability of the organism to oxidative challenges by acting as ROS scavengers, iron chelators, and enzyme modulators.\textsuperscript{23}

The actions of melatonin, the main product of the pineal gland, are not restricted to its role in the neuroendocrine physiology. Since 1993, melatonin has been known to be a radical scavenger with the ability to remove ROS\textsuperscript{24,25}; however, melatonin also acts as an antioxidant through the upregulation of antioxidant enzymes and the downregulation of pro-oxidant enzymes.\textsuperscript{26} Melatonin is distributed ubiquitously in organisms and, as far as is known, in all cellular compartments, and it quickly passes through all biological membranes.\textsuperscript{27} Antioxidant properties of melatonin, which could participate in the mechanism of its antihypertensive effect, were demonstrated in experimental hypertension.\textsuperscript{28} Anti-inflammatory actions of melatonin are related to the inhibition of prostaglandins effects, and, in particular, COX-2 downregulation.\textsuperscript{29} Moreover, melatonin offers protection against fibrosis by preventing oxidative damage caused by lipid peroxidation and glutathione depletion.\textsuperscript{30}

Pycnogenol, a standardized and patented extract from the bark of the French maritime pine (Pinus pinaster Aiton), consists of a concentrate of polyphenols.\textsuperscript{31} Pycnogenol is marketed worldwide as a food supplement or as an herbal drug. Its components are nutritionally important constituents of fruit, recognized by the food industry as powerful antioxidants. Pycnogenol may protect against oxidative stress in several cell systems by doubling the intracellular synthesis of antioxidative enzymes and by acting as a potent scavenger of free radicals. In addition to stimulation of antioxidative enzymes and radical scavenging, there are other mechanisms that contribute to the anti-inflammatory effects of Pycnogenol. It may modify the concentration of mRNA coding for iNOS resulting in a lower concentration of iNOS and a reduction in the generation of inflammatory NO radicals. In vitro experiments show that Pycnogenol inhibits angiotensin-converting enzyme, which consequent possible antihypertensive activity.\textsuperscript{32}

On the basis of all of the data mentioned previously, the aim of our study was to extensively investigate the antioxidant properties of melatonin and Pycnogenol in an experimental model of genetic hypertension.

**Materials and Methods**

**Experimental Design**

Twenty-eight male rats (21 SHRs and 7 Wistar-Kyoto rats [WKYs]) at 6 week of age were included in the study. The animals were obtained from Charles River Laboratory (Calco, Italy). Food and water were supplied ad libitum, and the water consumption of the animals was continuously monitored. Seven SHRs were treated with melatonin (Sigma) and 7 with Pycnogenol (Horphag Research Ltd). Both drugs were administered in the drinking water from week 6 to week 12 of age at a dose of 10 mg/kg per day. The concerned dose was chosen on the basis of previous published studies.\textsuperscript{21,33} Melatonin was administered in containers wrapped in aluminum foil because melatonin is light sensitive. Seven WKY rats and 7 SHRs were kept untreated as controls.

Systolic blood pressure values were measured noninvasively by a tail-cuff method (ITTC Life Science Instrument) at the end of the treatment period. At the end of the treatment, the animals were killed by decapitation. The aorta was removed and washed briefly in PBS (0.1 mol/L [pH 7.4]) and cut in 2 parts: one slice was frozen and the other slice was immediately fixed in 4% paraformaldehyde and embedded in paraffin wax. Paraffin-embedded slices (5 μm thick) were treated with Sirius red for collagen content evaluation, DNA fragmentation (TUNEL staining) for morphological studies, and with metalloproteinase 2, Bax, iNOS, and COX-2 for immunohistochemical studies.

Small mesenteric arteries were isolated and used for structural and functional evaluation by a micromyography. All of the procedures followed were in accordance with the guidelines of our institution (Medical School, University of Brescia).

**Micromyography**

Second-order branches of the mesenteric artery (∼150 to 250 μm of average diameter; 2-mm long) were dissected and mounted as a ring preparation on an isotonic myograph (Danish Myo Technology) by threading onto 2 stainless steel wires (40 μm diameter).

The following morphological parameters were evaluated: media thickness, normalized internal diameter, media: lumen ratio, and media cross-sectional area. Details about the micromyographic technique of evaluation of small artery morphology were reported previously.\textsuperscript{34,35} Endothelium-dependent relaxation was assessed by measuring the vasodilator response to acetylcholine (cumulative dose-response curve from 10\textsuperscript{-9} to 10\textsuperscript{-5} mol/L) after precontraction with norepinephrine (5 μmol/L). Endothelium-independent relaxation was assessed with sodium nitroprusside (10\textsuperscript{-5} to 10\textsuperscript{-7} mol/L) in norepinephrine-precontracted vessels. The response to acetylcholine and sodium nitroprusside was expressed as the percentage of decrease of the wall tension. The average values obtained from 2 vessels in each experiment were considered.

**Collagen Content Evaluation of Aorta**

Aorta sections were stained with phosphomolybdic acid 0.1%, washed in water, and then immersed in Picrosiris red (Sigma red 0.1% in picric acid). Collagen fibers were detected by polarized light microscopy (Olympus), which allows for visualization of collagen fibers of different thicknesses with different colors. Type I collagen fibers are orange to red, whereas the thinner type III collagen fibers appear yellow to green.\textsuperscript{36}

For quantitative analysis of total collagen content, after Sirius red staining digital pictures at ×40 magnification were taken using an optical microscope (Olympus) and then analyzed using an image...
Values for systolic blood pressure at the end of the treatment period, systolic blood pressure was significantly higher in untreated SHRs than in WKY controls \((P<0.001)\). The SHRs treated with melatonin or Pycnogenol showed a small but significant reduction in systolic blood pressure \((P<0.001\) and \(P<0.05\) versus untreated SHRs, respectively).

### Micromyography

Morphological results from mesenteric small resistance arteries are reported in Table 1. No significant differences in internal diameter were observed between groups. Media thickness and media:lumen ratio were significantly increased in untreated SHRs compared with WKY control rats. SHRs treated with Pycnogenol showed a significant reduction in the media:lumen ratio, whereas in those treated with melatonin a nonstatistically significant trend to observe a smaller media: lumen ratio compared with untreated SHR was observed. Media cross-section and media thickness of mesenteric small arteries were significantly reduced by both melatonin and Pycnogenol. A significant improvement in mesenteric small resistance artery endothelial function, assessed as vasodilatation to acetylcholine, was observed in SHRs treated with Pycnogenol or melatonin \((P<0.001)\) compared with untreated SHRs. However, the response to acetylcholine was not fully normalized compared with that observed in WKY control rats \((P<0.001)\). No difference among groups in the vasodilator response to sodium nitroprusside \((endothelium-independent vasodilatation)\) was observed (data not shown).

### Collagen Content

Total aortic collagen content was significantly greater in untreated SHRs \((P<0.001)\) compared with WKY control rats and with SHRs treated with melatonin or Pycnogenol. Polarized light microscopy evidenced a prevalence of red fibers in untreated SHRs corresponding with type I collagen \((fibrotic\) collagen\); on the contrary, in the remaining groups a prevalence of green fibers was observed because of the presence of type III collagen \((constitutive\) collagen; Figure 3A).

### TUNEL Staining

The number of TUNEL-positive cells was greater in untreated SHRs compared with WKY control rats. In SHRs treated with melatonin or Pycnogenol, the number of apo-
ptotic cells was significantly increased even compared with untreated SHRs. The quantitative data are reported in Figure 4.

### Immunohistochemical Studies for MMP2, Bax, iNOS, and COX-2

In untreated SHRs, the expression of MMP2 was lower compared with WKY controls. Both melatonin and Pycnogenol treatments increased MMP2 expression toward that observed in WKY control rats (Figure 3B). The semiquantitative analysis of Bax, reported in Table 2, shows that Bax was more expressed in untreated SHRs compared with WKY control rats, and a further increase was observed in SHRs treated with melatonin or Pycnogenol compared with untreated SHRs (Figure 5A). A greater iNOS expression was observed in untreated SHRs compared with the WKY control group (Figure 5B). Melatonin or Pycnogenol administration decreased iNOS protein expression compared with untreated SHRs (Figure 5B). A higher COX-2 expression was observed in untreated SHRs compared with WKY controls (Figure 5C), which was prevented by both melatonin and Pycnogenol administration (Figure 5C). Semiquantitative data are reported in Table 2.

Please see the online Data Supplement at [http://hyper.ahajournals.org](http://hyper.ahajournals.org) for supplemental data about immunohistochemical evaluation of cytochrome c, endothelial NO synthase, vascular endothelial growth factor expression, and Von Kossa staining for calcium.

### Discussion

Several pieces of evidence indicate that melatonin may have an antihypertensive effect. The potential use of melatonin in the treatment of hypertension represents a stimulating and
to normal values.41 In hypertensive rats, Pycnogenol dose-dependently decreased systolic blood pressure after intravenous injection. In a double-blind, placebo-controlled, crossover study in hypertensive patients, oral administration of Pycnogenol reduced systolic blood pressure after intravenous injection.10,14,18,42,43 Similarly, in hypertensive rats, Pycnogenol dose-dependently decreased systolic blood pressure.44

Blood pressure could conceivably be reduced by Pycnogenol by other mechanisms in addition to inhibition of angiotensin-converting enzyme or thromboxane B2.32 The present study demonstrated that melatonin and Pycnogenol may slightly decrease blood pressure and improve vessel morphology, as well as endothelial function, probably through a decrease of oxidative stress. As reported previously, hypertension has been associated with changes in vascular responses, such as impairment of endothelium-dependent vasodilator responses or enhancement of vasoconstrictor responses to different agonists, an excessive deposition of fibrillar collagen, a decrease of the proteins related to collagen degradation (MMP2), with changes in the regulation of apoptotic process, and with an increase of oxidative stress (iNOS) and inflammation (COX-2).10,14,18,42,43

Interesting interactions between vascular COX-2 and iNOS were demonstrated.44,45 In rat mesenteric arteries, a reduced NO availability may be partly attributable to an iNOS-dependent enhanced COX-2 expression.44 Atorvastatin restored NO availability by increasing iNOS levels and by abrogating vascular NADPH oxidase–driven superoxide production, which also results in a downregulation of COX-2–dependent 8-isoprostanate generation.45 Endothelial-independent responses to sodium nitroprusside may be influenced by microvascular structural alterations. However, in our study no difference was observed between untreated SHRs and WKY controls, despite the presence of a significantly different microvascular structure. On this basis, no changes should have been expected after treatment.

Clinical and experimental studies have shown that hypertension is associated with vascular remodeling of small resistance arteries, mainly characterized by an increase in the media:lumen ratio.5,9,18 After 6 weeks of treatment, we have observed that melatonin and Pycnogenol significantly improved mesenteric small resistance artery morphology. Other than changes in vascular smooth muscle cells, extracellular matrix deposition is an important feature of hypertensive vascular remodeling, and it may contribute to structural changes through reorganization of vascular wall components.46 In our study we have observed a pronounced vascular fibrosis, together with a decrease in MMP2 expression. We have demonstrated previously that interstitial matrix, and, namely, collagen content, as well as changes induced by treatment, are similar in aortas and in mesenteric small resistance arteries of SHRs and WKY rats.18 Therefore, small and large rat vessels show similar behaviors in terms of indices of fibrosis, and it is possible to postulate that our observations in the aortas might be extended also to smaller vessels.

An increase in ROS production and, consequently, in oxidative stress may lead to the development of hypertension47 and to an altered regulation of MMPs.17 Our data show that both an MMP2 decrease and an increase of collagen content in the vascular wall are simultaneously present in untreated SHRs, as demonstrated previously for other organs.48 The results obtained in treated SHRs confirm that melatonin and Pycnogenol prevent collagen accumulation and MMP2 decrease probably through their free radical scavenger activity and antioxidant properties.25,26,31

Sharifi and Schiffrin42 hypothesized an involvement of apoptosis in hypertension, suggesting that both apoptosis and growth processes may be involved in the development of vascular remodeling. Our results show an increase of aortic apoptotic cells in treated SHRs compared with WKY controls. The apoptosis progression in the vessels may help us to understand vascular remodeling processes. The amount of vascular apoptotic cells in SHRs appears to parallel the development of hypertension.57 NO upregulates Fas, a mediator of cell death, and, through cGMP production, it may induce apoptosis of vascular smooth muscle cells.52 We have observed that oxidative stress increases iNOS expression in untreated SHRs, confirming previous reports.10,43 The increased activity of iNOS may also induce a marked production of NO that can contribute to Fas upregulation and to the increase of apoptosis in untreated SHRs.49 The increased apoptosis rate in treated SHRs suggests that cell death may have a role in the antihypertensive and antioxidant actions of some compounds. The increased Bax expression in SHRs treated with melatonin and Pycnogenol may promote programmed cell death and, consequently, regression of vascular

<table>
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<tr>
<th>Parameter</th>
<th>Untreated SHRs</th>
<th>Untreated WKY Rats</th>
<th>SHRs Treated With Pycnogenol</th>
<th>SHRs Treated With Melatonin</th>
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<tr>
<td>MMP2</td>
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<td>+</td>
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<td>Bax</td>
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<td>iNOS</td>
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<td>COX-2</td>
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Table 2. Semiquantitative Evaluation of Protein Expression
remodeling through antigrowth effects. In fact, an increase in apoptotic cells may compensate excessive cellular proliferation, possibly observed in hypertension.\textsuperscript{8,37} This concept, which some authors refer to as “therapeutic apoptosis,”\textsuperscript{750} may play a major role in the regression of vascular alterations.\textsuperscript{37} The mechanism by which melatonin and Pycnogenol induce an increase of apoptosis is not known; a plausible hypothesis is that these substances are involved in the mitochondrial pathways and may activate caspase 9, the trigger of the intrinsic apoptotic pathway.\textsuperscript{51,52} Therefore, in untreated SHRs and in WKY controls an increased apoptosis rate may help in maintaining eutrophic (rather than hypertrophic) the vascular remodeling process. Because apoptosis might also be one of the mechanisms underlying the beneficial effects of melatonin and Pycnogenol on small artery structure, it is theoretically possible that the apoptosis rate may remain persistently high in treated rats. However, there is no doubt that relationships between apoptosis and vascular structure are complex, and it is not completely clear whether vascular apoptosis may be regarded as a mainly protective or a harmful process.

Hypertension may also be considered, to some extent, an inflammatory disease.\textsuperscript{53} We have observed an increase in COX-2 expression in untreated SHRs, which may be related to oxidative stress and ROS production. COX-2 contributes to vascular remodeling, and it has been associated with changes in vascular functional responses, such as impairment of endothelium-dependent vasodilator responses and/or an increase in vasoconstrictor responses.\textsuperscript{14} The anti-inflammatory and antioxidant actions of melatonin and Pycnogenol may reduce COX-2 expression in treated SHRs. Melatonin probably acts through its metabolite (AFMK) that inhibits the synthesis of a COX-2–dependent product (prostaglandin), a prostaglandin involved in vascular responses.\textsuperscript{15,29} Also, Pycnogenol seems to be effective in inhibiting both COX-1 and COX-2.\textsuperscript{54}

Limitation of the Study
Because of the semiquantitive nature of our approach, it is impossible to make reliable correlations between our findings and, therefore, to speculate about the specific pathophysiological mechanisms and relationships between our reported observations. In addition, we did not directly measure polyphenols contained in Pycnogenol or evaluated plasma levels of polyphenols. However, previous studies have shown that phenolic acids and procyanidins are the most represented polyphenols\textsuperscript{31} and that, after 6 weeks of supplementation with Pycnogenol, a significant increase in plasma polyphenol levels was detectable, which was reversed after therapeutic washout.\textsuperscript{55}

Perspectives
Melatonin and Pycnogenol may prevent structural and functional vascular alterations, which are the hallmark of hypertension. These substances, therefore, seem to possess beneficial properties, probably secondary to antioxidant, antiinflammatory, and free radical scavenging effects. It may be hypothesized that melatonin and Pycnogenol may, at least in part, act as antihypertensive compounds. The observation of
a regression of hypertension-related vascular alterations may have important therapeutic consequences.

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Disclosures

None.

References


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Online supplement


The first two Authors have equally contributed to the manuscript

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Immunohistochemical evaluation of aortic cytochrome C expression in the different groups.

SHR: untreated SHR; WKY: Wistar-Kyoto normotensive controls; SHR+PYC: SHR treated with pycnogenol; SHR+MEL: SHR treated with melatonin. Bar: 4 μm.
Immunohistochemical evaluation of aortic eNOS expression in the different groups.

SHR: untreated SHR; WKY: Wistar-Kyoto normotensive controls; SHR+PYC: SHR treated with pycnogenol; SHR+MEL: SHR treated with melatonin. Bar: 10 μm.
Immunohistochemical evaluation of aortic VGEF expression in the different groups.

SHR: untreated SHR; WKY: Wistar-Kyoto normotensive controls; SHR+PYC: SHR treated with pycnogenol; SHR+MEL: SHR treated with melatonin. Bar: 10 μm.
Figure S4: Von Kossa

Immunohistochemical evaluation of aortic Von Kossa staining for calcium in the different groups. SHR: untreated SHR; WKY: Wistar-Kyoto normotensive controls; SHR+PYC: SHR treated with pycnogenol; SHR+MEL: SHR treated with melatonin. Bar: 10 μm.
RESULTS

Regarding Cytocrome C expression we observed a dot positivity (brown spots) for the protein in control rats (Figure S1). This positivity became moderate and cytoplasmic in untreated SHR and remained cytoplasmic also after antioxidants treatment (Figure S1).

Immunohistochemical analysis of endothelial nitric oxide synthase (eNOS) localization showed an increase in labelling intensity in vessels from untreated SHR rats compared with WKY controls (Figure S2). Antioxidant administration maintained the increase of eNOS expression compared to WKY controls (Figure S2). Immunohistochemical analysis of vascular endothelial growth factor (VEGF) localization showed a weak positivity in WKY controls. In untreated SHR there was an increase in VEGF protein expression (Figure S3). After melatonin and pycnogenol treatment VEGF expression remain increased compared with WKY controls (Figure S3).

Von Kossa staining for calcium showed an increase in calcium deposits in the tunica media of vessels from SHR animals compared with WKY controls (Figure S4). After antioxidant treatment the calcium level became similar to that observed in WKY controls (Figure S4).
DISCUSSION

Our results showed cytochrome c translocation from mitochondria to cytoplasm in SHR animals. Once released, cytochrome c triggers the activation of caspase cascade activating the initiator caspases, such as caspase-3 induction. Finally, caspase-3, in turn cleaves various proteins leading to morphological and biochemical features characteristic for apoptosis (Gupta and Knowlton 2005, Orrenius 2004).

Over-production of reactive oxygen species induces concomitant release of cytochrome from mitochondria (Chirou MV et al, 2009). Reactive oxygen species generated from the mitochondrial electron transport chain may also induce cytochrome c dissociation (Petrosillo G et, 2001)

In our study we observed in untreated SHR an upregulation of eNOS expression. Aortic eNOS levels remained elevated after antioxidants treatment. The increase of eNOS expression might be interpreted as a compensatory mechanism to maintain the production of bioactive NO in the presence of an increased oxidative stress (Vera et al. 2007).

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<th>Semiquantitative evaluation of protein expression</th>
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<tr>
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<tr>
<td>Untreated SHR</td>
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<tr>
<td>eNOS</td>
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<tr>
<td>Cyt C</td>
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<td>VEGF</td>
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Semiquantitative data:

(+) weak

(++) moderate

(+++) strong
The results from the morphological and immunohistochemical study could be interpreted as supporting a protective role for VEGF in the development of hypertension. This is consistent with VEGF acting as an endogenous regulator of endothelial integrity in the vessel wall. This viewpoint has been supported by several studies, including those that show that administration of transgenic or recombinant VEGF to injured arteries can lead to accelerated re-endothelialization (Tsurumi et al., 1997, Van Belle et al. 1997). An increased expressions of VGEF in SHR was previously observed (Jesmin S. et al. 2007).

Von Kossa staining for calcium showed an increase in calcium deposits in the tunica media of vessels from SHR animals compared with WKY controls. After antioxidant treatment the calcium level became similar to that observed in WKY controls. Vascular calcification is enhanced in SHR rats and can be the result of alterations in mineral metabolism.
References